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METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

CROSS-REFERENCE

[0001] This application is a continuation of U.S. Application No. 18/941,832, filed November 8, 2024, which is a continuation of U.S. Application No. 17/667,285, filed on February 8, 2022, which is a continuation of U.S. Application No. 17/081,694 filed on October 27, 2020, which issued as U.S. Pat. No. 11,304,950 on April 19, 2022, and which is a continuation of International Application No. PCT/US2019/029486 filed on April 26, 2019, which claims the benefit of U.S. Provisional Patent Application No. 62/663,951, filed April 27, 2018, and U.S. Provisional Patent Application No. 62/822,815, filed March 23, 2019, each of which is entirely incorporated herein by reference.

BACKGROUND

[0002] Corticotropes are basophilic cells in the anterior pituitary that produce melanocyte-stimulating hormone, adrenocorticotropic hormone (ACTH) and lipotropin. The cells produce pro-opiomelanocortin (POMC) which undergoes cleavage to ACTH and β-lipotropin (β-LPH). These cells respond to corticotropin-releasing factor (CRF) and make up about 20% of the cells in the anterior pituitary. CRF is a releasing hormone that belongs to the corticotropin-releasing factor family and is the primary physiological regulator of proopiomelanocortin (POMC).

[0003] CRF is produced by parvocellular neuroendocrine cells within the paraventricular nucleus of the hypothalamus and is released at the median eminence from neurosecretory terminals of these neurons into the primary capillary plexus of the hypothalamo-hypophyseal portal system. The portal system carries the CRF to the anterior lobe of the pituitary, where it stimulates corticotropes to secrete ACTH and other biologically-active substances. ACTH, in turn, stimulates the synthesis of cortisol, glucocorticoids, mineralocorticoids and DHEA.

[0004] Testicular adrenal rest tumors (TART) and ovarian adrenal rest tumors (OART) are ACTH-responsive lesions of the testes and ovaries derived from adrenal tissue interchelated within these organs during embryogenesis. In response to high ACTH, hyperplasia of this tissue occurs, resulting in single or multiple lesions that may cause pain and infertility. The identification of compounds that modulate CRF function and downstream processes is an ongoing challenge. Currently, there are no non-steroidal, non-surgical solutions to control, shrink, or reduce TART. Disclosed herein, *inter alia*, are solutions to these and other problems in the art.

SUMMARY

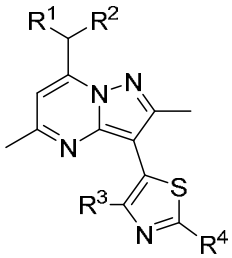
[0005] Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is a serious genetic disorder characterized by impaired adrenal synthesis of cortisol and consequent overproduction of adrenocorticotropic hormone (ACTH), 17-hydroxyprogesterone (17OHP), and adrenal androgens such as androstenedione (A4). CAH is an orphan condition with no FDA-approved therapy. To suppress androgen production, patients are often prescribed long-term supraphysiologic doses of glucocorticoid (GC), which has adverse effects and fail to suppress 17OHP and A4 in the majority of patients.

[0006] Compound 1 may be a potent, selective, nonsteroidal, oral corticotropin-releasing factor type-1 (CRF1) receptor antagonist that reduces excess ACTH, 17OHP, and A4 to improve clinical and biochemical sequelae of CAH. This may obviate the need for high dose steroids.

[0007] One aspect provided herein is a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF_1) antagonist or a pharmaceutically acceptable salt thereof.

[0008] In some embodiments, the subject has congenital adrenal hyperplasia (CAH). In some embodiments, the size and/or number of the tumors is decreased or reduced.

[0009] One aspect provided herein is a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a compound of structural Formula (I):

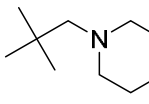
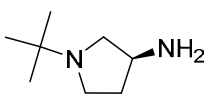
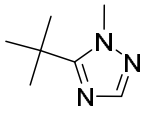
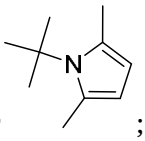


(I), or a pharmaceutically acceptable salt thereof,

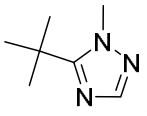
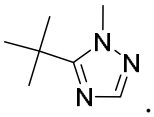
wherein:

R^1 and R^2 are independently ethyl or n-propyl;

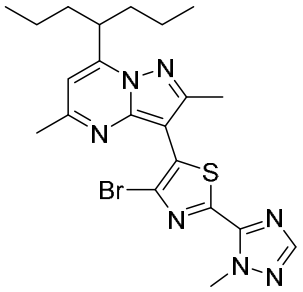
R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy; and

R^4 is hydrogen, Br, R^aR^bN–, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl,  ,  ,  , or  ;

R^a and R^b are independently hydrogen, C_1-C_3alkyl, $H_2NCH_2CH_2$–, $(CH_3)_3COC(O)NHCH_2CH_2$–, or $CH_3CH_2CH_2NHCH_2CH_2$–.

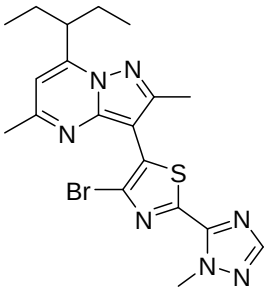
[0010] In some embodiments, R^3 is Cl, Br, methyl, or trifluoromethyl. In some embodiments, R^3 is Cl, Br, or methyl. In some embodiments, R^4 is Br, R^aR^bN–, pyridin-4-yl, morpholin-4-yl, or  . In some embodiments, R^4 is morpholin-4-yl or  .

[0011] In some embodiments, R^4 is hydrogen, Br, R^aR^bN– and R^a and R^b are independently C_1-C_3alkyl. In some embodiments, the compound is



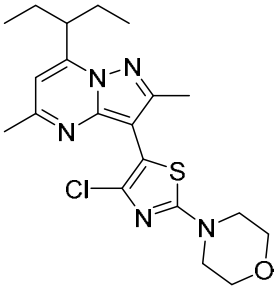
, or a pharmaceutically acceptable salt thereof.

[0012] In some embodiments, the compound is



, or a pharmaceutically acceptable salt thereof.

[0013] In some embodiments, the compound is



, or a pharmaceutically acceptable salt thereof.

[0014] In some embodiments, the subject has congenital adrenal hyperplasia (CAH). In some embodiments, the size and/or number of the tumors is decreased or reduced.

[0015] In some embodiments, from about 5 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In some embodiments, from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In some embodiments, from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically

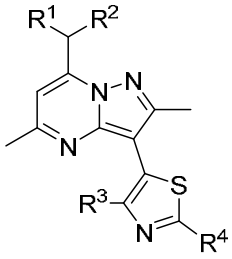
acceptable salt thereof, is administered to the subject. In some embodiments, from about 100 mg to about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject.

[0016] In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 5 mg/day to about 1000 mg/day is administered to the subject. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose from about 5 mg/day to about 25 mg/day is administered to the subject. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose from about 10 mg/day to about 400 mg/day is administered to the subject. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 100 mg/day to about 600 mg/day is administered to the subject.

[0017] In some embodiments, the compound is administered orally. In some embodiments, the compound is formulated as a capsule or tablet. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the fed state. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the fasted state.

[0018] In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at least once per day. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at bedtime. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 4 hours before sleep. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 2 hours before sleep. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 30 minutes before sleep. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the evening. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at about 10 pm at night.

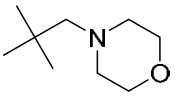
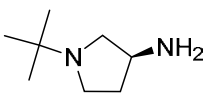
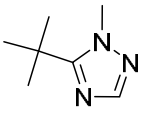
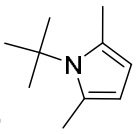
[0019] One aspect described herein is a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a pharmaceutical composition, comprising a compound of structural Formula (I) and a pharmaceutically acceptable excipient:


(I), or a pharmaceutically acceptable salt thereof,

wherein:

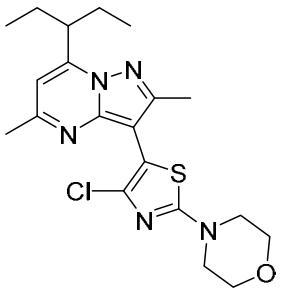
R^1 and R^2 are independently ethyl or n-propyl;

R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy; and

R^4 is hydrogen, Br, R^aR^bN–, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl,  ,  ,  , or  ;

R^a and R^b are independently hydrogen, C_1-C_3alkyl, $H_2NCH_2CH_2$–, $(CH_3)_3COC(O)NHCH_2CH_2$–, or $CH_3CH_2CH_2NHCH_2CH_2$–.

[0020] In some embodiments, the compound of Formula (I) has the structure:


, or a pharmaceutically acceptable salt thereof.

[0021] In some embodiments, the pharmaceutical composition comprises from about 5 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the pharmaceutical composition comprises from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the pharmaceutical composition comprises from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the pharmaceutical composition comprises from about 100 mg to about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[0022] In some embodiments, the pharmaceutical composition comprises about 5 mg, about 10 mg, about 15 mg, about 20 mg, 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265

mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg about 500 mg, or about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

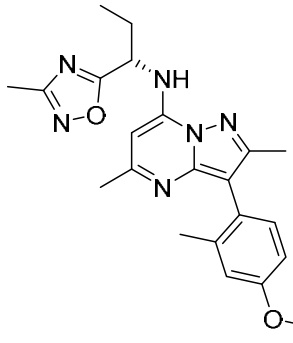
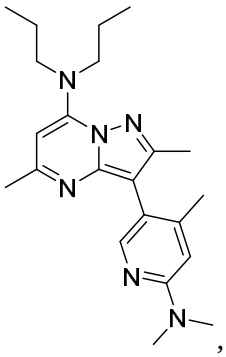
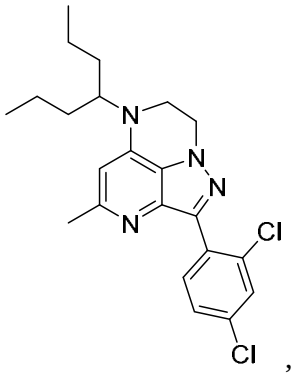
[0023] In some embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg/day to about 1000 mg/day. In some embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg/day to about 25 mg/day. In some embodiments, the pharmaceutical composition is administered at a dose of from about 10 mg/day to about 400 mg/day. In some embodiments, the pharmaceutical composition is administered at a dose of from about 100 mg/day to about 600 mg/day.

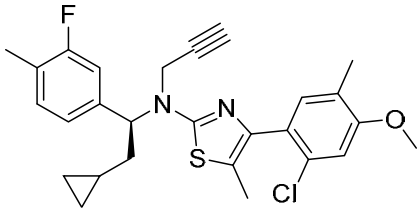
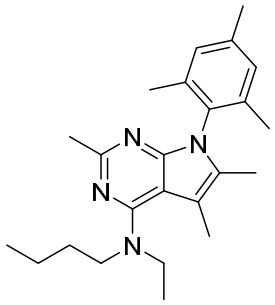
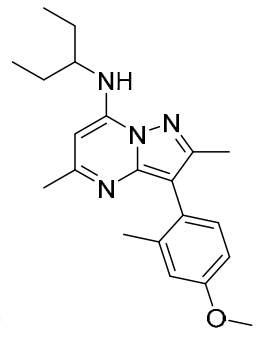
[0024] In some embodiments, the pharmaceutical composition is administered orally. In some embodiments, the pharmaceutical composition is formulated as capsule or a tablet. In some embodiments, the pharmaceutical composition is administered in the fed state. In some embodiments, the pharmaceutical composition is administered in the fasted state. In some embodiments, the pharmaceutical composition is administered at least once per day.

[0025] In some embodiments, the pharmaceutical composition is administered at bedtime. In some embodiments, the pharmaceutical composition is administered less than about 4 hours before sleep. In some embodiments, the pharmaceutical composition is administered less than about 2 hours before sleep. In some embodiments, the pharmaceutical composition is administered less than about 30 minutes before sleep. In some embodiments, the pharmaceutical composition is administered in the evening. In some embodiments, the pharmaceutical composition is administered at about 10 pm at night.

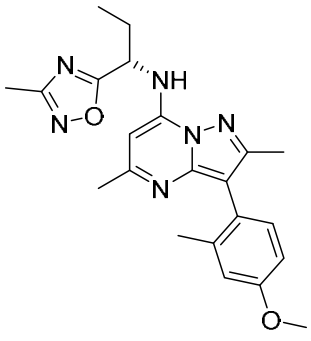
[0026] In some embodiments, the method further comprises administering to the subject an additional chemotherapeutic agent. In some embodiments, the additional chemotherapeutic agent is a glucocorticoid, a mineralocorticoid, an ACAT1 inhibitor, or an anti-androgen. In some embodiments, the glucocorticoid is beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone. In some embodiments, the mineralocorticoid is fludrocortisone.

[0027] In some embodiments, the additional chemotherapeutic agent is another CRF_1 antagonist. In some embodiments, the CRF_1 antagonist is

 ,  ,  , SSR-126374,

 ,  , or  , or a pharmaceutically acceptable salt thereof.

[0028] In some embodiments, the CRF_1 antagonist is

 , or a pharmaceutically acceptable salt thereof.

[0029] In some embodiments, the method further comprises an additional treatment selected from surgical resection of the tumors and radiation therapy, or a combination thereof.

[0030] In some embodiments, the additional therapy is surgical resection and the surgical resection is prior to, after, and/or concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition, or any combination thereof.

[0031] In some embodiments, the surgical resection is prior to administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In some embodiments, the surgical resection is after administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In some embodiments, the surgical resection is concurrent with

administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

[0032] In some embodiments, the additional treatment is radiation therapy and the radiation therapy is prior to, after, and/or concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition, or any combination thereof. In some embodiments, the radiation therapy is prior to administration of the CRF_1 antagonist of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

[0033] In some embodiments, the radiation therapy is after administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In some embodiments, the radiation therapy is concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

[0034] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0035] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also “Figure” and “FIG.” herein), of which:

[0037] FIG. 1A-1B. A schematic representation of intra-patient dose escalation study to evaluate safety, pharmacokinetics, and pharmacodynamics **(A).** A schematic representation of inhibition of CRF_1 with a CRF_1 antagonist (Compound 1) **(B)**.

[0038] FIG. 2. Graphic representation of pharmacokinetics (dose-proportional, adequate exposure at safe doses) in patients with CAH following 14-days of once daily dosing at each dose level with a CRF_1 antagonist (Compound 1).

[0039] FIG. 3. Graphic representation of pharmacokinetics (dose-proportional, adequate exposure at safe doses) in patients with CAH following 14-days of once daily dosing at each dose level with a CRF_1 antagonist (Compound 1).

[0040] FIG. 4. Graphic representation of patient-level response (measuring ACTH levels) to a CRF_1 antagonist (Compound 1).

[0041] FIG. 5A-5B. Graphic representation of patient-level response of sample responders (measuring ACTH levels) to a CRF_1 antagonist [Subject 003001 **(A)**; Subject 007002 **(B)**].

[0042] FIG. 6. Graphic representation of patient-level response (measuring 17-OHP levels) to a CRF_1 antagonist (Compound 1).

[0043] FIG. 7A-7B. Graphic representation of patient-level response of sample responders (measuring 17-OHP levels) to a CRF_1 antagonist [Subject 003001 **(A)**; Subject 007002 **(B)**].

[0044] FIG. 8. Graphic representation of patient-level response (measuring androstenedione levels) to a CRF_1 antagonist (Compound 1).

[0045] FIG. 9A-9B. Graphic representation of patient-level response of sample responders (measuring androstenedione levels) to a CRF_1 antagonist (Compound 1) [Subject 003001 **(A)**; Subject 007002 **(B)**].

[0046] FIG. 10A-10B. Images of a patient with TART pre-treatment with a CRF_1 antagonist (Compound 1) **(A)** and 6-weeks post-treatment with a CRF_1 antagonist (Compound 1) **(B)**.

[0047] FIG. 11A-11B. Graphic representation of an *in vitro* binding assay of a CRF_1 antagonist (Compound 1); ^{125}I-sauvagine binding to rat cerebellar membranes one hour after oral administration of increasing Compound 1 doses **(A)** and *in vivo* inhibition of CRF-induced activity after treatment with a CRF_1 antagonist; Compound 1 (oral, 10 mg/kg) **(B)**.

[0048] FIG. 12A-12B. Graphic representation of ACTH levels **(A)** and 17-OHP levels **(B)** after treatment with a CRF_1 antagonist (Compound 1).

[0049] FIG. 13. Graphic representation of hormone responses after treatment with a CRF_1 antagonist (Compound 1).

[0050] FIG. 14 depicts the proof of concept and escalating QD dosing at 10 pm.

[0051] FIG. 15A-15D are graphic representations of changes in androstenedione seen in participants in Cohort A and Cohort B.

[0052] FIG. 16A-16D are graphic representations of changes in 17-hydroxyprogesterone seen in participants in Cohort A and Cohort B.

[0053] FIG. 17A-17D are graphic representations of changes in adrenocorticotropic hormone seen in participants in Cohort A and Cohort B.

DETAILED DESCRIPTION

[0054] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

I. Definitions

[0055] The abbreviations used herein have their conventional meaning within the chemical and biological arts. The chemical structures and formulae set forth herein are constructed according to the standard rules of chemical valency known in the chemical arts.

[0056] Where substituent groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, e.g., $-CH_2O-$ is equivalent to $-OCH_2-$.

[0057] The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a straight (i.e., unbranched) or branched carbon chain (or carbon), or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include mono-, di- and multivalent radicals, having the number of carbon atoms designated (i.e., C_1-C_{10} means one to ten carbons). Alkyl is an uncyclized chain. Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, (cyclohexyl)methyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butynyl, and the higher homologs and isomers. An alkoxy is an alkyl attached to the remainder of the molecule via an oxygen linker (-O-).

[0058] The term "alkylene," by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from an alkyl, as exemplified, but not limited by, $-CH_2CH_2CH_2CH_2-$. Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred herein. A "lower alkyl" or "lower alkylene" is a shorter chain alkyl or alkylene group, generally having eight or fewer

carbon atoms. The term “alkenylene,” by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from an alkene.

[0059] The term “heteroalkyl,” by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or combinations thereof, including at least one carbon atom and at least one heteroatom (e.g., O, N, P, Si, and S), and wherein the nitrogen and sulfur atoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) (e.g., N, S, Si, or P) may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. Heteroalkyl is an uncyclized chain. Examples include, but are not limited to: $-CH_2-CH_2-O-CH_3$, $-CH_2-CH_2-NH-CH_3$, $-CH_2-CH_2-N(CH_3)-CH_3$, $-CH_2-S-CH_2-CH_3$, $-CH_2-CH_2$, $-S(O)-CH_3$, $-CH_2-CH_2-S(O)_2-CH_3$, $-CH=CH-O-CH_3$, $-Si(CH_3)_3$, $-CH_2-CH=N-OCH_3$, $-CH=CH-N(CH_3)-CH_3$, $-O-CH_3$, $-O-CH_2-CH_3$, and -CN. Up to two or three heteroatoms may be consecutive, such as, for example, $-CH_2-NH-OCH_3$ and $-CH_2-O-Si(CH_3)_3$. A heteroalkyl moiety may include one heteroatom (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include two optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include three optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include four optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include five optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include up to 8 optionally different heteroatoms (e.g., O, N, S, Si, or P).

[0060] Similarly, the term “heteroalkylene,” by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from heteroalkyl, as exemplified, but not limited by, $-CH_2-CH_2-S-CH_2-CH_2-$ and $-CH_2-S-CH_2-CH_2-NH-CH_2-$. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (e.g., alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula $-C(O)_2R'-$ represents both $-C(O)_2R'-$ and $-R'C(O)_2-$. As described above, heteroalkyl groups, as used herein, include those groups that are attached to the remainder of the molecule through a heteroatom, such as -C(O)R', -C(O)NR', -NR'R", -OR', -SR', and/or $-SO_2R'$. Where “heteroalkyl” is recited, followed by recitations of specific heteroalkyl groups, such as -NR'R" or the like, it will be understood that the terms heteroalkyl and -NR'R" are not redundant or mutually exclusive. Rather, the specific heteroalkyl groups are recited to add clarity. Thus, the term “heteroalkyl” should not be interpreted herein as excluding specific heteroalkyl groups, such as -NR'R" or the like.

[0061] The terms “cycloalkyl” and “heterocycloalkyl,” by themselves or in combination with other terms, mean, unless otherwise stated, cyclic versions of “alkyl” and “heteroalkyl,”

respectively. Cycloalkyl and heterocycloalkyl are not aromatic. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like. A “cycloalkylene” and a “heterocycloalkylene,” alone or as part of another substituent, means a divalent radical derived from a cycloalkyl and heterocycloalkyl, respectively. “Cycloalkyl” is also meant to refer to bicyclic and polycyclic hydrocarbon rings such as, for example, bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane, etc.

[0062] The terms “halo” or “halogen,” by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as “haloalkyl” are meant to include monohaloalkyl and polyhaloalkyl. For example, the term “halo(C_1-C_4)alkyl” includes, but is not limited to, fluoromethyl, difluoromethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

[0063] The term “acyl” means, unless otherwise stated, -C(O)R where R is a substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

[0064] The term “aryl” means, unless otherwise stated, a polyunsaturated, aromatic, hydrocarbon substituent, which can be a single ring or multiple rings (preferably from 1 to 3 rings) that are fused together (i.e., a fused ring aryl) or linked covalently. A fused ring aryl refers to multiple rings fused together wherein at least one of the fused rings is an aryl ring. The term “heteroaryl” refers to aryl groups (or rings) that contain at least one heteroatom such as N, O, or S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. Thus, the term “heteroaryl” includes fused ring heteroaryl groups (i.e., multiple rings fused together wherein at least one of the fused rings is a heteroaromatic ring). A 5,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 5 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. Likewise, a 6,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. And a 6,5-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 5 members, and wherein at least one ring is a heteroaryl ring. A heteroaryl group can be attached to the remainder of the molecule through a carbon or heteroatom. Non-

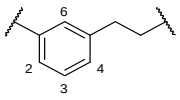
limiting examples of aryl and heteroaryl groups include phenyl, naphthyl, pyrrolyl, pyrazolyl, pyridazinyl, triazinyl, pyrimidinyl, imidazolyl, pyrazinyl, purinyl, oxazolyl, isoxazolyl, thiazolyl, furyl, thienyl, pyridyl, pyrimidyl, benzothiazolyl, benzoxazoyl benzimidazolyl, benzofuran, isobenzofuranyl, indolyl, isoindolyl, benzothiophenyl, isoquinolyl, quinoxalinyl, quinolyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalinyl, 5-quinoxalinyl, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below. An “arylene” and a “heteroarylene,” alone or as part of another substituent, mean a divalent radical derived from an aryl and heteroaryl, respectively. A heteroaryl group substituent may be -O- bonded to a ring heteroatom nitrogen.

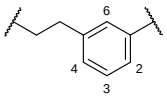
[0065] Spirocyclic rings are two or more rings wherein adjacent rings are attached through a single atom. The individual rings within spirocyclic rings may be identical or different. Individual rings in spirocyclic rings may be substituted or unsubstituted and may have different substituents from other individual rings within a set of spirocyclic rings. Possible substituents for individual rings within spirocyclic rings are the possible substituents for the same ring when not part of spirocyclic rings (e.g. substituents for cycloalkyl or heterocycloalkyl rings). Spirocylic rings may be substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkyl or substituted or unsubstituted heterocycloalkylene and individual rings within a spirocyclic ring group may be any of the immediately previous list, including having all rings of one type (e.g. all rings being substituted heterocycloalkylene wherein each ring may be the same or different substituted heterocycloalkylene). When referring to a spirocyclic ring system, heterocyclic spirocyclic rings means a spirocyclic rings wherein at least one ring is a heterocyclic ring and wherein each ring may be a different ring. When referring to a spirocyclic ring system, substituted spirocyclic rings means that at least one ring is substituted and each substituent may optionally be different.

[0066] The symbol “” denotes the point of attachment of a chemical moiety to the remainder of a molecule or chemical formula.

[0067] The term “oxo,” as used herein, means an oxygen that is double bonded to a carbon atom.

[0068] The term “alkylarylene” as an arylene moiety covalently bonded to an alkylene moiety (also referred to herein as an alkylene linker). In embodiments, the alkylarylene group has the formula:



or


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[0069] An alkylarylene moiety may be substituted (e.g. with a substituent group) on the alkylene moiety or the arylene linker (e.g. at carbons 2, 3, 4, or 6) with halogen, oxo, $-N_3$, $-CF_3$, $-CCl_3$, $-CBr_3$, $-CI_3$, -CN, -CHO, -OH, $-NH_2$, -COOH, $-CONH_2$, $-NO_2$, -SH, $-SO_2CH_3$ $-SO_3H$, $-OSO_3H$, $-SO_2NH_2$, –$NHNH_2$, –ONH_2, –$NHC(O)NHNH_2$, substituted or unsubstituted C_1-C_5 alkyl or substituted or unsubstituted 2 to 5 membered heteroalkyl). In embodiments, the alkylarylene is unsubstituted.

[0070] Each of the above terms (e.g., “alkyl,” “heteroalkyl,” “cycloalkyl,” “heterocycloalkyl,” “aryl,” and “heteroaryl”) includes both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

[0071] Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be one or more of a variety of groups selected from, but not limited to, -OR', =O, =NR', =N-OR', -NR'R", -SR', -halogen, -SiR'R"R'", -OC(O)R', -C(O)R', $-CO_2R'$, -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R'", $-NR"C(O)_2R'$, -NR-C(NR'R"R'")=NR"", -NR-C(NR'R")=NR'", $-S(O)R'$, $-S(O)_2R'$, $-S(O)_2NR'R"$, $-NRSO_2R'$, –NR'NR"R'", –ONR'R", –NR'C(O)NR"NR'"R"", -CN, $-NO_2$, $-NR'SO_2R"$, -NR'C(O)R", -NR'C(O)-OR", -NR'OR", in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R, R', R", R'", and R"" each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl (e.g., aryl substituted with 1-3 halogens), substituted or unsubstituted heteroaryl, substituted or unsubstituted alkyl, alkoxy, or thioalkoxy groups, or arylalkyl groups. When a compound described herein includes more than one R group, for example, each of the R groups is independently selected as are each R', R", R'", and R"" group when more than one of these groups is present. When R' and R" are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 4-, 5-, 6-, or 7-membered ring. For example, -NR'R" includes, but is not limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term “alkyl” is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (e.g., $-CF_3$ and $-CH_2CF_3$) and acyl (e.g., $-C(O)CH_3$, $-C(O)CF_3$, $-C(O)CH_2OCH_3$, and the like).

[0072] Similar to the substituents described for the alkyl radical, substituents for the aryl and heteroaryl groups are varied and are selected from, for example: -OR', -NR'R", -SR', -halogen, -

SiR'R"R'", -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R'", -NR"C$(O)_2$R', -NR-C(NR'R"R'")=NR"", -NR-C(NR'R")=NR'", -S(O)R', -$S(O)_2$R', -$S(O)_2$NR'R", -$NRSO_2$R', –NR'NR"R'", –ONR'R", –NR'C(O)NR"NR'"R"", -CN, -NO_2, -R', -N_3, -$CH(Ph)_2$, fluoro(C_1-C_4)alkoxy, and fluoro(C_1-C_4)alkyl, -NR'SO_2R", -NR'C(O)R", -NR'C(O)-OR", -NR'OR", in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R', R", R'", and R"" are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl. When a compound described herein includes more than one R group, for example, each of the R groups is independently selected as are each R', R", R'", and R"" groups when more than one of these groups is present.

[0073] Substituents for rings (e.g. cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene) may be depicted as substituents on the ring rather than on a specific atom of a ring (commonly referred to as a floating substituent). In such a case, the substituent may be attached to any of the ring atoms (obeying the rules of chemical valency) and in the case of fused rings or spirocyclic rings, a substituent depicted as associated with one member of the fused rings or spirocyclic rings (a floating substituent on a single ring), may be a substituent on any of the fused rings or spirocyclic rings (a floating substituent on multiple rings). When a substituent is attached to a ring, but not a specific atom (a floating substituent), and a subscript for the substituent is an integer greater than one, the multiple substituents may be on the same atom, same ring, different atoms, different fused rings, different spirocyclic rings, and each substituent may optionally be different. Where a point of attachment of a ring to the remainder of a molecule is not limited to a single atom (a floating substituent), the attachment point may be any atom of the ring and in the case of a fused ring or spirocyclic ring, any atom of any of the fused rings or spirocyclic rings while obeying the rules of chemical valency. Where a ring, fused rings, or spirocyclic rings contain one or more ring heteroatoms and the ring, fused rings, or spirocyclic rings are shown with one more floating substituents (including, but not limited to, points of attachment to the remainder of the molecule), the floating substituents may be bonded to the heteroatoms. Where the ring heteroatoms are shown bound to one or more hydrogens (e.g. a ring nitrogen with two bonds to ring atoms and a third bond to a hydrogen) in the structure or formula with the floating substituent, when the heteroatom is bonded to the floating substituent, the substituent will be understood to replace the hydrogen, while obeying the rules of chemical valency.

[0074] Two or more substituents may optionally be joined to form aryl, heteroaryl, cycloalkyl, or heterocycloalkyl groups. Such so-called ring-forming substituents are typically, though not

necessarily, found attached to a cyclic base structure. In one embodiment, the ring-forming substituents are attached to adjacent members of the base structure. For example, two ring-forming substituents attached to adjacent members of a cyclic base structure create a fused ring structure. In another embodiment, the ring-forming substituents are attached to a single member of the base structure. For example, two ring-forming substituents attached to a single member of a cyclic base structure create a spirocyclic structure. In yet another embodiment, the ring-forming substituents are attached to non-adjacent members of the base structure.

[0075] Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally form a ring of the formula -T-C(O)-$(CRR')_q$-U-, wherein T and U are independently -NR-, -O-, -CRR'-, or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -A-$(CH_2)_r$-B-, wherein A and B are independently -CRR'-, -O-, -NR-, -S-, -S(O) -, -$S(O)_2$-, -$S(O)_2$NR'-, or a single bond, and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -$(CRR')_s$-X'- $(C''R''R''')_d$-, where s and d are independently integers of from 0 to 3, and X' is -O-, -NR'-, -S-, -S(O)-, -$S(O)_2$-, or -$S(O)_2$NR'-. The substituents R, R', R", and R''' are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

[0076] As used herein, the terms “heteroatom” or “ring heteroatom” are meant to include oxygen (O), nitrogen (N), sulfur (S), phosphorus (P), and silicon (Si).

[0077] A “substituent group,” as used herein, means a group selected from the following moieties:

(A) oxo, halogen, -CF_3, -CN, -OH, -NH_2, -COOH, -$CONH_2$, -NO_2, -SH, -SO_3H, -SO_4H, -SO_2NH_2, –$NHNH_2$, –ONH_2, –NHC=(O)$NHNH_2$, –NHC=(O) NH_2, -$NHSO_2H$, -NHC= (O)H, -NHC(O)-OH, -NHOH, -OCF_3, -$OCHF_2$, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(B) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from:

(i) oxo, halogen, -CF_3, -CN, -OH, -NH_2, -COOH, -$CONH_2$, -NO_2, -SH, -SO_3H, -SO_4H, -SO_2NH_2, –$NHNH_2$, –ONH_2, –NHC=(O)$NHNH_2$, –NHC=(O) NH_2, -$NHSO_2H$, -NHC= (O)H, -NHC(O)-OH, -NHOH, -OCF_3, -$OCHF_2$, unsubstituted alkyl, unsubstituted heteroalkyl,

unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(ii) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from:

(a) oxo, halogen, $-CF_3$, -CN, -OH, $-NH_2$, -COOH, $-CONH_2$, $-NO_2$, -SH, $-SO_3H$, $-SO_4H$, $-SO_2NH_2$, $-NHNH_2$, $-ONH_2$, $-NHC(=O)NHNH_2$, $-NHC(=O)NH_2$, $-NHSO_2H$, -NHC(=O)H, -NHC(O)-OH, -NHOH, $-OCF_3$, $-OCHF_2$, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(b) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from: oxo, halogen, $-CF_3$, -CN, -OH, $-NH_2$, -COOH, $-CONH_2$, $-NO_2$, -SH, $-SO_3H$, $-SO_4H$, $-SO_2NH_2$, $-NHNH_2$, $-ONH_2$, $-NHC(=O)NHNH_2$, $-NHC(=O)NH_2$, $-NHSO_2H$, -NHC(=O)H, -NHC(O)-OH, -NHOH, $-OCF_3$, $-OCHF_2$, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl.

[0078] A “size-limited substituent” or “ size-limited substituent group,” as used herein, means a group selected from all of the substituents described above for a “substituent group,” wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C_1-C_{20} alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_8 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 8 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 10 membered heteroaryl.

[0079] A “lower substituent” or “ lower substituent group,” as used herein, means a group selected from all of the substituents described above for a “substituent group,” wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C_1-C_8 alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_7 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 7 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 9 membered heteroaryl.

[0080] In some embodiments, each substituted group described in the compounds herein is substituted with at least one substituent group. More specifically, in some embodiments, each substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene described in the compounds herein are substituted with at least one substituent group. In other embodiments, at least one or all of these groups are substituted with at least one size-limited substituent group. In other embodiments, at least one or all of these groups are substituted with at least one lower substituent group.

[0081] In other embodiments of the compounds herein, each substituted or unsubstituted alkyl may be a substituted or unsubstituted C_1-C_{20} alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_8 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 8 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and/or each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 10 membered heteroaryl. In some embodiments of the compounds herein, each substituted or unsubstituted alkylene is a substituted or unsubstituted C_1-C_{20} alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 20 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C_3-C_8 cycloalkylene, each substituted or unsubstituted heterocycloalkylene is a substituted or unsubstituted 3 to 8 membered heterocycloalkylene, each substituted or unsubstituted arylene is a substituted or unsubstituted C_6-C_{10} arylene, and/or each substituted or unsubstituted heteroarylene is a substituted or unsubstituted 5 to 10 membered heteroarylene.

[0082] In some embodiments, each substituted or unsubstituted alkyl is a substituted or unsubstituted C_1-C_8 alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_7 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 7 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and/or each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 9 membered heteroaryl. In some embodiments, each substituted or unsubstituted alkylene is a substituted or unsubstituted C_1-C_8 alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 8 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C_3-C_7 cycloalkylene, each substituted or unsubstituted heterocycloalkylene is a

substituted or unsubstituted 3 to 7 membered heterocycloalkylene, each substituted or unsubstituted arylene is a substituted or unsubstituted C_6-C_{10} arylene, and/or each substituted or unsubstituted heteroarylene is a substituted or unsubstituted 5 to 9 membered heteroarylene. In some embodiments, the compound is a chemical species set forth in the Examples section, figures, or tables below.

[0083] Certain compounds of the present invention possess asymmetric carbon atoms (optical or chiral centers) or double bonds; the enantiomers, racemates, diastereomers, tautomers, geometric isomers, stereoisometric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)- or, as (D)- or (L)- for amino acids, and individual isomers are encompassed within the scope of the present invention. The compounds of the present invention do not include those that are known in art to be too unstable to synthesize and/or isolate. The present invention is meant to include compounds in racemic and optically pure forms. Optically active (R)- and (S)-, or (D)- and (L)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers.

[0084] As used herein, the term “isomers” refers to compounds having the same number and kind of atoms, and hence the same molecular weight, but differing in respect to the structural arrangement or configuration of the atoms.

[0085] The term “tautomer,” as used herein, refers to one of two or more structural isomers which exist in equilibrium and which are readily converted from one isomeric form to another.

[0086] It will be apparent to one skilled in the art that certain compounds of this invention may exist in tautomeric forms, all such tautomeric forms of the compounds being within the scope of the invention.

[0087] Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of the invention.

[0088] Unless otherwise stated, structures depicted herein are also meant to include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon by ^{13}C- or ^{14}C-enriched carbon are within the scope of this invention.

[0089] The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the

compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^{3}H), iodine-125 (^{125}I), or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are encompassed within the scope of the present invention.

[0090] It should be noted that throughout the application that alternatives are written in Markush groups, for example, each amino acid position that contains more than one possible amino acid. It is specifically contemplated that each member of the Markush group should be considered separately, thereby comprising another embodiment, and the Markush group is not to be read as a single unit.

[0091] "Analog" or "analogue" is used in accordance with its plain ordinary meaning within Chemistry and Biology and refers to a chemical compound that is structurally similar to another compound (i.e., a so-called "reference" compound) but differs in composition, e.g., in the replacement of one atom by an atom of a different element, or in the presence of a particular functional group, or the replacement of one functional group by another functional group, or the absolute stereochemistry of one or more chiral centers of the reference compound. Accordingly, an analog is a compound that is similar or comparable in function and appearance but not in structure or origin to a reference compound.

[0092] The terms "a" or "an," as used in herein means one or more. In addition, the phrase "substituted with a[n]," as used herein, means the specified group may be substituted with one or more of any or all of the named substituents. For example, where a group, such as an alkyl or heteroaryl group, is "substituted with an unsubstituted C_1-C_{20} alkyl, or unsubstituted 2 to 20 membered heteroalkyl," the group may contain one or more unsubstituted C_1-C_{20} alkyls, and/or one or more unsubstituted 2 to 20 membered heteroalkyls.

[0093] Moreover, where a moiety is substituted with an R substituent, the group may be referred to as "R-substituted." Where a moiety is R-substituted, the moiety is substituted with at least one R substituent and each R substituent is optionally different. Where a particular R group is present in the description of a chemical genus (such as Formula (I)), a Roman alphabetic symbol may be used to distinguish each appearance of that particular R group. For example, where multiple R^{13} substituents are present, each R^{13} substituent may be distinguished as R^{13A}, R^{13B}, R^{13C}, R^{13D}, etc., wherein each of R^{13A}, R^{13B}, R^{13C}, R^{13D}, etc. is defined within the scope of the definition of R^{13} and optionally differently.

[0094] A "detectable moiety" as used herein refers to a moiety that can be covalently or noncovalently attached to a compound or biomolecule that can be detected for instance, using techniques known in the art. In embodiments, the detectable moiety is covalently attached. The detectable moiety may provide for imaging of the attached compound or biomolecule. The detectable moiety may indicate the contacting between two compounds. Exemplary detectable

moieties are fluorophores, antibodies, reactive dies, radio-labeled moieties, magnetic contrast agents, and quantum dots. Exemplary fluorophores include fluorescein, rhodamine, GFP, coumarin, FITC, Alexa fluor, Cy3, Cy5, BODIPY, and cyanine dyes. Exemplary radionuclides include Fluorine-18, Gallium-68, and Copper-64. Exemplary magnetic contrast agents include gadolinium, iron oxide and iron platinum, and manganese.

[0095] Descriptions of compounds of the present invention are limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to give compounds which are not inherently unstable and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions, such as aqueous, neutral, and several known physiological conditions. For example, a heterocycloalkyl or heteroaryl is attached to the remainder of the molecule via a ring heteroatom in compliance with principles of chemical bonding known to those skilled in the art thereby avoiding inherently unstable compounds.

[0096] The term “pharmaceutically acceptable salts” is meant to include salts of the active compounds that are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, oxalic, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (*see*, for example, Berge *et al.*, “Pharmaceutical Salts”, *Journal of Pharmaceutical Science*, **1977**, *66*, 1-19). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

[0097] Thus, the compounds of the present invention may exist as salts, such as with pharmaceutically acceptable acids. The present invention includes such salts. Non-limiting examples of such salts include hydrochlorides, hydrobromides, phosphates, sulfates, methanesulfonates, nitrates, maleates, acetates, citrates, fumarates, proprionates, tartrates (e.g., (+)-tartrates, (-)-tartrates, or mixtures thereof including racemic mixtures), succinates, benzoates, and salts with amino acids such as glutamic acid, and quaternary ammonium salts (e.g. methyl iodide, ethyl iodide, and the like). These salts may be prepared by methods known to those skilled in the art.

[0098] The neutral forms of the compounds are preferably regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound may differ from the various salt forms in certain physical properties, such as solubility in polar solvents. In embodiments, compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts. The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in a conventional manner. The parent form of the compounds differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but, unless specifically indicated, the salts disclosed herein are equivalent to the parent form of the compound for the purposes of the present invention.

[0099] In addition to salt forms, the present invention provides compounds, which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present invention. Prodrugs of the compounds described herein may be converted *in vivo* after administration. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an *ex vivo* environment, such as, for example, when contacted with a suitable enzyme or chemical reagent.

[0100] Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

[0101] "Pharmaceutically acceptable excipient" and "pharmaceutically acceptable carrier" refer to a substance that aids the administration of a compound to and absorption by a subject and can be included in the compositions of the present invention without causing a significant adverse toxicological effect on the patient. Non-limiting examples of pharmaceutically acceptable

excipients include water, NaCl, normal saline solutions, lactated Ringer's, normal sucrose, normal glucose, binders, fillers, disintegrants, lubricants, coatings, sweeteners, flavors, salt solutions (such as Ringer's solution), alcohols, oils, gelatins, carbohydrates such as lactose, amylose or starch, fatty acid esters, hydroxymethycellulose, polyvinyl pyrrolidine, and colors, and the like. Such preparations can be sterilized and, if desired, mixed with auxiliary agents such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, and/or aromatic substances and the like that do not deleteriously react with the compounds of the invention. One of skill in the art will recognize that other pharmaceutical excipients are useful in the present invention.

[0102] The term "preparation" is intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component with or without other carriers, is surrounded by a carrier, which is thus in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be used as solid dosage forms suitable for oral administration.

[0103] A "CRF1 inhibitor" refers to a compound (e.g., compounds described herein) that reduces the activity of CRF1 when compared to a control, such as absence of the compound or a compound with known inactivity.

[0104] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues, wherein the polymer may optionally be conjugated to a moiety that does not consist of amino acids. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer. In embodiments, the terms "polypeptide," "peptide," and "protein", used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include genetically coded and non-genetically coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified polypeptide backbones. The terms include fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence; fusion proteins with heterologous and homologous leader sequences, with or without N-terminus methionine residues; immunologically tagged proteins; and the like.

[0105] A polypeptide, or a cell is "recombinant" when it is artificial or engineered, or derived from or contains an artificial or engineered protein or nucleic acid (*e.g.* non-natural or not wild type). For example, a polynucleotide that is inserted into a vector or any other heterologous location, *e.g.*, in a genome of a recombinant organism, such that it is not associated with nucleotide sequences that normally flank the polynucleotide as it is found in nature is a

recombinant polynucleotide. A protein expressed *in vitro* or *in vivo* from a recombinant polynucleotide is an example of a recombinant polypeptide. Likewise, a polynucleotide sequence that does not appear in nature, for example a variant of a naturally occurring gene, is recombinant.

[0106] "Contacting" is used in accordance with its plain ordinary meaning and refers to the process of allowing at least two distinct species (e.g. chemical compounds including biomolecules or cells) to become sufficiently proximal to react, interact or physically touch. It should be appreciated; however, the resulting reaction product can be produced directly from a reaction between the added reagents or from an intermediate from one or more of the added reagents that can be produced in the reaction mixture.

[0107] The term "contacting" may include allowing two species to react, interact, or physically touch, wherein the two species may be a compound as described herein and a protein or enzyme. In some embodiments contacting includes allowing a compound described herein to interact with a protein or enzyme that is involved in a signaling pathway (e.g., MAP kinase pathway).

[0108] As defined herein, the term "activation", "activate", "activating" and the like in reference to a protein refers to conversion of a protein into a biologically active derivative from an initial inactive or deactivated state. The terms reference activation, or activating, sensitizing, or up-regulating signal transduction or enzymatic activity or the amount of a protein decreased in a disease.

[0109] The terms "agonist," "activator," "upregulator," etc. refer to a substance capable of detectably increasing the expression or activity of a given gene or protein. The agonist can increase expression or activity 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in comparison to a control in the absence of the agonist. In certain instances, expression or activity is 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or higher than the expression or activity in the absence of the agonist. In embodiments, an agonist is a molecule that interacts with a target to cause or promote an increase in the activation of the target. In embodiments, activators are molecules that increase, activate, facilitate, enhance activation, sensitize, or up-regulate, e.g., a gene, protein, ligand, receptor, or cell.

[0110] As defined herein, the term "inhibition", "inhibit", "inhibiting" and the like in reference to a protein-inhibitor interaction means negatively affecting (e.g. decreasing) the activity or function of the protein relative to the activity or function of the protein in the absence of the inhibitor. In embodiments inhibition means negatively affecting (e.g. decreasing) the concentration or levels of the protein relative to the concentration or level of the protein in the absence of the inhibitor. In embodiments inhibition refers to reduction of a disease or symptoms of disease. In embodiments, inhibition refers to a reduction in the activity of a particular protein

target. Thus, inhibition includes, at least in part, partially or totally blocking stimulation, decreasing, preventing, or delaying activation, or inactivating, desensitizing, or down-regulating signal transduction or enzymatic activity or the amount of a protein. In embodiments, inhibition refers to a reduction of activity of a target protein resulting from a direct interaction (e.g. an inhibitor binds to the target protein). In embodiments, inhibition refers to a reduction of activity of a target protein from an indirect interaction (e.g. an inhibitor binds to a protein that activates the target protein, thereby preventing target protein activation).

[0111] The terms “inhibitor,” “repressor” or “antagonist” or “downregulator” interchangeably refer to a substance capable of detectably decreasing the expression or activity of a given gene or protein. The antagonist can decrease expression or activity 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in comparison to a control in the absence of the antagonist. In certain instances, expression or activity is 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or lower than the expression or activity in the absence of the antagonist. An antagonist prevents, reduces, inhibits, or neutralizes the activity of an agonist, and an antagonist can also prevent, inhibit, or reduce constitutive activity of a target, e.g., a target receptor, even where there is no identified agonist. In embodiments, inhibitors are molecules that decrease, block, prevent, delay activation, inactivate, desensitize, or down-regulate, e.g., a gene, protein, ligand, receptor, or cell. An inhibitor may also be defined as a molecule that reduces, blocks, or inactivates a constitutive activity. An “antagonist” is a molecule that opposes the action(s) of an agonist.

[0112] The terms “corticotropin-releasing factor type-1,” “CRF_1,” “corticotropin-releasing hormone receptor 1,” and “CHHR1” refer to a protein (including homologs, isoforms, and functional fragments thereof) and is a receptor for corticotropin releasing-hormone. The terms include any recombinant or naturally-occurring form of CRF_1 variants thereof that maintain CRF_1 activity (e.g. within at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% activity compared to wildtype CRF1). The terms include any mutant form of CRF_1 variants (e.g., frameshift mutations) thereof that maintain CRF_1 activity (e.g. within at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% activity compared to wildtype CRF_1). In embodiments, the CRF_1 protein encoded by the *CRHR1* gene has the amino acid sequence set forth in or corresponding to Entrez 1233, UniProt P34998.

[0113] The term “expression” includes any step involved in the production of the polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion. Expression can be detected using conventional techniques for detecting protein (*e.g.*, ELISA, Western blotting, flow cytometry, immunofluorescence, immunohistochemistry, *etc.*).

[0114] The terms “disease” or “condition” refer to a state of being or health status of a patient or subject capable of being treated with the compounds or methods provided herein. The disease may be a malignant or benign tumor (e.g., testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART)). The disease may be cancer. In some further instances, “cancer” refers to human cancers and carcinomas, sarcomas, adenocarcinomas, lymphomas, leukemias, etc., including solid and lymphoid cancers, kidney, breast, lung, bladder, colon, ovarian, prostate, pancreas, adrenal, stomach, brain, head and neck, skin, uterine, testicular, glioma, esophagus, and liver cancer, including hepatocarcinoma, lymphoma, including B-acute lymphoblastic lymphoma, non-Hodgkin’s lymphomas (*e.g.*, Burkitt’s, Small Cell, and Large Cell lymphomas), Hodgkin’s lymphoma, leukemia (including MDS, AML, ALL, ATLL and CML), or multiple myeloma.

[0115] As used herein, the term “cancer” refers to all types of cancer, neoplasm or malignant tumors found in mammals (e.g. humans), including leukemia, carcinomas and sarcomas. Exemplary cancers that may be treated with a compound or method provided herein include adrenal cancer and tumors (e.g., testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART)) brain cancer, glioma, glioblastoma, neuroblastoma, prostate cancer, colorectal cancer, pancreatic cancer, cervical cancer, gastric cancer, ovarian cancer, lung cancer, and cancer of the head. Exemplary cancers that may be treated with a compound or method provided herein include cancer of the thyroid, endocrine system, brain, breast, cervix, colon, head & neck, liver, kidney, lung, non-small cell lung, melanoma, mesothelioma, ovary, sarcoma, stomach, uterus, Medulloblastoma, colorectal cancer, pancreatic cancer. Additional examples include, thyroid carcinoma, cholangiocarcinoma, pancreatic adenocarcinoma, skin cutaneous melanoma, colon adenocarcinoma, rectum adenocarcinoma, stomach adenocarcinoma, esophageal carcinoma, head and neck squamous cell carcinoma, breast invasive carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, glioma, glioblastoma multiforme, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine or exocrine pancreas, medullary thyroid cancer, medullary thyroid carcinoma, melanoma, colorectal cancer, papillary thyroid cancer, hepatocellular carcinoma, or prostate cancer.

[0116] The term “congenital adrenal hyperplasia” or “CAH” are any of several autosomal recessive diseases resulting from mutations of genes for enzymes mediating the biochemical

steps of production of mineralocorticoids, glucocorticoids or sex steroids from cholesterol by the adrenal glands (steroidogenesis). These conditions involve excessive or deficient production of sex steroids and can alter development of primary or secondary sex characteristics in some affected infants, children, or adults.

[0117] "Adrenal rest tumor" refers to a neoplasm of the testis (or ovaries) consisting of adrenal tissue. It may be secondary to hyperplasia of an adrenal gland.

[0118] "Testicular adrenal rest tumor(s)" or "TART" and/or ovarian adrenal rest tumor(s) are a complication of congenital adrenal hyperplasia (CAH), which may develop from ectopic remnants of intra-testicular adrenal tissue stimulated by adrenocorticotropic hormone (ACTH) hypersecretion. TART lesions are typically located within the rete testis and are bilateral, synchronous, nodular and multiple. TART can lead to testicular structural damage, spermatogenesis disorders, infertility and mass-forming lesions.

[0119] The term "leukemia" refers broadly to progressive, malignant diseases of the blood-forming organs and is generally characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. Leukemia is generally clinically classified on the basis of (1) the duration and character of the disease-acute or chronic; (2) the type of cell involved; myeloid (myelogenous), lymphoid (lymphogenous), or monocytic; and (3) the increase or non-increase in the number abnormal cells in the blood-leukemic or aleukemic (subleukemic). Exemplary leukemias that may be treated with a compound or method provided herein include, for example, acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemic leukemia, basophylic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, multiple myeloma, plasmacytic leukemia, promyelocytic leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, or undifferentiated cell leukemia.

[0120] The term "sarcoma" generally refers to a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Sarcomas that may be treated with a compound or method

provided herein include a chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, or telangiectaltic sarcoma.

[0121] The term “melanoma” is taken to mean a tumor arising from the melanocytic system of the skin and other organs. Melanomas that may be treated with a compound or method provided herein include, for example, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, nodular melanoma, subungal melanoma, or superficial spreading melanoma.

[0122] The term “carcinoma” refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas that may be treated with a compound or method provided herein include, for example, thyroid carcinoma, cholangiocarcinoma, pancreatic adenocarcinoma, skin cutaneous melanoma, colon adenocarcinoma, rectum adenocarcinoma, stomach adenocarcinoma, esophageal carcinoma, head and neck squamous cell carcinoma, breast invasive carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, medullary thyroid carcinoma, familial medullary thyroid carcinoma, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniforni carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypernephroid carcinoma,

infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, or carcinoma villosum.

[0123] The terms “treating” or “treatment” refer to any indicia of success in the therapy or amelioration of an injury, disease, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to the patient; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving a patient’s physical or mental well-being. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of a physical examination, neuropsychiatric exams, and/or a psychiatric evaluation. The term “treating” and conjugations thereof, may include prevention of an injury, pathology, condition, or disease. In embodiments, treating is preventing. In embodiments, treating does not include preventing.

[0124] “Treating” or “treatment” as used herein (and as well-understood in the art) also broadly includes any approach for obtaining beneficial or desired results in a subject’s condition, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of the extent of a disease, stabilizing (*i.e.*, not worsening) the state of disease, prevention of a disease’s transmission or spread, delay or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission, whether partial or total and whether detectable or undetectable. In other words, “treatment” as used herein includes any cure, amelioration, or prevention of a disease. Treatment may prevent the disease from occurring; inhibit the disease’s spread; relieve the disease’s symptoms (*e.g.*, ocular pain, seeing

halos around lights, red eye, very high intraocular pressure), fully or partially remove the disease's underlying cause, shorten a disease's duration, or do a combination of these things.

[0125] "Treating" and "treatment" as used herein include prophylactic treatment. Treatment methods include administering to a subject a therapeutically effective amount of a compound described herein. The administering step may consist of a single administration or may include a series of administrations. The length of the treatment period depends on a variety of factors, such as the severity of the condition, the age of the patient, the concentration of the compound, the activity of the compositions used in the treatment, or a combination thereof. It will also be appreciated that the effective dosage of an agent used for the treatment or prophylaxis may increase or decrease over the course of a particular treatment or prophylaxis regime. Changes in dosage may result and become apparent by standard diagnostic assays known in the art. In some instances, chronic administration may be required. For example, the compositions are administered to the subject in an amount and for a duration sufficient to treat the patient.

[0126] The term "prevent" refers to a decrease in the occurrence of disease symptoms in a patient. As indicated above, the prevention may be complete (no detectable symptoms) or partial, such that fewer symptoms are observed than would likely occur absent treatment. In embodiments, prevent refers to slowing the progression of the disease, disorder or condition or inhibiting progression thereof to a harmful or otherwise undesired state.

[0127] "Patient" or "subject in need thereof" refers to a living organism suffering from or prone to a disease or condition that can be treated by administration of a pharmaceutical composition as provided herein. Non-limiting examples include humans, other mammals, bovines, rats, mice, dogs, monkeys, goat, sheep, cows, deer, and other non-mammalian animals. In some embodiments, a patient is human.

[0128] An "effective amount" is an amount sufficient for a compound to accomplish a stated purpose relative to the absence of the compound (e.g., achieve the effect for which it is administered, treat a disease, reduce enzyme activity, increase enzyme activity, reduce a signaling pathway, or reduce one or more symptoms of a disease or condition). An example of an "effective amount" is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which could also be referred to as a "therapeutically effective amount." A "reduction" of a symptom or symptoms (and grammatical equivalents of this phrase) means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). A "prophylactically effective amount" of a drug is an amount of a drug that, when administered to a subject, will have the intended prophylactic effect, e.g., preventing or delaying the onset (or reoccurrence) of an injury, disease, pathology or condition, or reducing the likelihood of the onset (or reoccurrence) of an injury, disease, pathology, or

condition, or their symptoms. The full prophylactic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a prophylactically effective amount may be administered in one or more administrations. An "activity decreasing amount," as used herein, refers to an amount of antagonist required to decrease the activity of an enzyme relative to the absence of the antagonist. A "function disrupting amount," as used herein, refers to the amount of antagonist required to disrupt the function of an enzyme or protein relative to the absence of the antagonist. The exact amounts will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*see, e.g.*, Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); Pickar, *Dosage Calculations* (1999); and *Remington: The Science and Practice of Pharmacy*, 20th Edition, 2003, Gennaro, Ed., Lippincott, Williams & Wilkins). The therapeutically effective amount can be ascertained by measuring relevant physiological effects, and it can be adjusted in connection with the dosing regimen and diagnostic analysis of the subject's condition, and the like. By way of example, measurement of the serum level of a CRF1 inhibitor (or, e.g., a metabolite thereof) at a particular time post-administration may be indicative of whether a therapeutically effective amount has been administered.

[0129] For any compound described herein, the therapeutically effective amount can be initially determined from cell culture assays. Target concentrations will be those concentrations of active compound(s) that are capable of achieving the methods described herein, as measured using the methods described herein or known in the art.

[0130] Therapeutically effective amounts for use in humans can also be determined from animal models. For example, a dose for humans can be formulated to achieve a concentration that has been found to be effective in animals. The dosage in humans can be adjusted by monitoring compounds effectiveness and adjusting the dosage upwards or downwards, as described above. Adjusting the dose to achieve maximal efficacy in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan. Adjusting the dose to achieve maximal therapeutic window efficacy or toxicity in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan.

[0131] The term "therapeutically effective amount," as used herein, refers to that amount of the therapeutic agent sufficient to ameliorate the disorder, as described above. For example, for the given parameter, a therapeutically effective amount will show an increase or decrease of at least 5%, 10%, 15%, 20%, 25%, 40%, 50%, 60%, 75%, 80%, 90%, or at least 100%. Therapeutic efficacy can also be expressed as "-fold" increase or decrease. For example, a therapeutically

effective amount can have at least a 1.2-fold, 1.5-fold, 2-fold, 5-fold, or more effect over a control.

[0132] The terms “QD,” “qd,” and “q.d.” refer to once a day, i.e., a drug or therapeutic agent is administered once a day for the duration of the therapeutic regimen.

[0133] The terms “BD,” “bd,” and “b.i.d.” refer to twice a day, i.e., a drug or therapeutic agent is administered two times a day for the duration of the therapeutic regimen.

[0134] The terms “QID,” “qid,” and “q.i.d.” refer to three times a day, i.e., a drug or therapeutic agent is administered three times a day for the duration of the therapeutic regimen.

[0135] Dosages may be varied depending upon the requirements of the patient and the compound being employed. The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial therapeutic response in the patient over time. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects. Determination of the proper dosage for a particular situation is within the skill of the practitioner. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under circumstances is reached. Dosage amounts and intervals can be adjusted individually to provide levels of the administered compound effective for the particular clinical indication being treated. This will provide a therapeutic regimen that is commensurate with the severity of the individual's disease state.

[0136] As used herein, the term “administering” means oral administration, administration as a suppository, topical contact, intravenous, parenteral, intraperitoneal, intramuscular, intralesional, intrathecal, intracranial, intranasal or subcutaneous administration, or the implantation of a slow-release device, *e.g.,* a mini-osmotic pump, to a subject. Administration is by any route, including parenteral and transmucosal (*e.g.,* buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, *e.g.,* intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, *etc.* By “co-administer” it is meant that a composition described herein is administered at the same time, just prior to, or just after the administration of one or more additional therapies (e.g. anti-cancer agent, chemotherapeutic, or treatment for a neurodegenerative disease). The compound of the invention can be administered alone or can be coadministered to the patient. Coadministration is meant to include simultaneous or sequential administration of the compound individually or in combination (more than one compound or agent). Thus, the preparations can also be combined, when desired, with other active substances (e.g. to reduce metabolic degradation). The compositions of the present invention can be

delivered by transdermally, by a topical route, formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols. Oral preparations include tablets, pills, powder, dragees, capsules, liquids, lozenges, cachets, gels, syrups, slurries, suspensions, etc., suitable for ingestion by the patient. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water/propylene glycol solutions. The compositions of the present invention may additionally include components to provide sustained release and/or comfort. Such components include high molecular weight, anionic mucomimetic polymers, gelling polysaccharides and finely-divided drug carrier substrates. These components are discussed in greater detail in U.S. Pat. Nos. 4,911,920; 5,403,841; 5,212,162; and 4,861,760. The entire contents of these patents are incorporated herein by reference in their entirety for all purposes. The compositions of the present invention can also be delivered as microspheres for slow release in the body. For example, microspheres can be administered via intradermal injection of drug-containing microspheres, which slowly release subcutaneously (see Rao, *J. Biomater Sci. Polym. Ed*. 7:623-645, 1995; as biodegradable and injectable gel formulations (see, e.g., Gao *Pharm. Res*. 12:857-863, 1995); or, as microspheres for oral administration (see, e.g., Eyles, *J. Pharm. Pharmacol.* 49:669-674, 1997). In another embodiment, the formulations of the compositions of the present invention can be delivered by the use of liposomes which fuse with the cellular membrane or are endocytosed, *i.e.*, by employing receptor ligands attached to the liposome, that bind to surface membrane protein receptors of the cell resulting in endocytosis. By using liposomes, particularly where the liposome surface carries receptor ligands specific for target cells, or are otherwise preferentially directed to a specific organ, one can focus the delivery of the compositions of the present invention into the target cells in vivo. (See, *e.g.*, Al-Muhammed, *J. Microencapsul.* 13:293-306, 1996; Chonn, *Curr. Opin. Biotechnol.* 6:698-708, 1995; Ostro, *Am. J. Hosp. Pharm.* 46:1576-1587, 1989). The compositions of the present invention can also be delivered as nanoparticles.

[0137] By “co-administer” it is meant that a composition described herein is administered at the same time, just prior to, or just after the administration of one or more additional therapies. The compounds of the invention can be administered alone or can be coadministered to the patient. Coadministration is meant to include simultaneous or sequential administration of the compounds individually or in combination (more than one compound). The compositions of the present invention can be delivered transdermally, by a topical route, or formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols.

[0138] For any compound described herein, the therapeutically effective amount can be initially determined from cell culture assays. Target concentrations will be those concentrations of active compound(s) that are capable of achieving the methods described herein, as measured using the methods described herein or known in the art.

[0139] Utilizing the teachings provided herein, an effective prophylactic or therapeutic treatment regimen can be planned that does not cause substantial toxicity and yet is effective to treat the clinical symptoms demonstrated by the particular patient. This planning should involve the careful choice of active compound by considering factors such as compound potency, relative bioavailability, patient body weight, presence and severity of adverse side effects, preferred mode of administration and the toxicity profile of the selected agent.

[0140] The compounds described herein can be used in combination with one another, with other active agents known to be useful in treating cancer (e.g. colon cancer), cardiovascular disease, metabolic disease, immune or inflammatory disease or disorder.

[0141] In some embodiments, co-administration includes administering one active agent within 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24 hours, 2 days, 4 days, 1 week or 1 month of a second active agent. Co-administration includes administering two active agents simultaneously, approximately simultaneously (e.g., within about 1, 5, 10, 15, 20, or 30 minutes of each other), or sequentially in any order. In some embodiments, co-administration can be accomplished by co-formulation, i.e., preparing a single pharmaceutical composition including both active agents. In other embodiments, the active agents can be formulated separately. In another embodiment, the active and/or adjunctive agents may be linked or conjugated to one another. In some embodiments, the compounds described herein may be combined with treatments for cancer (e.g. colon cancer), cardiovascular disease, metabolic disease, immune or inflammatory disease or disorder.

[0142] The compounds described herein can be administered to treat a fertility disease or disorder and/or cancer. In this regard, the compounds disclosed herein may be administered either alone to treat such diseases or disorders or may be co-administered with another therapeutic agent to treat such diseases or disorders.

[0143] The compounds disclosed herein may be co-administered with an agonist or antagonist of CRF_1 function. The compounds described herein can be co-administered with conventional anti-cancer/chemotherapeutic.

[0144] "Anti-cancer agent" is used in accordance with its plain ordinary meaning and refers to a composition (e.g. compound, drug, antagonist, inhibitor, modulator) having antineoplastic properties or the ability to inhibit the growth or proliferation of cells. In some embodiments, an anti-cancer agent is a chemotherapeutic. In some embodiments, an anti-cancer agent is an agent

identified herein having utility in methods of treating cancer. In some embodiments, an anti-cancer agent is an agent approved by the FDA or similar regulatory agency of a country other than the USA, for treating cancer. Examples of anti-cancer agents include, but are not limited to, MEK (e.g. MEK1, MEK2, or MEK1 and MEK2) inhibitors (e.g. XL518, CI-1040, PD035901, selumetinib/ AZD6244, GSK1120212/ trametinib, GDC-0973, ARRY-162, ARRY-300, AZD8330, PD0325901, U0126, PD98059, TAK-733, PD318088, AS703026, BAY 869766), alkylating agents (*e.g.*, cyclophosphamide, ifosfamide, chlorambucil, busulfan, melphalan, mechlorethamine, uramustine, thiotepa, nitrosoureas, nitrogen mustards (e.g., mechloroethamine, cyclophosphamide, chlorambucil, meiphalan), ethylenimine and methylmelamines (e.g., hexamethlymelamine, thiotepa), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine, lomusitne, semustine, streptozocin), triazenes (decarbazine)), anti-metabolites (*e.g.*, 5-azathioprine, leucovorin, capecitabine, fludarabine, gemcitabine, pemetrexed, raltitrexed, folic acid analog (e.g., methotrexate), or pyrimidine analogs (e.g., fluorouracil, floxouridine, Cytarabine), purine analogs (e.g., mercaptopurine, thioguanine, pentostatin), *etc.*), plant alkaloids (*e.g.*, vincristine, vinblastine, vinorelbine, vindesine, podophyllotoxin, paclitaxel, docetaxel, *etc.*), topoisomerase inhibitors (*e.g.*, irinotecan, topotecan, amsacrine, etoposide (VP16), etoposide phosphate, teniposide, *etc.*), antitumor antibiotics (*e.g.*, doxorubicin, adriamycin, daunorubicin, epirubicin, actinomycin, bleomycin, mitomycin, mitoxantrone, plicamycin, *etc.*), platinum-based compounds (*e.g.* cisplatin, oxaloplatin, carboplatin), anthracenedione (e.g., mitoxantrone), substituted urea (e.g., hydroxyurea), methyl hydrazine derivative (e.g., procarbazine), adrenocortical suppressant (e.g., mitotane, aminoglutethimide), epipodophyllotoxins (e.g., etoposide), antibiotics (e.g., daunorubicin, doxorubicin, bleomycin), enzymes (e.g., L-asparaginase), inhibitors of mitogen-activated protein kinase signaling (e.g. U0126, PD98059, PD184352, PD0325901, ARRY-142886, SB239063, SP600125, BAY 43-9006, wortmannin, or LY294002, Syk inhibitors, mTOR inhibitors, antibodies (e.g., rituxan), gossyphol, genasense, polyphenol E, Chlorofusin, all trans-retinoic acid (ATRA), bryostatin, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), 5-aza-2'-deoxycytidine, all trans retinoic acid, doxorubicin, vincristine, etoposide, gemcitabine, imatinib (Gleevec.RTM.), geldanamycin, 17-N-Allylamino-17-Demethoxygeldanamycin (17-AAG), flavopiridol, LY294002, bortezomib, trastuzumab, BAY 11-7082, PKC412, PD184352, 20-epi-1, 25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense

oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; 9-dioxamycin; diphenyl spiromustine; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone;

miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylerie conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen-binding protein; sizofuran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem

cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; zinostatin stimalamer, Adriamycin, Dactinomycin, Bleomycin, Vinblastine, Cisplatin, acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; fluorocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; iimofosine; interleukin I1 (including recombinant interleukin II, or rlL.sub.2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-1a; interferon gamma-1b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazoie; nogalamycin; ormaplatin; oxisuran; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone

hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride, agents that arrest cells in the G2-M phases and/or modulate the formation or stability of microtubules, (e.g. Taxol.TM (i.e. paclitaxel), Taxotere.TM, compounds comprising the taxane skeleton, Erbulozole (i.e. R-55104), Dolastatin 10 (i.e. DLS-10 and NSC-376128), Mivobulin isethionate (i.e. as CI-980), Vincristine, NSC-639829, Discodermolide (i.e. as NVP-XX-A-296), ABT-751 (Abbott, i.e. E-7010), Altorhyrtins (e.g. Altorhyrtin A and Altorhyrtin C), Spongistatins (e.g. Spongistatin 1, Spongistatin 2, Spongistatin 3, Spongistatin 4, Spongistatin 5, Spongistatin 6, Spongistatin 7, Spongistatin 8, and Spongistatin 9), Cemadotin hydrochloride (i.e. LU-103793 and NSC-D-669356), Epothilones (e.g. Epothilone A, Epothilone B, Epothilone C (i.e. desoxyepothilone A or dEpoA), Epothilone D (i.e. KOS-862, dEpoB, and desoxyepothilone B), Epothilone E, Epothilone F, Epothilone B N-oxide, Epothilone A N-oxide, 16-aza-epothilone B, 21-aminoepothilone B (i.e. BMS-310705), 21-hydroxyepothilone D (i.e. Desoxyepothilone F and dEpoF), 26-fluoroepothilone, Auristatin PE (i.e. NSC-654663), Soblidotin (i.e. TZT-1027), LS-4559-P (Pharmacia, i.e. LS-4577), LS-4578 (Pharmacia, i.e. LS-477-P), LS-4477 (Pharmacia), LS-4559 (Pharmacia), RPR-112378 (Aventis), Vincristine sulfate, DZ-3358 (Daiichi), FR-182877 (Fujisawa, i.e. WS-9885B), GS-164 (Takeda), GS-198 (Takeda), KAR-2 (Hungarian Academy of Sciences), BSF-223651 (BASF, i.e. ILX-651 and LU-223651), SAH-49960 (Lilly/Novartis), SDZ-268970 (Lilly/Novartis), AM-97 (Armad/Kyowa Hakko), AM-132 (Armad), AM-138 (Armad/Kyowa Hakko), IDN-5005 (Indena), Cryptophycin 52 (i.e. LY-355703), AC-7739 (Ajinomoto, i.e. AVE-8063A and CS-39.HCl), AC-7700 (Ajinomoto, i.e. AVE-8062, AVE-8062A, CS-39-L-Ser.HCl, and RPR-258062A), Vitilevuamide, Tubulysin A, Canadensol, Centaureidin (i.e. NSC-106969), T-138067 (Tularik, i.e. T-67, TL-138067 and TI-138067), COBRA-1 (Parker Hughes Institute, i.e. DDE-261 and WHI-261), H10 (Kansas State University), H16 (Kansas State University), Oncocidin A1 (i.e. BTO-956 and DIME), DDE-313 (Parker Hughes Institute), Fijianolide B, Laulimalide, SPA-2 (Parker Hughes Institute), SPA-1 (Parker Hughes Institute, i.e. SPIKET-P), 3-IAABU (Cytoskeleton/Mt. Sinai School of Medicine, i.e. MF-569), Narcosine (also known as NSC-5366), Nascapine, D-24851 (Asta Medica), A-105972 (Abbott), Hemiasterlin, 3-BAABU (Cytoskeleton/Mt. Sinai School of Medicine, i.e. MF-191), TMPN (Arizona State University), Vanadocene acetylacetonate, T-138026 (Tularik), Monsatrol, lnanocine (i.e. NSC-698666), 3-IAABE (Cytoskeleton/Mt. Sinai

School of Medicine), A-204197 (Abbott), T-607 (Tuiarik, i.e. T-900607), RPR-115781 (Aventis), Eleutherobins (such as Desmethyleleutherobin, Desaetyleleutherobin, lsoeleutherobin A, and Z-Eleutherobin), Caribaeoside, Caribaeolin, Halichondrin B, D-64131 (Asta Medica), D-68144 (Asta Medica), Diazonamide A, A-293620 (Abbott), NPI-2350 (Nereus), Taccalonolide A, TUB-245 (Aventis), A-259754 (Abbott), Diozostatin, (-)-Phenylahistin (i.e. NSCL-96F037), D-68838 (Asta Medica), D-68836 (Asta Medica), Myoseverin B, D-43411 (Zentaris, i.e. D-81862), A-289099 (Abbott), A-318315 (Abbott), HTI-286 (i.e. SPA-110, trifluoroacetate salt) (Wyeth), D-82317 (Zentaris), D-82318 (Zentaris), SC-12983 (NCI), Resverastatin phosphate sodium, BPR-OY-007 (National Health Research Institutes), and SSR-250411 (Sanofi)), steroids (*e.g.*, dexamethasone), finasteride, aromatase inhibitors, gonadotropin-releasing hormone agonists (GnRH) such as goserelin or leuprolide, adrenocorticosteroids (e.g., prednisone), progestins (e.g., hydroxyprogesterone caproate, megestrol acetate, medroxyprogesterone acetate), estrogens (e.g., diethlystilbestrol, ethinyl estradiol), antiestrogen (e.g., tamoxifen), androgens (e.g., testosterone propionate, fluoxymesterone), antiandrogen (e.g., flutamide), immunostimulants (*e.g.*, Bacillus Calmette-Guérin (BCG), levamisole, interleukin-2, alpha-interferon, *etc.*), monoclonal antibodies (*e.g.*, anti-CD20, anti-HER2, anti-CD52, anti-HLA-DR, and anti-VEGF monoclonal antibodies), immunotoxins (*e.g.*, anti-CD33 monoclonal antibody-calicheamicin conjugate, anti-CD22 monoclonal antibody-pseudomonas exotoxin conjugate, *etc.*), radioimmunotherapy (*e.g.*, anti-CD20 monoclonal antibody conjugated to ^{111}In, ^{90}Y, or ^{131}I, *etc.*), triptolide, homoharringtonine, dactinomycin, doxorubicin, epirubicin, topotecan, itraconazole, vindesine, cerivastatin, vincristine, deoxyadenosine, sertraline, pitavastatin, irinotecan, clofazimine, 5-nonyloxytryptamine, vemurafenib, dabrafenib, erlotinib, gefitinib, EGFR inhibitors, epidermal growth factor receptor (EGFR)-targeted therapy or therapeutic (e.g. gefitinib (Iressa ™), erlotinib (Tarceva ™), cetuximab (Erbitux™), lapatinib (Tykerb™), panitumumab (Vectibix™), vandetanib (Caprelsa™), afatinib/BIBW2992, CI-1033/canertinib, neratinib/HKI-272, CP-724714, TAK-285, AST-1306, ARRY334543, ARRY-380, AG-1478, dacomitinib/PF299804, OSI-420/desmethyl erlotinib, AZD8931, AEE788, pelitinib/EKB-569, CUDC-101, WZ8040, WZ4002, WZ3146, AG-490, XL647, PD153035, BMS-599626), sorafenib, imatinib, sunitinib, dasatinib, or the like.

[0145] "Chemotherapeutic" or "chemotherapeutic agent" is used in accordance with its plain ordinary meaning and refers to a chemical composition or compound having antineoplastic properties or the ability to inhibit the growth or proliferation of cells.

[0146] The compounds disclosed herein may be co-administered with an antiproliferative/antineoplastic drug or a combination thereof, as used in medical oncology, such as an alkylating agent (for example cis-platin, carboplatin, cyclophosphamide, nitrogen mustard,

melphalan, chlorambucil, busulphan or a nitrosourea); an antimetabolite (for example an antifolate such as a fluoropyrimidine like 5-fluorouracil or tegafur, raltitrexed, methotrexate, cytosine arabinoside, hydroxyurea, gemcitabine or paclitaxel); an antitumour antibiotic (for example an anthracycline such as adriamycin, bleomycin, doxorubicin, daunomycin, epirubicin, idarubicin, mitomycin-C, dactinomycin or mithramycin); an antimitotic agent (for example a vinca alkaloid such as vincristine, vinblastine, vindesine or vinorelbine, or a taxoid such as taxol or taxotere); or a topoisomerase inhibitor (for example an epipodophyllotoxin such as etoposide, teniposide, amsacrine, topotecan or a camptothecin); (ii) a cytostatic agent such as an antioestrogen (for example tamoxifen, toremifene, raloxifene, droloxifene or iodoxyfene), an oestrogen receptor down regulator (for example fulvestrant), an antiandrogen (for example bicalutamide, flutamide, nilutamide or cyproterone acetate), a LHRH antagonist or LHRH agonist (for example goserelin, leuprorelin or buserelin), a progestogen (for example megestrol acetate), an aromatase inhibitor (for example as anastrozole, letrozole, vorazole or exemestane) or an inhibitor of 5.alpha.-reductase such as finasteride; (iii) an agent which inhibits cancer cell invasion (for example a metalloproteinase inhibitor like marimastat or an inhibitor of urokinase plasminogen activator receptor function); (iv) an inhibitor of growth factor function, for example: a growth factor antibody (for example the anti-erbb2 antibody trastuzumab, or the anti-erbb1 antibody cetuximab [C225]), a farnesyl transferase inhibitor, a tyrosine kinase inhibitor or a serine/threonine kinase inhibitor, an inhibitor of the epidermal growth factor family (for example an EGFR family tyrosine kinase inhibitor such as N-(3-chloro-4-fluorophenyl)-7-methoxy-6-(3-morpholinopropoxy)quinazoli- n-4-amine (gefitinib, AZD 1839), N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine (erlotinib, OSI-774) or 6-acrylamido-N-(3-chloro-4-fluorophenyl)-7-(3-morpholinopropoxy)quinazoli- n-4-amine (CI 1033)), an inhibitor of the platelet-derived growth factor family, or an inhibitor of the hepatocyte growth factor family; (v) an antiangiogenic agent such as one which inhibits the effects of vascular endothelial growth factor (for example the anti-vascular endothelial cell growth factor antibody bevacizumab, a compound disclosed in WO 97/22596, WO 97/30035, WO 97/32856 or WO 98/13354), or a compound that works by another mechanism (for example linomide, an inhibitor of integrin .alpha.v.beta.3 function or an angiostatin); (vi) a vascular damaging agent such as combretastatin A4, or a compound disclosed in WO 99/02166, WO 00/40529, WO 00/41669, WO 01/92224, WO 02/04434 or WO 02/08213; (vii) an agent used in antisense therapy, for example one directed to one of the targets listed above, such as ISIS 2503, an anti-ras antisense; (viii) an agent used in a gene therapy approach, for example approaches to replace aberrant genes such as aberrant p53 or aberrant BRCA1 or BRCA2, GDEPT (gene-directed enzyme pro-drug therapy) approaches such as those using cytosine deaminase, thymidine kinase or a

bacterial nitroreductase enzyme and approaches to increase patient tolerance to chemotherapy or radiotherapy such as multi-drug resistance gene therapy; or (ix) an agent used in an immunotherapeutic approach, for example ex-vivo and in-vivo approaches to increase the immunogenicity of patient tumour cells, such as transfection with cytokines such as interleukin 2, interleukin 4 or granulocyte-macrophage colony stimulating factor, approaches to decrease T-cell anergy, approaches using transfected immune cells such as cytokine-transfected dendritic cells, approaches using cytokine-transfected tumour cell lines and approaches using anti-idiotypic antibodies.

[0147] In embodiments, the compounds disclosed herein can be co-administered with an antibody, such as a monoclonal antibody targeting B-Lymphocytes (such as CD20 (rituximab), MRA-aIL16R and T-Lymphocytes, CTLA4-Ig, HuMax I1-15) or antibody modulating Ig function such as anti-IgE (for example omalizumab).

[0148] In embodiments, treatment of cancer includes administration of an effective amount of at least two of the following: a CRF1 inhibitor, an inhibitor of the PD-L1/PD-1 pathway, an inhibitor of CTLA-4, an agonistic antibody of CD137 (4-1BB). In some embodiments, the method may comprise the use of two or more combinations.

[0149] In a further embodiment, the compounds described herein can be co-administered with conventional radiotherapeutic agents including, but not limited to, radionuclides such as ^{47}Sc, ^{64}Cu, ^{67}Cu, ^{89}Sr, ^{86}Y, ^{87}Y, ^{90}Y, ^{105}Rh, ^{111}Ag, ^{111}In, ^{117m}Sn, ^{149}Pm, ^{153}Sm, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{211}At, and ^{212}Bi, optionally conjugated to antibodies directed against tumor antigens.

[0150] In addition, a CRF_1 inhibitor may be combined with the therapeutic administration of immune cells, sometimes referred to as adoptive cell transfer. These cells may be cells from the patient, a genetically related or unrelated donor, they may be genetically modified (e.g. CAR-T cells, NK cells, etc), cell lines, genetically modified cell lines and live or dead versions of the above. CRF1 inhibitors may also be combined with vaccines of any kind (e.g. protein/peptide, viral, bacterial, cellular) to stimulate immune responses to cancer.

[0151] The CRF_1 inhibitors/CRF_1 antagonists disclosed herein can be administered by any acceptable route, such oral, intraadiposal, intraarterial, intraarticular, intracranial, intradermal, intralesional, intramusculay, intranasal, intraocularal, intrapericardial, intraperitoneal, intrapleural, intraprostatical, intrarectal, intrathecal, intratracheal, intratumoral, intraumbilical, intravaginal, intravenousl, intravesicullar, intravitreal, liposomal, local, mucosal, parenteral, rectal, subconjunctival, subcutaneous, sublingual, topical, transbuccal, transdermal, vaginal, in cremes, in lipid compositions, via a catheter, via a lavage, via continuous infusion, via infusion, via inhalation, via injection, via local delivery, via localized perfusion, bathing target cells directly, or any combination thereof.

[0152] The CRF_1 inhibitors/CRF_1 antagonists disclosed herein can be administered by any acceptable route, such oral, intraadiposal, intraarterial, intraarticular, intracranial, intradermal, intralesional, intramusculay, intranasal, intraocularal, intrapericardial, intraperitoneal, intrapleural, intraprostatical, intrarectal, intrathecal, intratracheal, intratumoral, intraumbilical, intravaginal, intravenousl, intravesicullar, intravitreal, liposomal, local, mucosal, parenteral, rectal, subconjunctival, subcutaneous, sublingual, topical, transbuccal, transdermal, vaginal, in cremes, in lipid compositions, via a catheter, via a lavage, via continuous infusion, via infusion, via inhalation, via injection, via local delivery, via localized perfusion, bathing target cells directly, or any combination thereof.

[0153] The CRF_1 inhibitors/CRF_1 antagonists disclosed herein may be administered once daily until study reached endpoint. The CRF_1 inhibitors/CRF_1 antagonists disclosed herein may be administered at least three times but in some studies four or more times depending on the length of the study and/or the design of the study.

[0154] The methods disclosed herein may be used in combination with additional cancer therapy. In some embodiments, the distinct cancer therapy comprises surgery, radiotherapy, chemotherapy, toxin therapy, immunotherapy, cryotherapy or gene therapy. In some embodiments, the cancer is a chemotherapy-resistant or radio-resistant cancer.

[0155] A “cell” as used herein, refers to a cell carrying out metabolic or other function sufficient to preserve or replicate its genomic DNA. A cell can be identified by well-known methods in the art including, for example, presence of an intact membrane, staining by a particular dye, ability to produce progeny or, in the case of a gamete, ability to combine with a second gamete to produce a viable offspring. Cells may include prokaryotic and eukaroytic cells. Prokaryotic cells include but are not limited to bacteria. Eukaryotic cells include but are not limited to yeast cells and cells derived from plants and animals, for example mammalian, insect (*e.g.*, spodoptera) and human cells. Cells may be useful when they are naturally nonadherent or have been treated not to adhere to surfaces, for example by trypsinization.

[0156] “Control” or “control experiment” is used in accordance with its plain ordinary meaning and refers to an experiment in which the subjects or reagents of the experiment are treated as in a parallel experiment except for omission of a procedure, reagent, or variable of the experiment. In some instances, the control is used as a standard of comparison in evaluating experimental effects. In some embodiments, a control is the measurement of the activity of a protein in the absence of a compound as described herein (including embodiments and examples).

[0157] The term “modulator” refers to a composition that increases or decreases the level of a target molecule or the function of a target molecule or the physical state of the target of the molecule. In some embodiments, a CRF1 associated disease modulator is a compound that

reduces the severity of one or more symptoms of a disease associated with CRF_1 (e.g. an endocrine disorder, a cancer including but not limited to TART or OART). A CRF_1 modulator is a compound that increases or decreases the activity or function or level of activity or level of function of CRF_1. A modulator may act alone, or it may use a cofactor, e.g., a protein, metal ion, or small molecule. Examples of modulators include small molecule compounds and other bioorganic molecules. Numerous libraries of small molecule compounds (e.g., combinatorial libraries) are commercially available and can serve as a starting point for identifying a modulator. The skilled artisan is able to develop one or more assays (e.g., biochemical or cell-based assays) in which such compound libraries can be screened in order to identify one or more compounds having the desired properties; thereafter, the skilled medicinal chemist is able to optimize such one or more compounds by, for example, synthesizing and evaluating analogs and derivatives thereof. Synthetic and/or molecular modeling studies can also be utilized in the identification of an activator.

[0158] The term “modulate” is used in accordance with its plain ordinary meaning and refers to the act of changing or varying one or more properties. “Modulation” refers to the process of changing or varying one or more properties. For example, as applied to the effects of a modulator on a target protein, to modulate means to change by increasing or decreasing a property or function of the target molecule or the amount of the target molecule. In embodiments, the terms “modulate”, “modulation” and the like refer to the ability of a molecule (e.g., an activator or an inhibitor) to increase or decrease the function or activity of CRF_1, either directly or indirectly, relative to the absence of the molecule.

[0159] The term “associated” or “associated with” in the context of a substance or substance activity or function associated with a disease (e.g. a protein associated disease, an endocrine disorder, a disease associated with CRF_1 activity, a CRF_1 associated disease (e.g., testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART)) means that the disease (e.g., testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART)) is caused by (in whole or in part), or a symptom of the disease is caused by (in whole or in part) the substance or substance activity or function. For example, an endocrine disorder associated with CRF_1 activity or function may be an endocrine disorder that results (entirely or partially) from aberrant CRF_1 function (e.g. enzyme activity, protein-protein interaction, signaling pathway) or an endocrine disorder wherein a particular symptom of the disease is caused (entirely or partially) by aberrant CRF_1 activity or function. As used herein, what is described as being associated with a disease, if a causative agent, could be a target for treatment of the disease. For example, an endocrine disorder associated with CRF_1 activity or function or a CRF_1 associated disease (e.g., an endocrine disorder including but not limited to TART or OART), may be treated with a

compound described herein (e.g., CRF_1 modulator, CRF_1 inhibitor, or CRF_1 antagonist), in the instance where increased CRF_1 activity or function (e.g. signaling pathway activity) causes the disease (e.g., an endocrine disorder such as TART or OART). For example, an endocrine disorder associated with CRF_1 activity or function or a CRF_1 associated endocrine disorder, may be treated with an CRF_1 modulator or CRF_1 inhibitor, in the instance where increased CRF_1 activity or function (e.g. signaling pathway activity) causes the disease.

[0160] The term "aberrant" as used herein refers to different from normal. When used to describe enzymatic activity or protein function, aberrant refers to activity or function that is greater or less than a normal control or the average of normal non-diseased control samples. Aberrant activity may refer to an amount of activity that results in a disease, wherein returning the aberrant activity to a normal or non-disease-associated amount (e.g. by administering a compound or using a method as described herein), results in reduction of the disease or one or more disease symptoms.

[0161] The term "signaling pathway" as used herein refers to a series of interactions between cellular and optionally extra-cellular components (e.g. proteins, nucleic acids, small molecules, ions, lipids) that conveys a change in one component to one or more other components, which in turn may convey a change to additional components, which is optionally propogated to other signaling pathway components. For example, binding of a CRF_1 with a compound as described herein may reduce the level of a product of the CRF_1 catalyzed reaction or the level of a downstream derivative of the product or binding may reduce the interactions between the CRF_1 or a reaction product and downstream effectors or signaling pathway components (e.g., MAP kinase pathway), resulting in changes in cell growth, proliferation, or survival.

[0162] As used herein, the terms "corticotropin-releasing factor type-1," "CRF_1," "corticotropin-releasing hormone receptor 1," and "CHHR1" and all other related art-accepted terms, many of which are set forth below, refer to a compound capable of modulating, either directly or indirectly, the CRF_1 receptor in an *in vitro* assay, an *in vivo* model, and/or other means indicative of therapeutic efficacy. The terms also refer to a compound that exhibits at least some therapeutic benefit in a human subject.

[0163] The phrase "in a sufficient amount to effect a change" means that there is a detectable difference between a level of an indicator measured before (e.g., a baseline level) and after administration of a particular therapy. Indicators include any objective parameter (e.g., serum concentration) or subjective parameter (e.g., a subject's feeling of well-being).

[0164] The "activity" of a molecule may describe or refer to the binding of the molecule to a ligand or to a receptor; to catalytic activity; to the ability to stimulate gene expression or cell signaling, differentiation, or maturation; to antigenic activity; to the modulation of activities of

other molecules; and the like. The term "proliferative activity" encompasses an activity that promotes, that is necessary for, or that is specifically associated with, for example, normal cell division, as well as cancer, tumors, dysplasia, cell transformation, metastasis, and angiogenesis.

[0165] "Substantially pure" indicates that a component makes up greater than about 50% of the total content of the composition, and typically greater than about 60% of the total polypeptide content. More typically, "substantially pure" refers to compositions in which at least 75%, at least 85%, at least 90% or more of the total composition is the component of interest. In some cases, the polypeptide will make up greater than about 90%, or greater than about 95% of the total content of the composition (percentage in a weight per weight basis).

[0166] The terms "specifically binds" and "selectively binds," when referring to a ligand/receptor, antibody/antigen, or other binding pair, indicates a binding reaction which is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated conditions, a specified ligand binds to a particular receptor and does not bind in a significant amount to other proteins present in the sample. The antibody, or binding composition derived from the antigen-binding site of an antibody, of the contemplated method binds to its antigen, or a variant or mutein thereof, with an affinity that is at least two-fold greater, at least 10-times greater, at least 20-times greater, or at least 100-times greater than the affinity with any other antibody, or binding composition derived therefrom. In embodiments, the antibody will have an affinity that is greater than about 10^9 liters/mol, as determined by, e.g., Scatchard analysis (Munsen, et al. (1980) Analyt. Biochem. 107:220-239).

[0167] The terms "DNA," "nucleic acid," "nucleic acid molecule," "polynucleotide" and the like are used interchangeably herein to refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Non-limiting examples of polynucleotides include linear and circular nucleic acids, messenger RNA (mRNA), complementary DNA (cDNA), recombinant polynucleotides, vectors, probes, primers and the like.

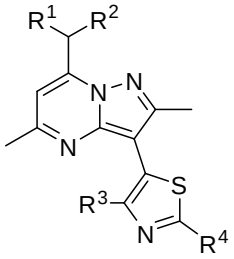
[0168] As used herein, the terms "variants" and "homologs" are used interchangeably to refer to amino acid or nucleic acid sequences that are similar to reference amino acid or nucleic acid sequences, respectively. The term encompasses naturally-occurring variants and non-naturally-occurring variants. Naturally-occurring variants include homologs (polypeptides and nucleic acids that differ in amino acid or nucleotide sequence, respectively, from one species to another), and allelic variants (polypeptides and nucleic acids that differ in amino acid or nucleotide sequence, respectively, from one individual to another within a species). Thus, variants and homologs encompass naturally occurring amino acid and nucleic acid sequences encoded thereby and their isoforms, as well as splice variants of a protein or gene. The terms also

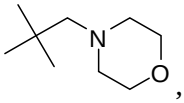
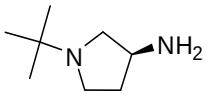
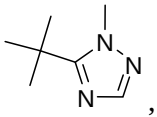
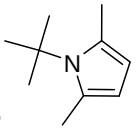
encompass nucleic acid sequences that vary in one or more bases from a naturally-occurring nucleic acid sequence but still translate into an amino acid sequence that corresponds to the naturally-occurring protein due to degeneracy of the genetic code. Non-naturally-occurring variants and homologs include polypeptides and nucleic acids that comprise a change in amino acid or nucleotide sequence, respectively, where the change in sequence is artificially introduced (e.g., muteins); for example, the change is generated in the laboratory by human intervention ("hand of man"). Therefore, non-naturally occurring variants and homologs may also refer to those that differ from the naturally-occurring sequences by one or more conservative substitutions and/or tags and/or conjugates.

[0169] The term "muteins" as used herein refers broadly to mutated recombinant proteins. These proteins usually carry single or multiple amino acid substitutions and are frequently derived from cloned genes that have been subjected to site-directed or random mutagenesis, or from completely synthetic genes.

II. Compounds

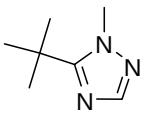
[0170] In an aspect provided herein, is a compound of Formula (I):

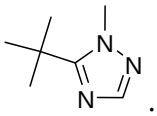


, or a pharmaceutically acceptable salt thereof. R^1 and R^2 are independently ethyl or n-propyl. R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy. R^4 is hydrogen, Br, R^aR^bN–, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl,  ,  ,  , or  . R^a and R^b are independently hydrogen, C_1-C_3alkyl, $H_2NCH_2CH_2$–, $(CH_3)_3COC(O)NHCH_2CH_2$–, or $CH_3CH_2CH_2NHCH_2CH_2$–In embodiments, the inhibitor forms an irreversible bond with the amino acid residue. In embodiments, the bond is a covalent bond.

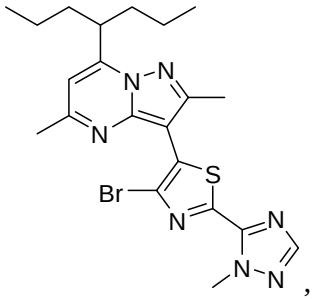
[0171] In certain embodiments, R^3 is Cl, Br, methyl, or trifluoromethyl.

[0172] In certain embodiments, R^3 is Cl, Br, or methyl.

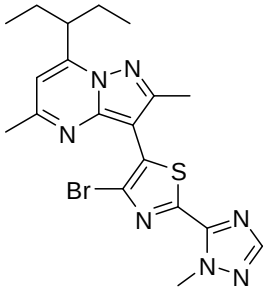
[0173] In certain embodiments, R^4 is Br, R^aR^bN–, pyridin-4-yl, morpholin-4-yl, or  .

[0174] In certain embodiments, R^4 is morpholin-4-yl or  .

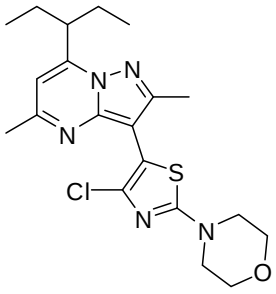
[0175] In certain embodiments, R^4 is hydrogen, Br, R^aR^bN– and R^a and R^b are independently C_1-C_3alkyl.

[0176] In certain embodiments, the compound is  , or a pharmaceutically acceptable salt thereof.

[0177] In certain embodiments, the compound is

 , or a pharmaceutically acceptable salt thereof.

[0178] In certain embodiments, the compound is

 , or a pharmaceutically acceptable salt thereof.

[0179] In one aspect described herein, there is a method of treating a subject in need thereof comprising administering an astressin or a pharmaceutically acceptable salt or solvate thereof. An astressin generally refers to a nonselective corticotropin releasing hormone antagonist that reduces the synthesis of ACTH and cortisol.

[0180] In one aspect described herein, there is a method of treating congenital adrenal hyperplasia (CAH) in a subject in need thereof, comprising administering an astressin or a variant having at least about 70% identity thereto or a pharmaceutically acceptable salt or solvate thereof. In one aspect described herein, there is a method of treating congenital adrenal hyperplasia (CAH) in a subject in need thereof, the method comprising: (i) measuring a hormone

level in the subject in need thereof; (ii) administering an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof; and repeating steps (i) and (ii) until the hormone level reaches a pre-determined range followed by a maintenance therapy of a daily dosing of said astressin.

[0181] In one aspect described herein, there is a method of improving hyperandrogenic symptoms in a subject in need thereof, comprising administering an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof. In one aspect described herein, there is a method of treating menstrual irregularity, ovulatory dysfunction or infertility, in a subject in need thereof, comprising administering an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof. In one aspect described herein, there is a method of improving metabolic symptoms in a subject in need thereof, comprising administering an astressin or a variant having at least about 70% identity thereto; or a pharmaceutically acceptable salt or solvate thereof.

[0182] In one aspect described herein, there is a method of improving quality of life in a subject in need thereof, comprising administering an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof.

III. Pharmaceutical Compositions

[0183] In an aspect, there is provided a pharmaceutical composition, including a compound as described herein, including embodiments, or the structural Formula (I), and a pharmaceutically acceptable excipient.

[0184] The compounds (e.g., CRF_1 inhibitors/antagonists) of the present disclosure may be in the form of compositions suitable for administration to a subject. In general, such compositions are "pharmaceutical compositions" comprising a compound (e.g., CRF_1 inhibitor(s)/ antagonist(s)) and one or more pharmaceutically acceptable or physiologically acceptable diluents, carriers or excipients. In certain embodiments, the compound (e.g., CRF_1 inhibitor/antagonist) are present in a therapeutically acceptable amount. The pharmaceutical compositions may be used in the methods of the present disclosure; thus, for example, the pharmaceutical compositions can be administered *ex vivo* or *in vivo* to a subject in order to practice the therapeutic and prophylactic methods and uses described herein.

[0185] In one aspect described herein, there is a pharmaceutical composition in the form of a capsule comprising an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof. In one aspect described herein, there is a pharmaceutical composition in the form of a tablet comprising an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof.

[0186] The pharmaceutical compositions of the present disclosure can be formulated to be compatible with the intended method or route of administration; exemplary routes of administration are set forth herein.

[0187] The pharmaceutical compositions containing the active ingredient (e.g., an inhibitor of CRF_1 function) may be in a form suitable for oral use, for example, as tablets, capsules, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups, solutions, microbeads or elixirs. Pharmaceutical compositions intended for oral use may be prepared for the manufacture of pharmaceutical compositions, and such compositions may contain one or more agents such as, for example, sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets, capsules and the like contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture thereof. These excipients may be, for example, diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc.

[0188] The tablets, capsules and the like suitable for oral administration may be uncoated or coated to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action. For example, a time-delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated to form osmotic therapeutic tablets for controlled release. Additional agents include biodegradable or biocompatible particles or a polymeric substance such as polyesters, polyamine acids, hydrogel, polyvinyl pyrrolidone, polyanhydrides, polyglycolic acid, ethylene-vinylacetate, methylcellulose, carboxymethylcellulose, protamine sulfate, or lactide/glycolide copolymers, polylactide/glycolide copolymers, or ethylenevinylacetate copolymers in order to control delivery of an administered composition. For example, the oral agent can be entrapped in microcapsules prepared by coacervation techniques or by interfacial polymerization, by the use of hydroxymethylcellulose or gelatin-microcapsules or poly(methylmethacrolate) microcapsules, respectively, or in a colloid drug delivery system. Colloidal dispersion systems include macromolecule complexes, nano-capsules, microspheres, microbeads, and lipid-based systems, including oil-in-water emulsions, micelles, mixed micelles, and liposomes. Methods for the preparation of the above-mentioned formulations will be apparent to those skilled in the art.

[0189] Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium

phosphate, kaolin or microcrystalline cellulose, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

[0190] Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture thereof. Such excipients can be suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxy-propylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents, for example a naturally-occurring phosphatide (e.g., lecithin), or condensation products of an alkylene oxide with fatty acids (e.g., polyoxy-ethylene stearate), or condensation products of ethylene oxide with long chain aliphatic alcohols (e.g., for heptadecaethyleneoxycetanol), or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol (e.g., polyoxyethylene sorbitol monooleate), or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides (e.g., polyethylene sorbitan monooleate). The aqueous suspensions may also contain one or more preservatives.

[0191] Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation.

[0192] Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, and optionally one or more suspending agents and/or preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified herein.

[0193] The pharmaceutical compositions of the present disclosure may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example, liquid paraffin, or mixtures of these. Suitable emulsifying agents may be naturally occurring gums, for example, gum acacia or gum tragacanth; naturally occurring phosphatides, for example, soy bean, lecithin, and esters or partial esters derived from fatty acids; hexitol anhydrides, for example, sorbitan monooleate; and condensation products of partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monooleate.

[0194] The pharmaceutical compositions typically comprise a therapeutically effective amount of a CRF_1 inhibitor/antagonist contemplated by the present disclosure and one or more pharmaceutically and physiologically acceptable formulation agents. Suitable pharmaceutically acceptable or physiologically acceptable diluents, carriers or excipients include, but are not limited to, antioxidants (e.g., ascorbic acid and sodium bisulfate), preservatives (e.g., benzyl

alcohol, methyl parabens, ethyl or n-propyl, p-hydroxybenzoate), emulsifying agents, suspending agents, dispersing agents, solvents, fillers, bulking agents, detergents, buffers, vehicles, diluents, and/or adjuvants. For example, a suitable vehicle may be physiological saline solution or citrate-buffered saline, possibly supplemented with other materials common in pharmaceutical compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Those skilled in the art will readily recognize a variety of buffers that can be used in the pharmaceutical compositions and dosage forms contemplated herein. Typical buffers include, but are not limited to, pharmaceutically acceptable weak acids, weak bases, or mixtures thereof. As an example, the buffer components can be water soluble materials such as phosphoric acid, tartaric acids, lactic acid, succinic acid, citric acid, acetic acid, ascorbic acid, aspartic acid, glutamic acid, and salts thereof. Acceptable buffering agents include, for example, a Tris buffer; N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES); 2-(N-Morpholino)ethanesulfonic acid (MES); 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES); 3-(N-Morpholino)propanesulfonic acid (MOPS); and N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS).

[0195] After a pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form, a lyophilized form requiring reconstitution prior to use, a liquid form requiring dilution prior to use, or other acceptable form. In some embodiments, the pharmaceutical composition is provided in a single-use container (e.g., a single-use vial, ampule, syringe, or autoinjector (similar to, e.g., an EpiPen®)), whereas a multi-use container (e.g., a multi-use vial) is provided in other embodiments.

[0196] Formulations can also include carriers to protect the composition against rapid degradation or elimination from the body, such as a controlled release formulation, including liposomes, hydrogels, prodrugs and microencapsulated delivery systems. For example, a time-delay material such as glyceryl monostearate or glyceryl stearate alone, or in combination with a wax, may be employed. Any drug delivery apparatus may be used to deliver a CRF_1 inhibitor/antagonist, including implants (e.g., implantable pumps) and catheter systems, slow injection pumps and devices, all of which are well known to the skilled artisan.

[0197] Depot injections, which are generally administered subcutaneously or intramuscularly, may also be utilized to release the compound (e.g., CRF_1 inhibitor/antagonist) disclosed herein over a defined period of time. Depot injections are usually either solid- or oil-based and generally comprise at least one of the formulation components set forth herein. One of ordinary skill in the art is familiar with possible formulations and uses of depot injections.

[0198] The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents mentioned herein. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Acceptable diluents, solvents and dispersion media that may be employed include water, Ringer's solution, isotonic sodium chloride solution, Cremophor® EL (BASF, Parsippany, NJ) or phosphate buffered saline (PBS), ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof. In addition, sterile fixed oils are conventionally employed as a solvent or suspending medium; for this purpose, any bland fixed oil may be employed, including synthetic mono- or diglycerides. Moreover, fatty acids, such as oleic acid, find use in the preparation of injectables. Prolonged absorption of particular injectable formulations can be achieved by including an agent that delays absorption (e.g., aluminum monostearate or gelatin).

[0199] The present disclosure contemplates the administration of the compound (e.g., CRF_1 inhibitor/antagonist) in the form of suppositories for rectal administration. The suppositories can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include, but are not limited to, cocoa butter and polyethylene glycols.

[0200] The compound (e.g., CRF_1 inhibitor/antagonist) contemplated by the present disclosure may be in the form of any other suitable pharmaceutical composition (e.g., sprays for nasal or inhalation use) currently known or developed in the future.

IV. Methods

[0201] In certain embodiments, provided herein are methods for treating or preventing a disease comprising administering an effective amount of a compound of Formula (I), or an isotopic variant thereof or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof.

[0202] In one embodiment, provided herein are methods for treating or preventing a tumor, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing a benign tumor, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing a malignant tumor, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing an adrenal

tumor, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing an adrenal rest tumor, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof.

[0203] In one embodiment, provided herein are methods for treating or preventing infertility, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing male infertility, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for improving or increasing sperm count, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for improving or increasing sperm motility, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for improving or increasing sperm morphology, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing female infertility, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof.

[0204] In one embodiment, provided herein are methods for treating or preventing a proliferative or hyperproliferative disease or disorder, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In certain embodiments, the proliferative disease is cancer. In certain embodiments, the proliferative disease is a cancer of the breast, skin, prostate, cervix, uterus, ovary, testes, bladder, lung, liver, larynx, oral cavity, colon and gastrointestinal tract (e.g., esophagus, stomach, pancreas), brain, thyroid, blood, and lymphatic system.

[0205] In certain embodiments, the cancer treatable with the methods provided herein includes, but is not limited to, (1) leukemias, including, but not limited to, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemias such as myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia leukemias and myelodysplastic syndrome or a symptom thereof (such as anemia, thrombocytopenia, neutropenia, bicytopenia or pancytopenia), refractory anemia (RA), RA with ringed sideroblasts (RARS), RA with excess blasts (RAEB),

RAEB in transformation (RAEB-T), preleukemia, and chronic myelomonocytic leukemia (CMML), (2) chronic leukemias, including, but not limited to, chronic rnyelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, and hairy cell leukemia; (3) polycythemia vera; (4) lymphomas, including, but not limited to, Hodgkin's disease and non-Hodgkin's disease; (5) multiple myelomas, including, but not limited to, smoldering multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma, and extramedullary plasmacytoma; (6) Waldenstrom's macroglobulinernia; (7) monoclonal gammopathy of undetermined significance; (8) benign monoclonal gammopathy; (9) heavy chain disease; (10) bone and connective tissue sarcomas, including, but not limited to, bone sarcoma, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, metastatic cancers, neurilemmoma, rhabdomyosarcoma, and synovial sarcoma; (11) brain tumors, including, but not limited to, glioma, astrocytoma, brain stem glioma, ependymoma, aligodendrogliorna, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma, pineocytoma, pineoblastoma, and primary brain lymphoma; (12) breast cancer, including, but not limited to, adenocarcinoma, lobular (small cell) carcinoma, intraductal carcinoma, medullary breast cancer, mutinous breast cancer, tubular breast cancer, papillary breast cancer, primary cancers, Paget's disease, and inflammatory breast cancer; (13) adrenal cancer, including, but not limited to, pheochromocytom and adrenocortical carcinoma; (14) thyroid cancer, including, but not limited to, papillary or follicular thyroid cancer, medullary thyroid cancer, and anaplastic thyroid cancer; (15) pancreatic cancer, including, but not limited to, insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, and carcinoid or islet cell tumor; (16) pituitary cancer, including, but limited to, Cushing's disease, prol actin-secreting tumor, acromegaly, and diabetes insipius; (17) eye cancer, including, but not limited, to ocular melanoma such as iris melanoma, choroidal melanoma, and cilliary body melanoma, and retinoblastoma; (18) vaginal cancer, including, but not limited to, squamous cell carcinoma, adenocarcinoma, and melanoma; (19) vulvar cancer, including, but not limited to, squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, sarcoma, and Paget's disease; (20) cervical cancers, including, but not limited to, squamous cell carcinoma, and adenocarcinoma; (21) uterine cancer, including, but not limited to, endometrial carcinoma and uterine sarcoma; (22) ovarian cancer, including, but not limited to, ovarian epithelial carcinoma, borderline tumor, germ cell tumor, and stromal tumor; (23) esophageal cancer, including, but not limited to, squamous cancer, adenocarcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, adenosquamous

carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and oat cell (small cell) carcinoma; (24) stomach cancer, including, but not limited to, adenocarcinoma, fungating (polypoid), ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma, fibrosarcoma, and carcinosarcoma; (25) colon cancer; (26) rectal cancer; (27) liver cancer, including, but not limited to, hepatocellular carcinoma and hepatoblastoma; (28) gallbladder cancer, including, but not limited to, adenocarcinoma; (29) cholangiocarcinomas, including, but not limited to, pappillary, nodular, and diffuse; (30) lung cancer, including, but not limited to, non-small cell lung cancer, squamous cell carcinoma (epidermoid carcinoma), adenocarcinoma, large-cell carcinoma, and small-cell lung cancer; (31) testicular cancer, including, but not limited to, germinal tumor, seminoma, anaplastic, classic (typical), spermatocytic, nonserninoma, embryonal carcinoma, teratoma carcinoma, and choriocarcinoma (yolk-sac tumor); (32) prostate cancer, including, but not limited to, adenocarcinoma, leiomyosarcoma, and rhabdomyosarcorna; (33) penal cancer; (34) oral cancer, including, but not limited to, squamous cell carcinoma; (35) basal cancer; (36) salivary gland cancer, including, but not limited to, adenocarcinoma, mucoepidermoid carcinoma, and adenoidcystic carcinoma; (37) pharynx cancer, including, but not limited to, squamous cell cancer and verrucous; (38) skin cancer, including, but not limited to, basal cell carcinoma, squamous cell carcinoma and melanoma, superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, and acral lentiginous melanoma; (39) kidney cancer, including, but not limited to, renal cell cancer, adenocarcinoma, hypernephroma, fibrosarcoma, and transitional cell cancer (renal pelvis and/or uterer); (40) Wilms' tumor; (41) bladder cancer, including, but not limited to, transitional cell carcinoma, squamous cell cancer, adenocarcinoma, and carcinosarcoma; and other cancer, including, not limited to, myxosarcoma, osteogenic sarcoma, endotheliosarcoma, lymphangio-endotheliosarcoma, mesothelioma, synovioma, hemangioblastoma, epithelial carcinoma, cystadenocarcinoma, bronchogenic carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, and papillary adenocarcinomas (*See* Fishman *et al.*, 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia and Murphy *et al.*, 1997, *Informed Decisions: The Complete Book of Cancer Diagnosis, Treatment, and Recovery*, Viking Penguin, Penguin Books U.S.A., Inc., United States of America).

[0206] Depending on the disorder, disease, or condition to be treated, and the subject's condition, the compounds or pharmaceutical compositions provided herein can be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracistemal injection or infusion, subcutaneous injection, or implant), inhalation, nasal, vaginal, rectal, sublingual, or topical (e.g., transdermal or local) routes of administration and can be formulated, alone or

together, in suitable dosage unit with pharmaceutically acceptable excipients, carriers, adjuvants, and vehicles appropriate for each route of administration as described elsewhere herein.

[0207] The suitability of a particular route of administration employed for a particular active agent will depend on the active agent itself (e.g., whether it can be administered orally without decomposing prior to entering the blood stream) and the disease being treated. Recommended routes of administration for the second active agents are known to those of ordinary skill in the art. See, e.g., Physicians' Desk Reference, 1755-1760 (56th ed., 2002).

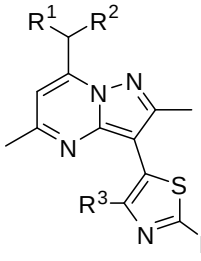
[0208] In one embodiment, provided herein are methods for treating or preventing testicular adrenal rest tumors (TART) in a subject, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof, wherein the subject has congenital adrenal hyperplasia (CAH).

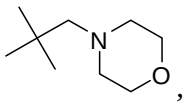
[0209] In one embodiment, provided herein are methods for treating or preventing ovarian adrenal rest tumors (OART) in a subject, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof, wherein the subject has congenital adrenal hyperplasia (CAH).

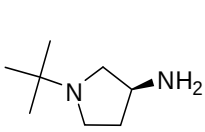
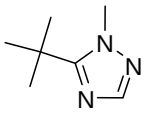
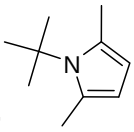
[0210] In an aspect provided herein, is a method of treating or preventing testicular adrenal rest tumors (TART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF_1) antagonist or a pharmaceutically acceptable salt thereof.

[0211] In an aspect provided herein, is a method of treating or preventing ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF_1) antagonist or a pharmaceutically acceptable salt thereof.

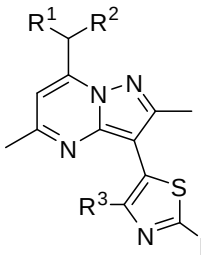
[0212] In an aspect provided herein, is a method of treating or preventing testicular adrenal rest tumors (TART), comprising administering to a subject in need thereof a compound of structural Formula (I):



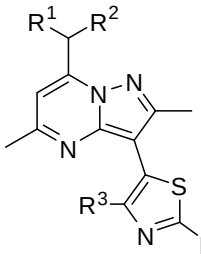
, or a pharmaceutically acceptable salt thereof. R^1 and R^2 are independently ethyl or n-propyl. R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy. R^4 is hydrogen, Br, R^aR^bN–, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl,  ,

 ,  , or  . R^a and R^b are independently hydrogen, C_1-C_3alkyl, $H_2NCH_2CH_2$–, $(CH_3)_3COC(O)NHCH_2CH_2$–, or $CH_3CH_2CH_2NHCH_2CH_2$–.

[0213] In an aspect provided herein, is a method of treating or preventing ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a compound of structural Formula (I):

 , or a pharmaceutically acceptable salt thereof. R^1, R^2, R^3 and R^4 are as described herein, including embodiments.

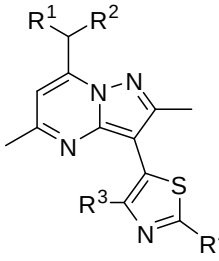
[0214] In an aspect provided herein, is a method of decreasing or reducing adrenocorticotropic hormone (ACTH) levels in a subject in need thereof, comprising administering to the subject contacting the ACTH with a compound of structural Formula (I):

 , or a pharmaceutically acceptable salt thereof. R^1, R^2, R^3 and R^4 are as described herein, including embodiments. R^1, R^2, R^3 and R^4 are as described herein, including embodiments. In certain embodiments, at least about 50% of the subjects demonstrate reduction in ACTH.In certain embodiments, at least about 60% of the subjects demonstrate reduction in ACTH.In certain embodiments, at least about 70% of the subjects demonstrate reduction in ACTH. In certain embodiments, at least about 80% of the subjects demonstrate reduction in ACTH. In embodiments, at least about 10% of subjects were in the standard or normal range for ACTH after treatment.

[0215] In embodiments, at least about 20% of subjects were in the standard or normal range for ACTH after treatment. In embodiments, at least about 30% of subjects were in the standard or normal range for ACTH after treatment. In certain embodiments, ACTH levels are decreased or reduced within about 30 minutes post dosing. In certain embodiments, ACTH levels are decreased or reduced within about 1 hour post dosing. In certain embodiments, ACTH levels are

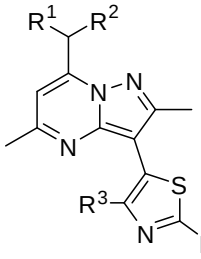
decreased or reduced within about 90 minutes post dosing. In certain embodiments, ACTH levels are decreased or reduced within about 2 hours post dosing.

[0216] In an aspect provided herein, is a method of decreasing or reducing 17α-hydroxyprogesterone (17-OHP) levels in a subject in need thereof, comprising administering to the subject a compound of structural Formula (I):



, or a pharmaceutically acceptable salt thereof. R^1, R^2, R^3 and R^4 are as described herein, including embodiments. R^1, R^2, R^3 and R^4 are as described herein, including embodiments. In certain embodiments, at least about 50% of the subjects demonstrate reduction in 17-OHP. In certain embodiments, at least about 60% of the subjects demonstrate reduction in 17-OHP. In certain embodiments, at least about 70% of the subjects demonstrate reduction in 17-OHP. In certain embodiments, at least about 80% of the subjects demonstrate reduction in 17-OHP. In embodiments, at least about 30% of subjects were in the standard or normal range of 1200 ng/dL after treatment. In embodiments, at least about 40% of subjects were in the standard or normal range of 1200 ng/dL after treatment. In embodiments, at least about 50% of subjects were in the standard or normal range of 1200 ng/dL after treatment. In embodiments, at least about 60% of subjects were in the standard or normal range of 1200 ng/dL after treatment. In embodiments, at least about 30% of subjects demonstrated an at least about 25% reduction in 17-OHP after treatment. In embodiments, at least about 40% of subjects demonstrated an at least about 25% reduction in 17-OHP after treatment. In embodiments, at least about 50% of subjects demonstrated an at least about 25% reduction in 17-OHP after treatment. In embodiments, at least about 60% of subjects demonstrated an at least about 25% reduction in 17-OHP after treatment.

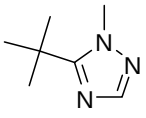
[0217] In an aspect provided herein, is a method of decreasing or reducing androstenedione levels in a subject in need thereof, comprising administering to the subject a compound of structural Formula (I):

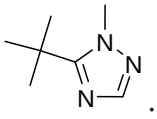


, or a pharmaceutically acceptable salt thereof. R^1, R^2, R^3 and R^4 are as described herein, including embodiments. R^1, R^2, R^3 and R^4 are as described herein, including embodiments. In certain embodiments, at least about 50% of the subjects demonstrate reduction in androstenedione.In certain embodiments, at least about 60% of the subjects demonstrate reduction in androstenedione.In certain embodiments, at least about 70% of the subjects demonstrate reduction in androstenedione. In certain embodiments, at least about 80% of the subjects demonstrate reduction in androstenedione. In certain embodiments, at least about 90% of the subjects demonstrate reduction in androstenedione. In certain embodiments, at least 100% of the subjects demonstrate reduction in androstenedione. In embodiments, at least about 30% of subjects demonstrated an at least about 25% reduction in androstenedione after treatment. In embodiments, at least about 40% of subjects demonstrated an at least about 25% reduction in androstenedione after treatment. In embodiments, at least about 50% of subjects demonstrated an at least about 25% reduction in androstenedione after treatment. In embodiments, at least 60% of subjects demonstrated an at least about 25% reduction in androstenedione after treatment. In embodiments, at least about 30% of subjects were in the standard or normal range for androstenedione after treatment. In embodiments, at least about 40% of subjects were in the standard or normal range for androstenedione after treatment. In embodiments, at least about 50% of subjects were in the standard or normal range for androstenedione after treatment.

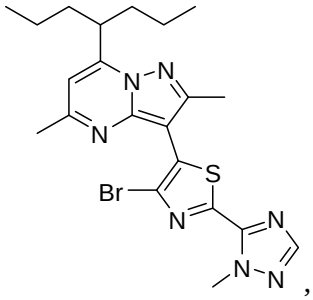
[0218] In certain embodiments, R^3 is Cl, Br, methyl, or trifluoromethyl.

[0219] In certain embodiments, R^3 is Cl, Br, or methyl.

[0220] In certain embodiments, R^4 is Br, R^aR^bN–, pyridin-4-yl, morpholin-4-yl, or  .

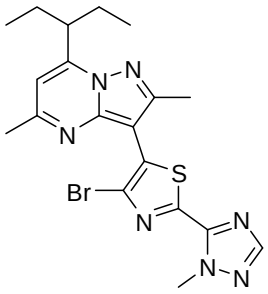
[0221] In certain embodiments, R^4 is morpholin-4-yl or  .

[0222] In certain embodiments, R^4 is hydrogen, Br, R^aR^bN– and R^a and R^b are independently C_1-C_3alkyl.



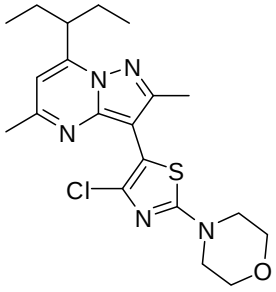
[0223] In certain embodiments, the compound is , or a pharmaceutically acceptable salt thereof.

[0224] In certain embodiments, the compound is



, or a pharmaceutically acceptable salt thereof.

[0225] In certain embodiments, the compound is



, or a pharmaceutically acceptable salt thereof.

[0226] In certain embodiments, a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is cyclically administered to a patient. Cycling therapy involves the administration of an active agent or a combination of active agents for a period of time, followed by a rest for a period of time, and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improves the efficacy of the treatment.

[0227] In certain embodiments, in the treatment, prevention, or amelioration of one or more symptoms of the disorders, diseases, or conditions described herein (e.g., treatment or prevention of testicular adrenal rest tumors (TART) or treatment or prevention of ovarian adrenal rest tumors (OART)), an appropriate dosage level of a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof generally is ranging from about 1 mg to 3000 mg, from about 1 mg to 2000 mg, from about 1 mg to 1000 mg, from about 1 mg to about 500 mg, from about 5 mg to about 500 mg,

from about 5 mg to about 400 mg, from about 5 mg to about 300 mg, from about 5 mg to about 250 mg, from about 5 mg to about 200 mg, from about 50 mg to about 200 mg, from about 10 mg to about 400 mg, from about 5 mg to about 25 mg, from about 10 mg to about 25 mg, or from about 25 to about 200 mg, which can be administered in single or multiple doses. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1 mg, about 5 mg, about 10 mg mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 105 mg, about 110 mg, about 115 mg, about 120 mg, about 125 mg, about 130 mg, about 135 mg, about 140 mg, about 145 mg, about 150 mg, about 155 mg, about 160 mg, about 165 mg, about 170 mg, about 175 mg, about 180 mg, about 185 mg, about 190 mg, about 195 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 450 mg, about 500 mg, about 600 mg, about 800 mg, or about 1000 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1 mg, about 5 mg, about 10 mg mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 105 mg, about 110 mg, about 115 mg, about 120 mg, about 125 mg, about 130 mg, about 135 mg, about 140 mg, about 145 mg, about 150 mg, about 155 mg, about 160 mg, about 165 mg, about 170 mg, about 175 mg, about 180 mg, about 185 mg, about 190 mg, about 195 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 450 mg, about 500 mg, about 600 mg, about 800 mg, or about 1000 mg/day.

[0228] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 5 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an

amount of about 270 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 280 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is

administered in an amount of about 600 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg.

[0229] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 105 mg, about 110 mg, about 115 mg, about 120 mg, about 125 mg, about 130 mg, about 135 mg, about 140 mg, about 145 mg, about 150 mg, about 155 mg, about 160 mg, about 165 mg, about 170 mg, about 175 mg, about 180 mg, about 185 mg, about 190 mg, about 195 mg, about 200 mg, or about 1000 mg.

[0230] For oral administration, the pharmaceutical compositions provided herein can be formulated in the form of tablets or capsules containing from about 1.0 mg to about 1,000 mg of a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In certain embodiments, for oral administration, the pharmaceutical compositions provided herein can be formulated in the form of tablets or capsules containing about 1 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 105 mg, about 110 mg, about 115 mg, about 120 mg, about 125 mg, about 130 mg, about 135 mg, about 140 mg, about 145 mg, about 150 mg, about 155 mg, about 160 mg, about 165 mg, about 170 mg, about 175 mg, about 180 mg, about 185 mg, about 190 mg, about 195 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about

350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 405 mg, about 410 mg, about 415 mg, about 420 mg, about 425 mg, about 430 mg, about 435 mg, about 440 mg, about 445 mg, about 450 mg, about 455 mg, about 460 mg, about 465 mg, about 470 mg, about 475 mg, about 480 mg, about 485 mg, about 490 mg, about 495 mg, about 500 mg, about 505 mg, about 510 mg, about 515 mg, about 520 mg, about 525 mg, about 530 mg, about 535 mg, about 540 mg, about 545 mg, about 550 mg, about 555 mg, about 560 mg, about 565 mg, about 570 mg, about 575 mg, about 580 mg, about 585 mg, about 590 mg, about 595 mg, about 600 mg, about 605 mg, about 610 mg, about 615 mg, about 620 mg, about 625 mg, about 630 mg, about 635 mg, about 640 mg, about 645 mg, about 650 mg, about 655 mg, about 660 mg, about 665 mg, about 670 mg, about 675 mg, about 680 mg, about 685 mg, about 690 mg, about 695 mg, about 700 mg, about 705 mg, about 710 mg, about 715 mg, about 720 mg, about 725 mg, about 730 mg, about 735 mg, about 740 mg, about 745 mg, about 750 mg, about 755 mg, about 760 mg, about 765 mg, about 770 mg, about 775 mg, about 780 mg, about 785 mg, about 790 mg, about 795 mg, about 800 mg, about 810 mg, about 820 mg, about 830 mg, about 840 mg, about 850 mg, about 860 mg, about 870 mg, about 880 mg, about 890 mg, about 900 mg, about 910 mg, about 920 mg, about 930 mg, about 940 mg, about 950 mg, about 960 mg, about 970 mg, about 990 mg, about 990 mg, or about 1000 mg of a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof for the symptomatic adjustment of the dosage to the patient to be treated. In certain embodiments, for oral administration, the pharmaceutical compositions provided herein can be formulated in the form of tablets or capsules containing about 50 mg of a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In certain embodiments, for oral administration, the pharmaceutical compositions provided herein can be formulated in the form of tablets or capsules containing about 200 mg of a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof.The pharmaceutical compositions can be administered on a regimen of one (1) to four (4) times per day, including once, twice, three times, and four times per day. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered once per day. In some embodiments, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about

310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 405 mg, about 410 mg, about 415 mg, about 420 mg, about 425 mg, about 430 mg, about 435 mg, about 440 mg, about 445 mg, about 450 mg, about 455 mg, about 460 mg, about 465 mg, about 470 mg, about 475 mg, about 480 mg, about 485 mg, about 490 mg, about 495 mg, about 500 mg, about 505 mg, about 510 mg, about 515 mg, about 520 mg, about 525 mg, about 530 mg, about 535 mg, about 540 mg, about 545 mg, about 550 mg, about 555 mg, about 560 mg, about 565 mg, about 570 mg, about 575 mg, about 580 mg, about 585 mg, about 590 mg, about 595 mg, about 600 mg, about 605 mg, about 610 mg, about 615 mg, about 620 mg, about 625 mg, about 630 mg, about 635 mg, about 640 mg, about 645 mg, about 650 mg, about 655 mg, about 660 mg, about 665 mg, about 670 mg, about 675 mg, about 680 mg, about 685 mg, about 690 mg, about 695 mg, about 700 mg, about 705 mg, about 710 mg, about 715 mg, about 720 mg, about 725 mg, about 730 mg, about 735 mg, about 740 mg, about 745 mg, about 750 mg, about 755 mg, about 760 mg, about 765 mg, about 770 mg, about 775 mg, about 780 mg, about 785 mg, about 790 mg, about 795 mg, about 800 mg, about 810 mg, about 820 mg, about 830 mg, about 840 mg, about 850 mg, about 860 mg, about 870 mg, about 880 mg, about 890 mg, about 900 mg, about 910 mg, about 920 mg, about 930 mg, about 940 mg, about 950 mg, about 960 mg, about 970 mg, about 990 mg, about 990 mg, or about 1000 mg of the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered once per day. In some embodiments, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 405 mg, about 410 mg, about 415 mg, about 420 mg, about 425 mg, about 430 mg, about 435 mg, about 440 mg, about 445 mg, about 450 mg, about 455 mg, about 460 mg, about 465 mg, about 470 mg, about 475 mg, about 480 mg, about 485 mg, about 490 mg, about 495 mg, about 500 mg, about 505 mg, about 510 mg, about 515 mg, about 520 mg, about 525 mg, about 530 mg, about 535 mg, about 540 mg, about 545 mg, about 550 mg, about 555 mg, about 560 mg, about 565 mg, about 570 mg, about 575 mg, about 580 mg, about 585 mg, about 590 mg, about 595 mg, about 600 mg, about 605 mg,

about 610 mg, about 615 mg, about 620 mg, about 625 mg, about 630 mg, about 635 mg, about 640 mg, about 645 mg, about 650 mg, about 655 mg, about 660 mg, about 665 mg, about 670 mg, about 675 mg, about 680 mg, about 685 mg, about 690 mg, about 695 mg, about 700 mg, about 705 mg, about 710 mg, about 715 mg, about 720 mg, about 725 mg, about 730 mg, about 735 mg, about 740 mg, about 745 mg, about 750 mg, about 755 mg, about 760 mg, about 765 mg, about 770 mg, about 775 mg, about 780 mg, about 785 mg, about 790 mg, about 795 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, or about 1000 mg of the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered twice per day. In some embodiments, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 405 mg, about 410 mg, about 415 mg, about 420 mg, about 425 mg, about 430 mg, about 435 mg, about 440 mg, about 445 mg, about 450 mg, about 455 mg, about 460 mg, about 465 mg, about 470 mg, about 475 mg, about 480 mg, about 485 mg, about 490 mg, about 495 mg, about 500 mg, about 505 mg, about 510 mg, about 515 mg, about 520 mg, about 525 mg, about 530 mg, about 535 mg, about 540 mg, about 545 mg, about 550 mg, about 555 mg, about 560 mg, about 565 mg, about 570 mg, about 575 mg, about 580 mg, about 585 mg, about 590 mg, about 595 mg, about 600 mg, about 605 mg, about 610 mg, about 615 mg, about 620 mg, about 625 mg, about 630 mg, about 635 mg, about 640 mg, about 645 mg, about 650 mg, about 655 mg, about 660 mg, about 665 mg, about 670 mg, about 675 mg, about 680 mg, about 685 mg, about 690 mg, about 695 mg, about 700 mg, about 705 mg, about 710 mg, about 715 mg, about 720 mg, about 725 mg, about 730 mg, about 735 mg, about 740 mg, about 745 mg, about 750 mg, about 755 mg, about 760 mg, about 765 mg, about 770 mg, about 775 mg, about 780 mg, about 785 mg, about 790 mg, about 795 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, or about 1000 mg of the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered three times per day.

[0231] In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered for a period of one day. In some embodiments, the compound of Formula (I), or an isotopic variant

thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of two days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of three days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of four days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of five days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of six days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of seven days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of eight days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of nine days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of ten days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 11 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 12 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 13 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 14 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered for a period of 15 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 16 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 17 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically

acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 18 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 19 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 20 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 21 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 22 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 23 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 24 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 25 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 26 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 27 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 28 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 29 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered for a period of 30 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 31 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 32 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 33 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate,

hydrate, or prodrug thereof is administered over a period of 34 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 35 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 36 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 37 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 38 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 39 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 40 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 41 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 42 days or more.

[0232] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg once daily for a period of up to at least about 14 days.

[0233] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically

acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 140 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg once daily for a period of up to at least

about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically

acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg once daily for a period of up to at least about 14 days.

[0234] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 5 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg twice daily

for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 140 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a

pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg twice daily

for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg twice daily for a period of up to at least about 14 days.

[0235] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 5 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant

thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 280 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330

mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant

thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg once daily for a period of up to at least about 28 days.

[0236] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 5 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an

amount of about 220 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 280 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula

(I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a

pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg twice daily for a period of up to at least about 28 days.

[0237] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 255 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230

mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 420 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant

thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg once daily for a period of up to at least about 42 days.

[0238] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an

amount of about 5 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula

(I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 420 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an

amount of about 370 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg twice daily for a period of up to at least about 42 days.

[0239] In some embodiments, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 75 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, or about 400 mg of the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered twice per day. In some embodiments, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 75 mg, about 100 mg, about 150 mg, about 200

mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, or about 800 mg of the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered at least once per day.

[0240] In certain embodiments, from about 5 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, from about 25 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, from about 100 mg to about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject.

[0241] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered orally.

[0242] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is formulated as a capsule or tablet.

[0243] In certain embodiments, from about 25 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject.

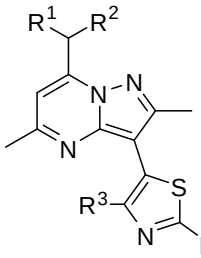
[0244] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 5 mg/day to about 1000 mg/day is administered to the subject. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 25 mg/day to about 1000 mg/day is administered to the subject. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 5 mg/day to about 25 mg/day is administered to the subject. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 10 mg/day to about 400 mg/day is administered to the subject.In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 100 mg/day to about 600 mg/day.

[0245] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the fed state. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the fasted state. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at least once per day.

[0246] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at bedtime. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 6 hours before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 5 hours before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 4 hours before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 3 hours before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 2 hours before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 1 hour before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 30 minutes before sleep.

[0247] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the evening. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at about 10 pm at night.

[0248] In another aspect is provided a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a pharmaceutical composition, comprising a compound of structural Formula (I): and a pharmaceutically acceptable excipientcomprising with a compound as described herein, including embodiments, or the structural Formulae (I) and a pharmaceutically acceptable excipient:



, or a pharmaceutically acceptable salt thereof. R^1, R^2, R^3, and R^4 are as described herein, including embodiments.

[0249] In certain embodiments, the pharmaceutical composition comprises from about 25 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments, the pharmaceutical composition comprises from about 25 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments, the pharmaceutical composition comprises from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments,

the pharmaceutical composition comprises from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments, the pharmaceutical composition comprises from about 100 mg to about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[0250] In certain embodiments, the pharmaceutical composition comprises about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg about 500 mg, or about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[0251] In certain embodiments, the pharmaceutical composition is administered orally.

[0252] In certain embodiments, the pharmaceutical composition is formulated as a capsule or tablet. In certain embodiments, the capsule or tablet comprises about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, or about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments, the capsule or tablet comprises about 50 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments, the capsule or tablet comprises about 200 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[0253] In certain embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 25 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically

acceptable salt thereof, is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject.

[0254] In certain embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg/day to about 1000 mg/day is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 25 mg/day to about 1000 mg/day is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg/day to about 25 mg/day is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 10 mg/day to about 400 mg/day is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 100 mg/day to about 600 mg/day.

[0255] In certain embodiments, the pharmaceutical composition is administered in the fed state. In certain embodiments, the pharmaceutical composition is administered in the fasted state. In certain embodiments, the pharmaceutical composition is administered at least once per day.

[0256] In certain embodiments, the pharmaceutical composition is administered at bedtime. In certain embodiments, the pharmaceutical composition is administered less than about 6 hours before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 5 hours before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 4 hours before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 3 hours before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 2 hours before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 1 hour before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 30 minutes before sleep.

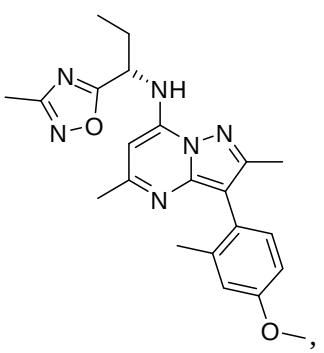
[0257] In certain embodiments, the pharmaceutical composition is administered in the evening. In certain embodiments, the pharmaceutical composition is administered at about 10 pm at night.

[0258] In certain embodiments, the subject has congenital adrenal hyperplasia (CAH).

[0259] In certain embodiments, the size and/or number of the tumors is decreased or reduced. In certain embodiments, the size of the tumors is decreased or reduced. In certain embodiments, the size of the tumors is decreased or reduced.

[0260] In certain embodiments of the methods disclosed herein, the method further comprises administering to the subject an additional chemotherapeutic agent.

[0261] In certain embodiments, the the additional chemotherapeutic agent is a glucocorticoid, a mineralocorticoid, an ACAT1 inhibitor, or an anti-androgen. In certain embodiments, the glucocorticoid is beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone. In certain embodiments, the mineralocorticoid is fludrocortisone. In certain embodiments, the additional chemotherapeutic agent is another CRF_1 antagonist. In certain embodiments, the CRF_1 antagonist is: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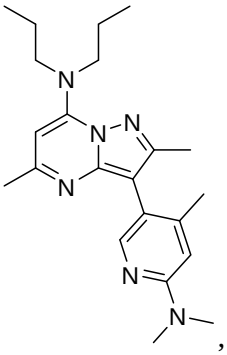
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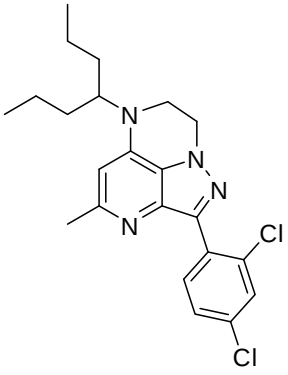
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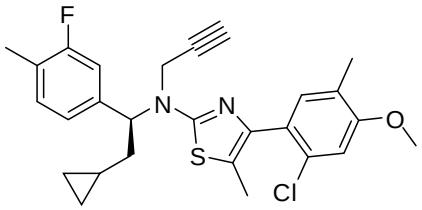
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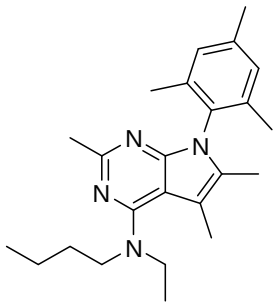
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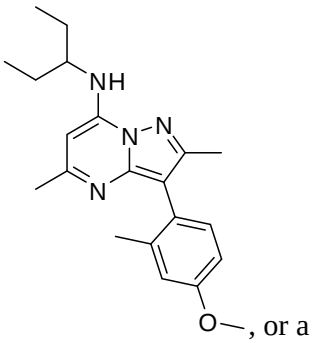
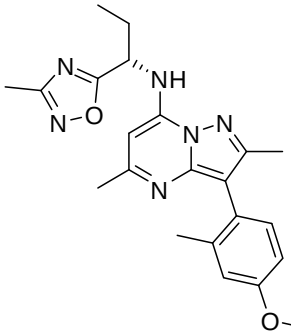

, or a pharmaceutically acceptable salt thereof. In certain embodiments, the CRF_1 antagonist is


, or a pharmaceutically acceptable salt thereof.

[0262] In certain embodiments of the methods disclosed herein, the method further comprises an additional treatment selected from surgical resection of the tumors and radiation therapy, or a combination thereof. In certain embodiments, the additional therapy is surgical resection and the surgical resection is prior to, after, and/or concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition, or any combination thereof. In certain embodiments, the surgical resection is prior

to administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In certain embodiments, the surgical resection is after administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In certain embodiments, the surgical resection is concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

[0263] In certain embodiments, the additional treatment is radiation therapy and the radiation therapy is prior to, after, and/or concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition, or any combination thereof. In certain embodiments, the radiation therapy is prior to administration of the CRF_1 antagonist of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In certain embodiments, the radiation therapy is after administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In certain embodiments, the radiation therapy is concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

[0264] In one embodiment, the pharmaceutical compositions are provided in a dosage form for oral administration, which comprise a compound provided herein, and one or more pharmaceutically acceptable excipients or carriers. The pharmaceutical compositions provided herein that are formulated for oral administration may be in tablet, capsule, powder, or liquid form. In some embodiments, a tablet comprises a solid carrier or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil, or synthetic oil. Physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol, or polyethylene glycol may be included. In some embodiments, a capsule comprises a solid carrier such as gelatin.

[0265] In another embodiment, the pharmaceutical compositions are provided in a dosage form for parenteral administration, which comprise a compound provided herein, and one or more pharmaceutically acceptable excipients or carriers. Where pharmaceutical compositions may be formulated for intravenous, cutaneous or subcutaneous injection, the active ingredient will be in the form of a parenterally acceptable aqueous solution, which is pyrogen-free and has a suitable pH, isotonicity, and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles, such as Sodium Chloride injection, Ringer's injection, or Lactated Ringer's injection. In some embodiments, preservatives, stabilisers, buffers, antioxidants, and/or other additives are included as required.

[0266] In yet another embodiment, the pharmaceutical compositions are provided in a dosage form for topical administration, which comprise a compound provided herein, and one or more pharmaceutically acceptable excipients or carriers.

[0267] The pharmaceutical compositions can also be formulated as modified release dosage forms, including delayed-, extended-, prolonged-, sustained-, pulsatile-, controlled-, accelerated-, fast-, targeted-, and programmed-release, and gastric retention dosage forms. These dosage forms can be prepared according to conventional methods and techniques known to those skilled in the art (*see, Remington: The Science and Practice of Pharmacy*, supra; *Modified-Release Drug Delivery Technology*, 2nd Edition, Rathbone *et al.*, Eds., Marcel Dekker, Inc.: New York, NY, 2008).

[0268] The pharmaceutical compositions provided herein can be provided in a unit-dosage form or multiple-dosage form. A unit-dosage form, as used herein, refers to physically discrete a unit suitable for administration to a human and animal subject, and packaged individually as is known in the art. Each unit-dose contains a predetermined quantity of an active ingredient(s) sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carriers or excipients. Examples of a unit-dosage form include an ampoule, syringe, and individually packaged tablet and capsule. A unit-dosage form may be administered in fractions or multiples thereof. A multiple-dosage form is a plurality of identical unit-dosage forms packaged in a single container to be administered in segregated unit-dosage form. Examples of a multiple-dosage form include a vial, bottle of tablets or capsules, or bottle of pints or gallons.

[0269] The pharmaceutical compositions provided herein can be administered at once, or multiple times at intervals of time. It is understood that the precise dosage and duration of treatment may vary with the age, weight, and condition of the patient being treated, and may be determined empirically using known testing protocols or by extrapolation from *in vivo* or *in vitro* test or diagnostic data. It is further understood that for any particular individual, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the formulations.

[0270] In certain embodiments, the pharmaceutical compositions provided herein further comprise one or more chemotherapeutic agents as defined herein.

A. Oral Administration

[0271] The pharmaceutical compositions provided herein for oral administration can be provided in solid, semisolid, or liquid dosage forms for oral administration. As used herein, oral administration also includes buccal, lingual, and sublingual administration. Suitable oral dosage

forms include, but are not limited to, tablets, fastmelts, chewable tablets, capsules, pills, strips, troches, lozenges, pastilles, cachets, pellets, medicated chewing gum, bulk powders, effervescent or non-effervescent powders or granules, oral mists, solutions, emulsions, suspensions, wafers, sprinkles, elixirs, and syrups. In addition to the active ingredient(s), the pharmaceutical compositions can contain one or more pharmaceutically acceptable carriers or excipients, including, but not limited to, binders, fillers, diluents, disintegrants, wetting agents, lubricants, glidants, coloring agents, dye-migration inhibitors, sweetening agents, flavoring agents, emulsifying agents, suspending and dispersing agents, preservatives, solvents, non-aqueous liquids, organic acids, and sources of carbon dioxide.

[0272] Binders or granulators impart cohesiveness to a tablet to ensure the tablet remaining intact after compression. Suitable binders or granulators include, but are not limited to, starches, such as corn starch, potato starch, and pre-gelatinized starch (e.g., STARCH 1500); gelatin; sugars, such as sucrose, glucose, dextrose, molasses, and lactose; natural and synthetic gums, such as acacia, alginic acid, alginates, extract of Irish moss, panwar gum, ghatti gum, mucilage of isabgol husks, carboxymethylcellulose, methylcellulose, polyvinylpyrrolidone (PVP), Veegum, larch arabogalactan, powdered tragacanth, and guar gum; celluloses, such as ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose, methyl cellulose, hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC), hydroxypropyl methyl cellulose (HPMC); microcrystalline celluloses, such as AVICEL-PH-101, AVICEL-PH-103, AVICEL RC-581, AVICEL-PH-105 (FMC Corp., Marcus Hook, PA); and mixtures thereof. Suitable fillers include, but are not limited to, talc, calcium carbonate, microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The amount of a binder or filler in the pharmaceutical compositions provided herein varies upon the type of formulation, and is readily discernible to those of ordinary skill in the art. The binder or filler may be present from about 50 to about 99% by weight in the pharmaceutical compositions provided herein.

[0273] Suitable diluents include, but are not limited to, dicalcium phosphate, calcium sulfate, lactose, sorbitol, sucrose, inositol, cellulose, kaolin, mannitol, sodium chloride, dry starch, and powdered sugar. Certain diluents, such as mannitol, lactose, sorbitol, sucrose, and inositol, when present in sufficient quantity, can impart properties to some compressed tablets that permit disintegration in the mouth by chewing. Such compressed tablets can be used as chewable tablets. The amount of a diluent in the pharmaceutical compositions provided herein varies upon the type of formulation, and is readily discernible to those of ordinary skill in the art.

[0274] Suitable disintegrants include, but are not limited to, agar; bentonite; celluloses, such as methylcellulose and carboxymethylcellulose; wood products; natural sponge; cation-exchange

resins; alginic acid; gums, such as guar gum and Veegum HV; citrus pulp; cross-linked celluloses, such as croscarmellose; cross-linked polymers, such as crospovidone; cross-linked starches; calcium carbonate; microcrystalline cellulose, such as sodium starch glycolate; polacrilin potassium; starches, such as corn starch, potato starch, tapioca starch, and pre-gelatinized starch; clays; aligns; and mixtures thereof. The amount of a disintegrant in the pharmaceutical compositions provided herein varies upon the type of formulation, and is readily discernible to those of ordinary skill in the art. The amount of a disintegrant in the pharmaceutical compositions provided herein varies upon the type of formulation, and is readily discernible to those of ordinary skill in the art. The pharmaceutical compositions provided herein may contain from about 0.5 to about 15% or from about 1 to about 5% by weight of a disintegrant.

[0275] Suitable lubricants include, but are not limited to, calcium stearate; magnesium stearate; mineral oil; light mineral oil; glycerin; sorbitol; mannitol; glycols, such as glycerol behenate and polyethylene glycol (PEG); stearic acid; sodium lauryl sulfate; talc; hydrogenated vegetable oil, including peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil; zinc stearate; ethyl oleate; ethyl laureate; agar; starch; lycopodium; silica or silica gels, such as AEROSIL® 200 (W.R. Grace Co., Baltimore, MD) and CAB-O-SIL® (Cabot Co. of Boston, MA); and mixtures thereof. The pharmaceutical compositions provided herein may contain about 0.1 to about 5% by weight of a lubricant.

[0276] Suitable glidants include, but are not limited to, colloidal silicon dioxide, CAB-O-SIL® (Cabot Co. of Boston, MA), and asbestos-free talc. Suitable coloring agents include, but are not limited to, any of the approved, certified, water soluble FD&C dyes, and water insoluble FD&C dyes suspended on alumina hydrate, and color lakes and mixtures thereof. A color lake is the combination by adsorption of a water-soluble dye to a hydrous oxide of a heavy metal, resulting in an insoluble form of the dye. Suitable flavoring agents include, but are not limited to, natural flavors extracted from plants, such as fruits, and synthetic blends of compounds which produce a pleasant taste sensation, such as peppermint and methyl salicylate. Suitable sweetening agents include, but are not limited to, sucrose, lactose, mannitol, syrups, glycerin, and artificial sweeteners, such as saccharin and aspartame. Suitable emulsifying agents include, but are not limited to, gelatin, acacia, tragacanth, bentonite, and surfactants, such as polyoxyethylene sorbitan monooleate (TWEEN® 20), polyoxyethylene sorbitan monooleate 80 (TWEEN® 80), and triethanolamine oleate. Suitable suspending and dispersing agents include, but are not limited to, sodium carboxymethylcellulose, pectin, tragacanth, Veegum, acacia, sodium carbomethylcellulose, hydroxypropyl methylcellulose, and polyvinylpyrrolidone. Suitable preservatives include, but are not limited to, glycerin, methyl and propylparaben, benzoic add,

sodium benzoate and alcohol. Suitable wetting agents include, but are not limited to, propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate, and polyoxyethylene lauryl ether. Suitable solvents include, but are not limited to, glycerin, sorbitol, ethyl alcohol, and syrup. Suitable non-aqueous liquids utilized in emulsions include, but are not limited to, mineral oil and cottonseed oil. Suitable organic acids include, but are not limited to, citric and tartaric acid. Suitable sources of carbon dioxide include, but are not limited to, sodium bicarbonate and sodium carbonate.

[0277] It should be understood that many carriers and excipients may serve several functions, even within the same formulation.

[0278] The pharmaceutical compositions provided herein for oral administration can be provided as compressed tablets, tablet triturates, chewable lozenges, rapidly dissolving tablets, multiple compressed tablets, or enteric-coating tablets, sugar-coated, or film-coated tablets. Enteric-coated tablets are compressed tablets coated with substances that resist the action of stomach acid but dissolve or disintegrate in the intestine, thus protecting the active ingredients from the acidic environment of the stomach. Enteric-coatings include, but are not limited to, fatty acids, fats, phenyl salicylate, waxes, shellac, ammoniated shellac, and cellulose acetate phthalates. Sugar-coated tablets are compressed tablets surrounded by a sugar coating, which may be beneficial in covering up objectionable tastes or odors and in protecting the tablets from oxidation. Film-coated tablets are compressed tablets that are covered with a thin layer or film of a water-soluble material. Film coatings include, but are not limited to, hydroxyethylcellulose, sodium carboxymethylcellulose, polyethylene glycol 4000, and cellulose acetate phthalate. Film coating imparts the same general characteristics as sugar coating. Multiple compressed tablets are compressed tablets made by more than one compression cycle, including layered tablets, and press-coated or dry-coated tablets.

[0279] The tablet dosage forms can be prepared from the active ingredient in powdered, crystalline, or granular forms, alone or in combination with one or more carriers or excipients described herein, including binders, disintegrants, controlled-release polymers, lubricants, diluents, and/or colorants. Flavoring and sweetening agents are especially useful in the formation of chewable tablets and lozenges.

[0280] The pharmaceutical compositions provided herein for oral administration can be provided as soft or hard capsules, which can be made from gelatin, methylcellulose, starch, or calcium alginate. The hard gelatin capsule, also known as the dry-filled capsule (DFC), consists of two sections, one slipping over the other, thus completely enclosing the active ingredient. The soft elastic capsule (SEC) is a soft, globular shell, such as a gelatin shell, which is plasticized by the addition of glycerin, sorbitol, or a similar polyol. The soft gelatin shells may contain a

preservative to prevent the growth of microorganisms. Suitable preservatives are those as described herein, including methyl- and propyl-parabens, and sorbic acid. The liquid, semisolid, and solid dosage forms provided herein may be encapsulated in a capsule. Suitable liquid and semisolid dosage forms include solutions and suspensions in propylene carbonate, vegetable oils, or triglycerides. Capsules containing such solutions can be prepared as described in U.S. Pat. Nos. 4,328,245; 4,409,239; and 4,410,545. The capsules may also be coated as known by those of skill in the art in order to modify or sustain dissolution of the active ingredient.

[0281] Coloring and flavoring agents can be used in all of the above dosage forms.

[0282] The pharmaceutical compositions provided herein for oral administration can be formulated as immediate or modified release dosage forms, including delayed-, sustained, pulsed-, controlled, targeted-, and programmed-release forms.

B. Parenteral Administration

[0283] The pharmaceutical compositions provided herein can be administered parenterally by injection, infusion, or implantation, for local or systemic administration. Parenteral administration, as used herein, include intravenous, intraarterial, intraperitoneal, intrathecal, intraventricular, intraurethral, intrasternal, intracranial, intramuscular, intrasynovial, intravesical, and subcutaneous administration.

[0284] The pharmaceutical compositions provided herein for parenteral administration can be formulated in any dosage forms that are suitable for parenteral administration, including solutions, suspensions, emulsions, micelles, liposomes, microspheres, nanosystems, and solid forms suitable for solutions or suspensions in liquid prior to injection. Such dosage forms can be prepared according to conventional methods known to those skilled in the art of pharmaceutical science (*see*, *Remington: The Science and Practice of Pharmacy*, supra).

[0285] The pharmaceutical compositions intended for parenteral administration can include one or more pharmaceutically acceptable carriers and excipients, including, but not limited to, aqueous vehicles, water-miscible vehicles, non-aqueous vehicles, antimicrobial agents or preservatives against the growth of microorganisms, stabilizers, solubility enhancers, isotonic agents, buffering agents, antioxidants, local anesthetics, suspending and dispersing agents, wetting or emulsifying agents, complexing agents, sequestering or chelating agents, cryoprotectants, lyoprotectants, thickening agents, pH adjusting agents, and inert gases.

[0286] Suitable aqueous vehicles include, but are not limited to, water, saline, physiological saline or phosphate buffered saline (PBS), sodium chloride injection, Ringers injection, isotonic dextrose injection, sterile water injection, dextrose and lactated Ringers injection. Suitable non-aqueous vehicles include, but are not limited to, fixed oils of vegetable origin, castor oil, corn oil, cottonseed oil, olive oil, peanut oil, peppermint oil, safflower oil, sesame oil, soybean oil,

hydrogenated vegetable oils, hydrogenated soybean oil, and medium-chain triglycerides of coconut oil, and palm seed oil. Suitable water-miscible vehicles include, but are not limited to, ethanol, 1,3-butanediol, liquid polyethylene glycol (e.g., polyethylene glycol 300 and polyethylene glycol 400), propylene glycol, glycerin, *N*-methy1-2-pyrrolidone, *N*,*N*-dimethylacetamide, and dimethyl sulfoxide.

[0287] Suitable antimicrobial agents or preservatives include, but are not limited to, phenols, cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoates, thimerosal, benzalkonium chloride (e.g., benzethonium chloride), methyl-and propyl-parabens, and sorbic acid. Suitable isotonic agents include, but are not limited to, sodium chloride, glycerin, and dextrose. Suitable buffering agents include, but are not limited to, phosphate and citrate. Suitable antioxidants are those as described herein, including bisulfite and sodium metabisulfite. Suitable local anesthetics include, but are not limited to, procaine hydrochloride. Suitable suspending and dispersing agents are those as described herein, including sodium carboxymethylcelluose, hydroxypropyl methylcellulose, and polyvinylpyrrolidone. Suitable emulsifying agents are those described herein, including polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate 80, and triethanolamine oleate. Suitable sequestering or chelating agents include, but are not limited to EDTA. Suitable pH adjusting agents include, but are not limited to, sodium hydroxide, hydrochloric acid, citric acid, and lactic acid. Suitable complexing agents include, but are not limited to, cyclodextrins, including α-cyclodextrin, β-cyclodextrin, hydroxypropyl-β-cyclodextrin, sulfobutylether-β-cyclodextrin, and sulfobutylether 7-β-cyclodextrin (CAPTISOL®, CyDex, Lenexa, KS).

[0288] When the pharmaceutical compositions provided herein are formulated for multiple dosage administration, the multiple dosage parenteral formulations must contain an antimicrobial agent at bacteriostatic or fungi static concentrations. All parenteral formulations must be sterile, as known and practiced in the art.

[0289] In one embodiment, the pharmaceutical compositions for parenteral administration are provided as ready-to-use sterile solutions. In another embodiment, the pharmaceutical compositions are provided as sterile dry soluble products, including lyophilized powders and hypodermic tablets, to be reconstituted with a vehicle prior to use. In yet another embodiment, the pharmaceutical compositions are provided as ready-to-use sterile suspensions. In yet another embodiment, the pharmaceutical compositions are provided as sterile dry insoluble products to be reconstituted with a vehicle prior to use. In still another embodiment, the pharmaceutical compositions are provided as ready-to-use sterile emulsions.

[0290] The pharmaceutical compositions provided herein for parenteral administration can be formulated as immediate or modified release dosage forms, including delayed-, sustained, pulsed-, controlled, targeted-, and programmed-release forms.

[0291] The pharmaceutical compositions provided herein for parenteral administration can be formulated as a suspension, solid, semi-solid, or thixotropic liquid, for administration as an implanted depot. In one embodiment, the pharmaceutical compositions provided herein are dispersed in a solid inner matrix, which is surrounded by an outer polymeric membrane that is insoluble in body fluids but allows the active ingredient in the pharmaceutical compositions diffuse through.

[0292] Suitable inner matrixes include, but are not limited to, polymethylmethacrylate, polybutyl-methacrylate, plasticized or unplasticized polyvinylchloride, plasticized nylon, plasticized polyethylene terephthalate, natural rubber, polyisoprene, polyisobutylene, polybutadiene, polyethylene, ethylene-vinyl acetate copolymers, silicone rubbers, polydimethylsiloxanes, silicone carbonate copolymers, hydrophilic polymers, such as hydrogels of esters of acrylic and metliacrylic acid, collagen, cross-linked polyvinyl alcohol, and cross-linked partially hydrolyzed polyvinyl acetate.

[0293] Suitable outer polymeric membranes include but are not limited to, polyethylene, polypropylene, ethylene/propylene copolymers, ethylene/ethyl acrylate copolymers, ethylene/vinyl acetate copolymers, silicone rubbers, polydimethyl siloxanes, neoprene rubber, chlorinated polyethylene, polyvinylchloride, vinyl chloride copolymers with vinyl acetate, vinylidene chloride, ethylene and propylene, ionomer polyethylene terephthalate, butyl rubber epichlorohydrin rubbers, ethylene/vinyl alcohol copolymer, ethylene/vinyl acetate/vinyl alcohol terpolymer, and ethylene/vinyloxyethanol copolymer.

V. Examples

[0294] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

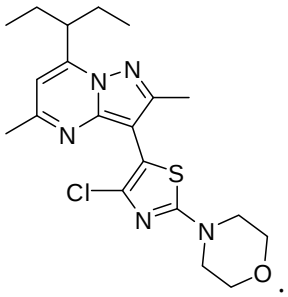
Example 1. A Phase 2, Open-Label Study to Evaluate the Efficacy and Safety of Compound 1 in Subjects with Testicular Adrenal Rest Tumors

[0295] Testicular adrenal rest tumors (TART) is a common complication in congenital adrenal hyperplasia (CAH) that is caused by adrenocorticotropin hormone (ACTH)–driven

overstimulation of aberrant adrenal cells within the testes and results in impaired spermatogenesis and infertility. TARTS can be found anywhere on the testes, though a typical (very common) location is within the mediastinum. This is a particularly problematic location. Because of the central location of TARTs being in the mediastinum, compression of the nearby seminiferous tubules is common and can lead to obstructive azoospermia, irreversible damage to surrounding tissue, and permanent infertility in males.

[0296] Current treatment for TART consists of chronic high-dose glucocorticoids, a problematic therapy with significant side effects and a narrow therapeutic window. Given the serious nature of TART and the limitations and risks of chronic steroid therapy, new treatment modalities are needed for patients with TART. Compound 1, a high-affinity and selective small-molecule antagonist of the corticotropin-releasing factor type-1 (CRF_1) receptor, may be effective in blocking ACTH synthesis, thereby reducing the size of testicular lesions in TART and reducing the need for chronic supraphysiologic doses of glucocorticoids. This mechanism of action has been validated in a previous proof-of-concept study using a similar small-molecule CRF_1 receptor antagonist, which produced dose-dependent reductions in ACTH and 17-hydroxyprogesterone (17-OHP) in subjects with CAH. Compound 1 has been shown to have an acceptable safety and tolerability profile in nonclinical toxicology studies and in 2 completed Phase 1 clinical studies in healthy volunteers. These promising results provide a rationale for investigating the use of Compound 1 in the treatment of TART, in combination with replacement glucocorticoids and mineralocorticoids.

[0297] The structure of Compound 1 is:



[0298] Building upon the 2 completed Phase 1 studies of Compound 1 in healthy volunteers and the ongoing Phase 2 proof-of-concept study of Compound 1 in subjects with CAH, this Phase 2 will study Compound 1 specifically in male CAH subjects with TART for the first time. A sample size of approximately 12 subjects was selected to provide sufficient data for an initial evaluation of efficacy and safety in subjects with TART while maintaining feasible subject recruitment goals for this rare condition. Given the rarity of the disease and thus the small sample size that can be recruited, this study will be open label, and all enrolled subjects will

receive active study drug. Subjects will serve as their own controls in that their outcome measures after 12 weeks of study drug dosing will be compared to their baseline measures.

[0299] The schedule of study visits (every 2 weeks through Week 4, then every 4 weeks for the remainder of the study) provides an appropriate balance between minimizing subject burden and closely monitoring subject safety and response to therapy. The 4-week post-treatment washout period will allow for adequate safety follow-up and analysis of any HPA axis rebound after the last dose of study drug.

[0300] The specification of study drug dosing at 10pm and after consumption of a snack is based on information from the Phase 1 studies in healthy volunteers. The plasma C_{max} (maximum concentration) for Compound 1 in the Phase 1 studies occurred approximately 5 hours after dosing. Therefore, Compound 1 will be dosed daily at 10pm in this study in order to achieve peak plasma study drug concentration at the time of day when most adrenal androgens are produced. Subjects will consume a standardized snack before taking study drug because the Phase 1 studies showed minimal study drug absorption when subjects were dosed in a fasted state. Specific dose reduction criteria, strict individual treatment stopping criteria, and appropriate laboratory and medical assessments have been established to ensure subject safety. (**FIGS. 1A-1B**).

Objectives	Endpoints
Primary	
• To assess the efficacy of Compound 1 in subjects with TART	• Reduction in the size/volume of TART on testicular ultrasound from screening to Week 12
Secondary	
• To evaluate the safety of Compound 1 in subjects with TART	• Adverse events (AEs), serious adverse events (SAEs), AEs leading to withdrawal, AEs of special interest (AESIs) • Physical examination findings • Vital signs • Changes in electrocardiogram (ECG) • Changes in clinical laboratory values
• To assess the pharmacodynamic (PD) biomarker effects of Compound 1	• Changes in plasma concentrations of key PD biomarkers, including ACTH, 17-OHP, testosterone, androstenedione, luteinizing hormone (LH),

	follicle-stimulating hormone (FSH), inhibin B, sex hormone–binding globulin (SHBG), renin, and aldosterone from baseline to Week 12
• To assess changes on semen analysis	• Changes in sperm count, morphology, and motility from baseline to Week 12
• To assess changes in metabolism	• Changes in metabolic parameters, including fasting glucose, HbA1c, insulin sensitivity, and lipid profile (including LDL cholesterol, HDL cholesterol, and triglycerides), from baseline to Week 12 • Changes in body weight, body mass index (BMI), waist circumference, and blood pressure
• To evaluate changes in quality of life (QoL) and mood	• Changes in QoL measured using the Short Form 36 (SF-36) • Changes in mood measured using the Hospital Anxiety and Depression Scale (HADS)

Study Population

[0301] Approximately 12 male subjects with TART will be enrolled at approximately 6 investigative sites in the United States, United Kingdom, and possibly other countries. Up to 12 additional subjects may be added to further evaluate specific safety, PD, or efficacy findings.

Eligibility Criteria

Inclusion Criteria

- Adult male subjects aged 18 to 30, inclusive
- Documented historical diagnosis of CAH based on documented genetic mutation or historical documentation of elevated 17-OHP
- Diagnosis of TART via testicular ultrasound and/or magnetic resonance imaging (MRI) at screening with ≥1 lesion(s) measuring ≥2 cm in diameter
- On a stable regimen of GC replacement for ≥30 days before baseline
- Agree to follow contraception guidelines and refrain from donating sperm throughout the treatment period and for 90 days after the last dose of study drug
- Subject is able to understand all study procedures and risks involved and provides written informed consent indicating willingness to comply with all aspects of the protocol

Exclusion Criteria

- Any clinically significant unstable medical condition, medically significant illness, or chronic disease within 30 days of screening, including but not limited to:

  - A malignancy or <3 years of remission history from any malignancy, other than successfully treated localized skin cancer
  - Presence of clinically significant renal disease, as evidenced by an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73m^2
  - Current or chronic history of liver disease or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones)
  - Confirmed positive test at screening for active hepatitis B, hepatitis C, or human immunodeficiency virus (HIV)
- Any clinically significant psychiatric disorders, including any current major depressive episode, bipolar disorder, schizophrenia, schizoaffective disorder, major depressive disorder with psychotic features, or any other psychotic disorder within the preceding 6 months
- HADS score >12 for either depression or anxiety
- At increased risk of suicide on the basis of the Investigator's judgment or the results of the Columbia-Suicide Severity Rating Scale (C-SSRS) conducted at screening and baseline (eg, C-SSRS Type 3, 4, or 5 ideation during the preceding 6 months or any suicidal behavior within the past 12 months)
- Clinically significant and abnormal clinical or laboratory assessments must be discussed with the Medical Monitor to determine eligibility for this study. Abnormal assessments that must be reviewed to determine eligibility include but are not limited to:
  - Clinically meaningful abnormal ECG results, in the opinion of the Investigator
  - Fridericia-corrected QT interval (QTcF) >450 msec
  - Alanine aminotransferase (ALT) >2x upper limit of normal (ULN)
  - Bilirubin >1.5x ULN (isolated bilirubin >1.5x ULN is acceptable if bilirubin is fractionated and direct bilirubin is <35%)
- A history that includes bilateral adrenalectomy or hypopituitarism
- Use of any other investigational drug within 30 days or 5 half-lives, whichever is longer, before initial screening
- Require use of prohibited concomitant medications, including strong inhibitors and/or inducers of CYP3A4 (with the exception of glucocorticoids, hydrocortisone, and dexamethasone) within 30 days or 5 half-lives (whichever is longer) of first dose of study drug. Medications that are sensitive substrates or substrates with narrow therapeutic ranges (metabolized by CYP3A4, 2C8, 2C9, or 2C19) should be discussed on a case-by-case basis with the Medical Monitor to determine whether the medication should be discontinued or may be continued with caution. If washout is feasible, then the medication should be

withdrawn at least 30 days or 5 half-lives (whichever is longer) before the first dose of study drug.

- Donation of blood within 60 days before first dose of study drug, or donation of platelets, white blood cells, or plasma within 15 days before first dose of study drug.

Study Duration

[0302] The expected duration of study participation for each subject is up to approximately 20 weeks. This includes a screening period of ≤4 weeks, a treatment period of 12 weeks, and a safety follow-up period of 4 weeks.

Study Design

[0303] This is a Phase 2, open-label efficacy and safety study of Compound 1 for the treatment of TART. All subjects will receive a daily oral dose of Compound 1 for 12 weeks.In-clinic study visits will occur for screening and at Day 1 (baseline) and Weeks 2, 4, 8, 12 (end of treatment), and 16 (follow-up).

Dose, Route, Regimen

[0304] All subjects will receive a daily oral dose of Compound 1 for 12 weeks. Subjects will receive 600 mg/day during the first 2 weeks and 1000 mg/day during the remaining 10 weeks of the treatment period. Study drug will be taken daily at 10pm (or bedtime, if earlier), 5 to 15 minutes after consumption of a standardized snack.

[0305] On mornings of study visits with 8am blood draws for laboratory assessments, subjects should take their morning glucocorticoid replacement after the blood draws. For all other days during the study, subjects may take their morning glucocorticoid replacement at their usual time (generally between 6am and 8am).

[0306] If a subject experiences a Grade 3 or higher TEAE considered at least possibly related to study drug, the Investigator may reduce the subject's daily dose of Compound 1 down in increments of 200 mg. Study drug will be discontinued in subjects who experience clinically significant liver chemistry, QTcF, or other individual treatment stopping criteria.

Investigational Drug

[0307] Compound 1 is a small-molecule CRF_1 receptor antagonist and will be supplied as 200-mg, size 1, white, hard gelatin capsules.

Example 2.

[0308] Since elevated ACTH is the primary driver for TART growth, blocking ACTH synthesis using Compound 1, a small-molecule antagonist of the CRF_1 receptor, may be effective in reducing ACTH levels, thereby reducing the size of testicular lesions in TART and reducing the need for chronic supraphysiologic doses of GCs. In CAH, this mechanism of action has been

validated in a previous proof-of-concept study using a similar small-molecule CRF_1 receptor antagonist, which produced dose-dependent reductions in ACTH and 17-OHP in subjects with CAH. The safety profile of Compound 1 has been established in nonclinical toxicology studies and in 2 completed Phase 1 clinical studies in healthy volunteers. A Phase 2 study in subjects with CAH is currently ongoing.

Nonclinical Toxicology of Compound 1

[0309] The nonclinical safety profile of Compound 1 has been evaluated in repeat-dose range-finding and definitive toxicology studies of up to 91 days in duration in rats and dogs using the clinically relevant route (oral) and schedule of administration (once daily). In rats and beagle dogs administered oral Compound 1 once daily for 91 consecutive days at doses up to 2000 mg/kg/day, toxicologic findings were all mild and limited to the male reproductive tract, and all Compound 1-related findings were reversible following a 6-week treatment-free recovery period.

[0310] Additionally, a fertility toxicology study was conducted in rats. In this study, male rats were administered oral Compound 1 once daily at 0, 5, 20, or 1000 mg/kg for 85 to 87 days (70 days before mating, during mating, and ≤4 days between mating and necropsy). Male reproductive performance (mean day of mating, mating and fertility indices, and conception rates) was unaffected by Compound 1 treatment. Untreated females mated with the Compound 1-treated males had no ovarian and uterine effects.

[0311] In addition, a complete battery of standard genotoxicity tests consisting of in vitro bacterial reverse mutation and chromosome aberration assays and an in vivo (rat) bone marrow micronucleus assay has been conducted. Exploratory and definitive embryo-fetal development studies in rabbits and a bovine corneal opacity and permeability assay have also been conducted.

Clinical Experience with Compound 1

[0312] To date, a total of 53 subjects, all healthy adults aged 21 to 65 years, have been treated with at least 1 dose of Compound 1 in 2 Phase 1 studies. The first-in-human study of Compound 1 (Study I3C-FW-BLAA) evaluated single ascending doses of 2 mg to 800 mg. In this study, a small number of subjects experienced diarrhea and headache of mild or moderate severity. A subsequent study (Study I3C-FW-BLAB) evaluated repeat doses of 50 mg, 100 mg, or 200 mg Compound 1 once daily for 14 consecutive days. A small number of subjects in the repeat-dose study experienced dyspnea, rhinorrhea, palpitations, and headache. The Phase 1 studies showed no clinically significant alterations in safety laboratory values, vital signs, or electrocardiograms (ECGs). Compound 1 has been well tolerated in healthy human subjects, with no SAEs, AEs leading to discontinuation, or dose-limiting toxicities (DLTs). A maximum tolerated dose has not been reached in humans.

[0313] An ongoing Phase 2 open-label study (Study Compound 2) is currently evaluating the safety and efficacy of Compound 1 dosed once daily for 6 weeks in adults with classic CAH. In the initial Cohort A of this study, all subjects are dosed starting at 200 mg/day for 14 days, escalating for each subject to 600 mg/day for 14 days, then 1000 mg/day for 14 days if the subject experiences no DLTs at the previous dose level. (**FIGS. 2-3**).

Known Potential Risks

[0314] Because the reversible testicular effects (degeneration/atrophy) observed in rats and dogs treated with high-dose Compound 1 (2,000 mg/kg/day) are likely associated with on-target (mechanism-driven) effects, these effects are likely to be relevant to human risk. However, male reproductive studies in rodents showed no impairment in fertility at the highest dose tested (1,000 mg/kg/day). Additionally, there were no effects on clinical laboratory markers of testicular function (LH and FSH) when Compound 1 was administered to healthy male volunteers at doses up to 200 mg per day for 14 consecutive days. Clinical laboratory measures of testicular function (LH, FSH, inhibin B, and SHBG) will be assessed throughout this study to monitor the risk of testicular injury. Testicular ultrasounds and semen analysis will also be performed at screening/baseline and the end of the treatment period.

[0315] Laboratory abnormalities in liver function tests (LFTs) have been observed at significantly higher doses in nonclinical studies. However, these events were not observed in the Phase 1 clinical studies. Clinical laboratory measures of liver chemistry will be assessed throughout this study, and strict individual treatment stopping criteria for liver chemistry findings are in place.

[0316] Compound 1-induced effects on the thyroid gland were noted in dogs after 91 days of high-dose (2,000 mg/kg/day) treatment but not in any other nonclinical studies. Clinical pathology parameters of thyroid function (eg, T4, T3, thyroid-stimulating hormone [TSH]) were not evaluated in the Phase 1 studies but will be monitored throughout this study.

Known Potential Benefits

[0317] Early clinical studies suggests that Compound 1 is effective as a CRF_1 receptor antagonist, a mechanism of action that supports the clinical investigation of Compound 1 for treatment of TART. In CAH, this mechanism of action has been validated in a previous proof-of-concept study using a similar small-molecule CRF_1 receptor antagonist, which produced dose-dependent reductions in ACTH and 17-OHP in subjects with CAH.

Assessment of Potential Risks and Benefits

[0318] The mild and reversible toxicologic effects of Compound 1 on the male reproductive tract in nonclinical studies occurred in the context of animals with normal, rather than elevated, baseline hormone function. As a CRF_1 receptor antagonist, Compound 1 is intended to reduce

abnormally elevated ACTH levels, which in TART drives overstimulation of aberrant adrenal cells within the testes. In nonclinical models where baseline hormone levels are within normal ranges, clinical benefits are not expected, and indeed, toxicities may be expected as a result of androgen reduction outside of the normal range. In TART, where baseline ACTH and androgen levels are pathologically elevated, androgen reduction would be considered a positive, therapeutic effect.

[0319] Given the serious nature of TART and the limitations (and risks) of chronic steroid therapy, new treatment modalities are needed for patients with TART. Given the acceptable safety and tolerability profile of Compound 1 in healthy volunteers from the Phase 1 program, the low probability of demonstrating meaningful PD marker changes in healthy subjects, and the lack of any clear benefit to exposing more healthy subjects to study drug, the Sponsor believes the benefit-to-risk profile favors the entry of Compound 1 into Phase 2 in subjects with TART.

Example 3. Congenital Andrenal Hyperplasia (CAH)

[0320] CAH results from a genetic defect in the 21-hydroxylase enzyme. CAH patients are thus unable to produce cortisol. Without cortisol to provide negative feedback, patients over-produce ACTH. High ACTH promotes the overproduction of potent androgens (male sex hormones). Without wishing to be bound by theory, Compound 1 blocks CRF_1 receptor to prevent ACTH release by the pituitary and associated drive on the hypothalamic-pituitary-adrenal axis. Replacement of cortisol to physiological levels will correct cortisol deficiency, however, will not lower ACTH, resulting in hyperplasia of adrenal gland and associated overproduction of adrenal androgens.

[0321] As shown in **FIGS. 4** and **5A-5B**, the attenuation of ACTH demonstrates target engagement and functional CRF_1 receptor antagonism. 80% of subjects demonstrated reduction in ACTH. 70% of subjects demonstrated > 25% reduction in ACTH. 30% of subjects were in the normal range (*) after treatment with a CRF_1 receptor antagonist.

[0322] **FIGS. 6** and **7A-7B** demonstrate “control” of disease based on standard guidelines, which would then allow steroid taper. 80% of subjects demonstrated reduction in 17-OHP. 50% of subjects demonstrated > 25% reduction in ACTH. 50% of subjects were in the guideline range (*; 1200 ng/dL) after treatment with a CRF_1 receptor antagonist.

[0323] **FIGS. 8** and **9A-9B** demonstrate attenuation of morning rise in A4 upon treatment with a CRF1 antagonist, indicative of its ability to control excess andreogen production and associated symptoms. 100% of subjects demonstrated reduction in androstenedione (at various doses). 60% of subjects demonstrated > 25% reduction in androstenedione. 50% of subjects were within the normal reference range after treatment with a CRF_1 receptor antagonist.

[0324] FIGS. 10A-10B show approximately 25% shrinkage in TART in a 25 year old male with classic congenital adrenal hyperplasia following 6 weeks treatment with a CRF_1 receptor antagonist.

[0325] FIGS. 11A-11B demonstrate Compound 1 is a potent, selective, and functional CRF_1 receptor antagonist. In in vitro and ex vivo studies, Compound 1 was determined to be a potent and selective CRF_1 receptor antagonist with high affinity and selective for the $hCRF_1$ receptor. Compound 1 was found to have functional potency to inhibit adenylate cyclase activity (CRF_1 receptor-mediated downstream cascade), demonstrating in vitro efficacy as a CRF_1 receptor antagonist. Compound 1 was found to significantly inhibit CRF-induced locomotor behavior in response to i.c.v. administration of CRF (**FIG. 11B**).

[0326] FIGS. 12A-12B demonstrate single pill of CRF_1 receptor antagonist improves ACTH and 17-OHP in CAH patients.

[0327] FIG. 13 shows the subject level hormone changes (ACTH, 17-OHP, and androstenedione) by dose of a CRF_1 receptor antagonist.

Example 4. Assess reduction in excess ACTH and adrenal steroid production

[0328] The objectives of the study are: to assess the ability of Compound 1 to reduce excess ACTH and adrenal steroid production in participants with classic CAH on a stable gluococorticoid regimen; to evaluate the safety and tolerability of tildacerfont in participants with classic CAH; and to evaluate the pharmacokinetics (PK) of tildacerfont.

ELIGIBILITY CRITERIA

[0329] Inclusion Criteria:

- Adult males and females 18 years or older
- Diagnosis of classic CAH due to 21-hydroxylase deficiency
- Morning 17OHP ≥800 ng/dL, before taking any morning GC dose
- On a stable regimen of gluococorticoid replacement for ≥30 days before enrollment

[0330] Exclusion Criteria:

- Clinically significant unstable medical condition, illness, or chronic disease within 30 days of screening
- Clinically significant psychiatric disorder
- History of bilateral adrenalectomy or hypopituitarism

STUDY DESIGN

[0331] FIG. 14 depicts the proof of concept and escalating QD dosing at 10 PM for Cohort A and the single dose level and BID dosing at 10 AM and 10 pm for Cohort B.

BASELINE CHARACTERISTICS

[0332] Table 1 provides a description of the baseline characteristics displayed by Cohort A and Cohort B. In Cohort A, 10 partcipants were enrolled and received at least 1 dose of Compound 1. One participant withdrew early because of an occupation-related scheduling conflict. In Cohort B, 9 participants were enrolled and received at least one dose of Compound 1. One participant was withdrawn upon discovery of a major protocol violation at Baseline and did not have postbaseline efficacy/PK data. Thus, baseline characteristics are calculated for n=8 in Cohort B. One participant in Cohort B was taking both HC and prednisone.

Table 1 – Baseline characteristics of each cohort

Parameter	**Cohort A (n=10)**	**Cohort B (n=8)**
Demographics		
Age (years), mean (range)	39.3 (24-66)	36.8 (19-66)
Sex	5M, 5F	1M, 7F
Bmi (kg/m^2), mean ± SD	31.5 ± 12.0	31.1 ± 6.7
Daily GC dose (mg HC equivalents), mean range	28 (13-40)	35 (20-50)
Types of GCs	4 JC, 3 pred, 3 dex	2HC, 4 pred, 3 dex
17OHP at screening[a] (ng/dl), mean ± SD	5,985 ± 6,347	4,592 ±4,624

Abbreviations: 17OHP: 17-hydroxyprogesterone; BMI: body mass index; dex: dexamethasone; F: female; GC: glucocorticoid; HC: hydrocortisone; M: male; pred: prednisone; SD: standard deviation.
a 17OHP reference ranges: 27-199 ng/dL males, 15-290 ng/dL females.

SAFETY

[0333] Across both cohorts, Compound 1 was generally well tolerated. The most common type of adverse event (AE) was gastrointestinal disorders There were no serious AEs and no AEs leading to withdrawal.

PHARMACOKINETICS

[0334] Over a wide range of doses (200 to 1000 mg QD), Compound 1exhibited a predictable, dose-dependent PK profile.

EFFICACY

[0335] FIG. 15A-15D show the changes in levels of Androstenedione (A4) across both cohorts. Levels of A4 improved at ≥1 dose level for 10 out of 10 participants in Cohort A and 6 out of 8 participants in Cohort B. Excess A4 is associated with clinical sequelae in CAH. Attenuation of A4 demonstrates control of adrenal androgen.

[0336] FIG. 16A-16D show the changes in levels of 17-hydroxyprogesterone (17-OHP) across both cohorts. Levels of 17OHP improved at ≥1 dose level for 8 out of 10 participants in Cohort A and 6 out of 7 participants in Cohort B. 17OHP is a critical hormone in CAH diagnosis and management. Attenuation of 17OHP demonstrates control of adrenal steroid production.

[0337] FIG. 17A-17D show the changes in levels of adrenocorticotropic hormone (ACTH) across both cohorts. Levels of ACTH improved at ≥1 dose level for 8 out of 10 participants in

Cohort A and 5 out of 7 participants in Cohort B. Attenuation of ACTH demonstrates target engagement and fucntional CRF_1 receptor antagonism.

Table 2 – Percentage change from baseline in ACTH, 17OHP and A4.

	Cohort A			Cohort B
	200 mg QD (Week 2)	600 mg QD (Week 4)	1000 mg QD (Week 6)	200 mg BID (Weed 2)
ACTH	-25.7 (±51.6)	-60.1 (±37.5)	-32.7 (±47.0)	-36.5 (±58.0)
17OHP	+22.2 (±172.3)	-23.7 (±64.2)	-13.8 (±74.7)	-47.8 (±63.3)
A4	-12.1 (±47.4)	-33.2 (±36.5)	-28.6 (±44.6)	-16.0 (±81.4)

CONCLUSIONS

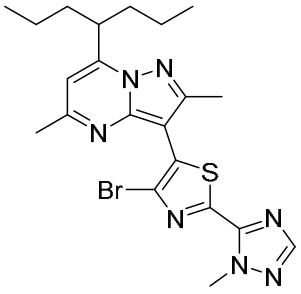
[0338] Compound 1, a potent and selective CRF_1 receptor antagonist, offers a novel nonsteroidal oral treatment approach for CAH. Compound 1 was well tolerated and efficacious in reducing excess ACTH, 17OHP, and androstenedione in patients with classic CAH. Compound 1 exhibited a predictable, dose-dependent PK profile suitable for the target population.

[0339] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby

CLAIMS

WHAT IS CLAIMED IS:

1. A method of treating an endocrine disorder associated with corticotropin-releasing factor type-1 (CRF_1) activity, comprising administering to a subject in need thereof a small molecule CRF_1 antagonist, or a pharmaceutically acceptable salt thereof, wherein the small molecule CRF_1 antagonist is: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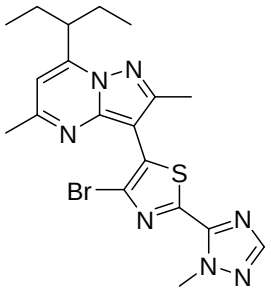
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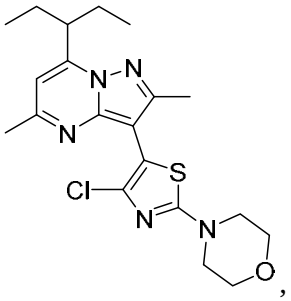
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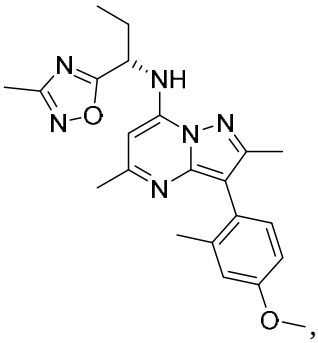
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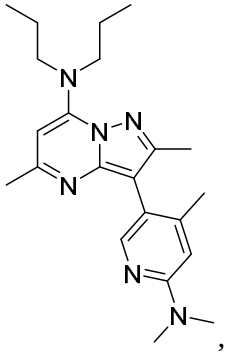
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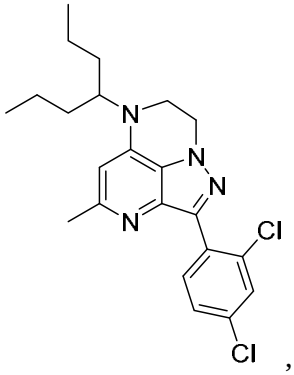
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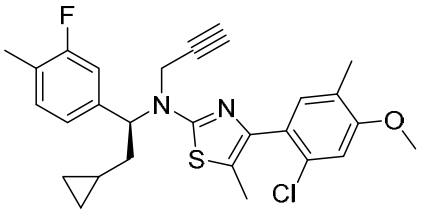
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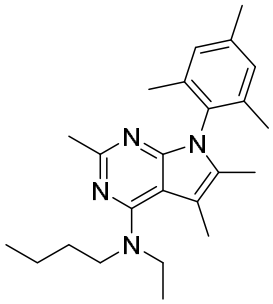
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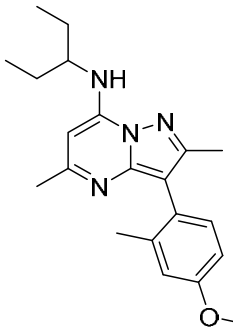
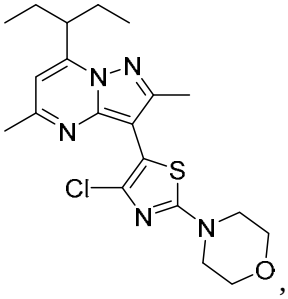
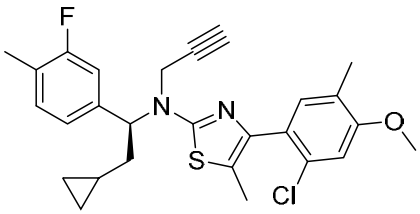

, or a pharmaceutically acceptable salt thereof.

2. The method of claim 1, wherein the small molecule CRF_1 antagonist is:



, or a pharmaceutically acceptable salt thereof.

3. The method of claim 1, wherein the small molecule CRF_1 antagonist is:



, or a pharmaceutically acceptable salt thereof.

4. The method of claim 1, wherein the endocrine disorder associated with CRF_1 activity is a testicular adrenal rest tumor (TART) or an ovarian adrenal rest tumor (OART).
5. The method of claim 1, wherein the subject has congenital adrenal hyperplasia (CAH).
6. The method of claim 1, wherein the subject is receiving a glucocorticoid in conjunction with the administering of the small molecule CRF_1 antagonist.
7. The method of claim 6, wherein the glucocorticoid comprises beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone, or a combination thereof.
8. The method of claim 7, wherein the glucocorticoid is hydrocortisone.
9. The method of claim 1, wherein the subject is an adolescent.
10. The method of claim 1, wherein the subject is at least 18 years old.
11. The method of claim 1, wherein the small molecule CRF_1 antagonist is administered at a dose of about 10 mg/day to about 400 mg/day to the subject.
12. The method of claim 11, wherein the small molecule CRF_1 antagonist is administered at a dose of about 50 mg.
13. The method of claim 11, wherein the small molecule CRF_1 antagonist is administered at a dose of about 100 mg.
14. The method of claim 11, wherein the small molecule CRF_1 antagonist is administered at a dose of about 200 mg.
15. The method of claim 11, wherein the small molecule CRF_1 antagonist is administered at a dose of about 400 mg.
16. The method of claim 1, wherein the small molecule CRF_1 antagonist is administered to the subject once per day.

17. The method of claim 1, wherein the small molecule CRF_1 antagonist is administered to the subject twice per day.
18. The method of claim 16, wherein the subject is in a fed state.
19. The method of claim 17, wherein the subject is in a fed state.
20. The method of claim 1, wherein the small molecule CRF_1 antagonist is administered orally.
21. The method of claim 1, wherein the small molecule CRF_1 antagonist is formulated as a capsule or tablet.

ABSTRACT OF THE DISCLOSURE

Provided herein are compounds and pharmaceutical compositions for the prevention and treatment of testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART).

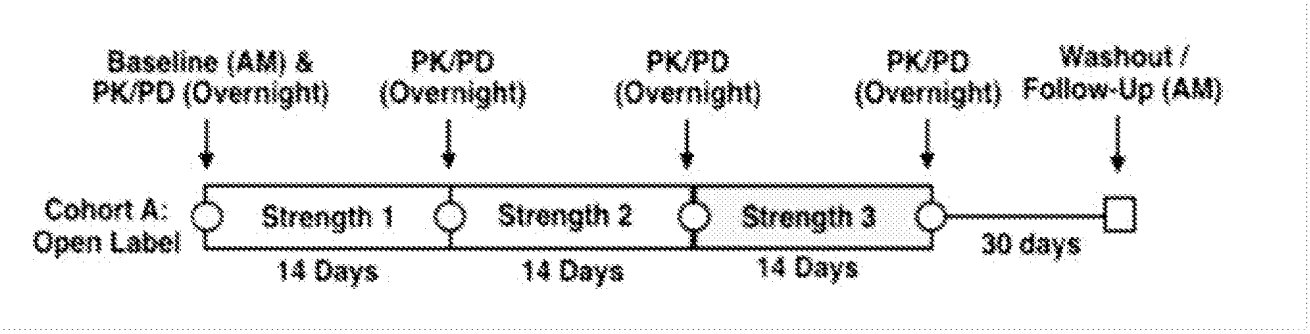

Baseline (AM) & PK/PD (Overnight)
PK/PD (Overnight)
PK/PD (Overnight)
PK/PD (Overnight)
Washout / Follow-Up (AM)
Cohort A: Open Label
Strength 1
Strength 2
Strength 3
30 days
14 Days
14 Days
14 Days

FIG. 1A

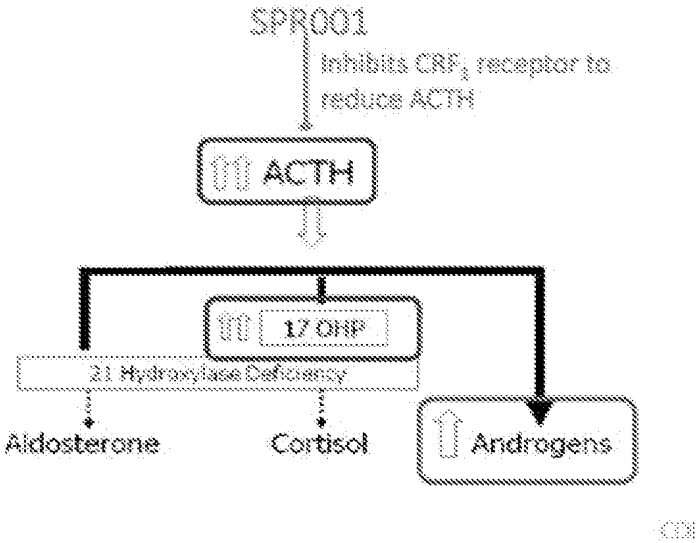

SPR001
Inhibits CRF1 receptor to reduce ACTH
ACTH
17 OHP
21 Hydroxylase Deficiency
Aldosterone
Cortisol
Androgens

FIG. 1B

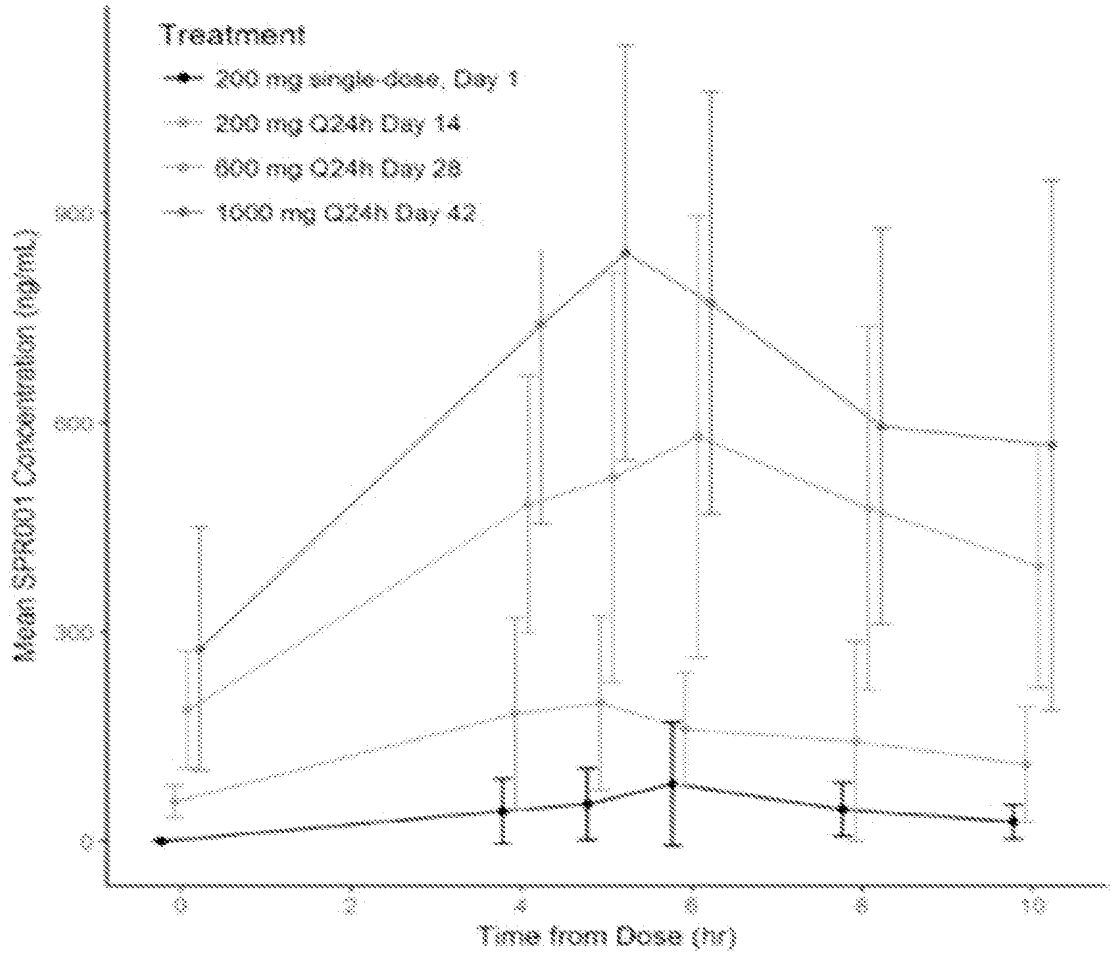

Treatment
200 mg single dose, Day 1
200 mg Q24h Day 14
800 mg Q24h Day 28
1000 mg Q24h Day 42
Mean SPR001 Concentration (ng/mL)
0
30
60
90
0
2
4
6
8
10
Time from Dose (hr)

FIG. 2

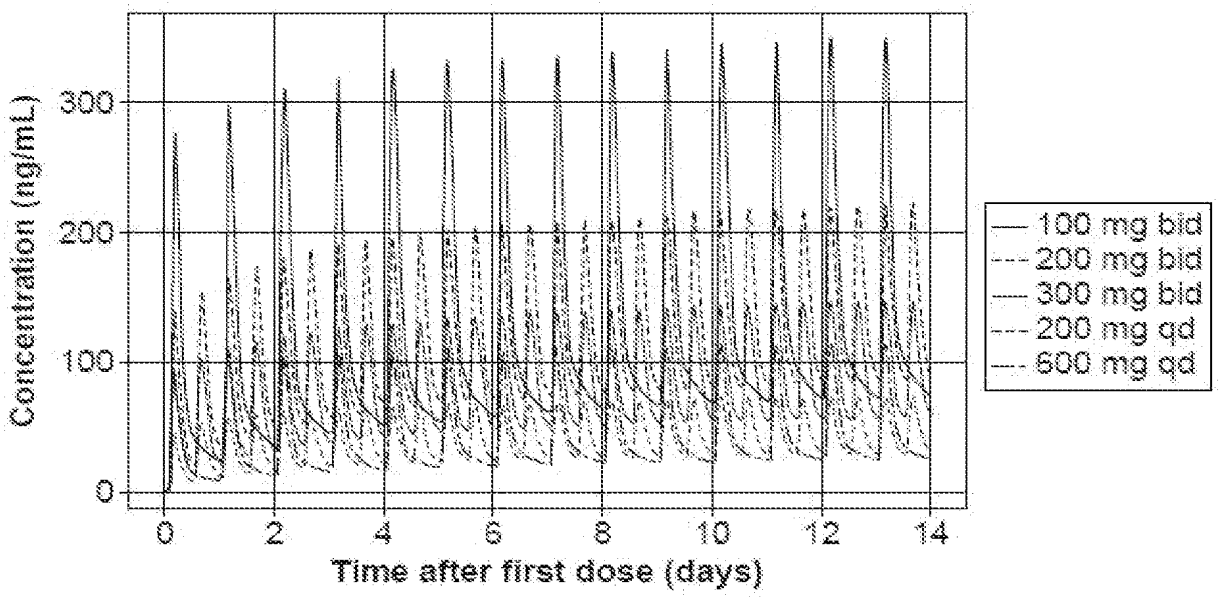

Concentration (ng/mL)
300
200
100
0
0
2
4
6
8
10
12
14
Time after first dose (days)
100 mg bid
200 mg bid
300 mg bid
200 mg qd
600 mg qd

FIG. 3

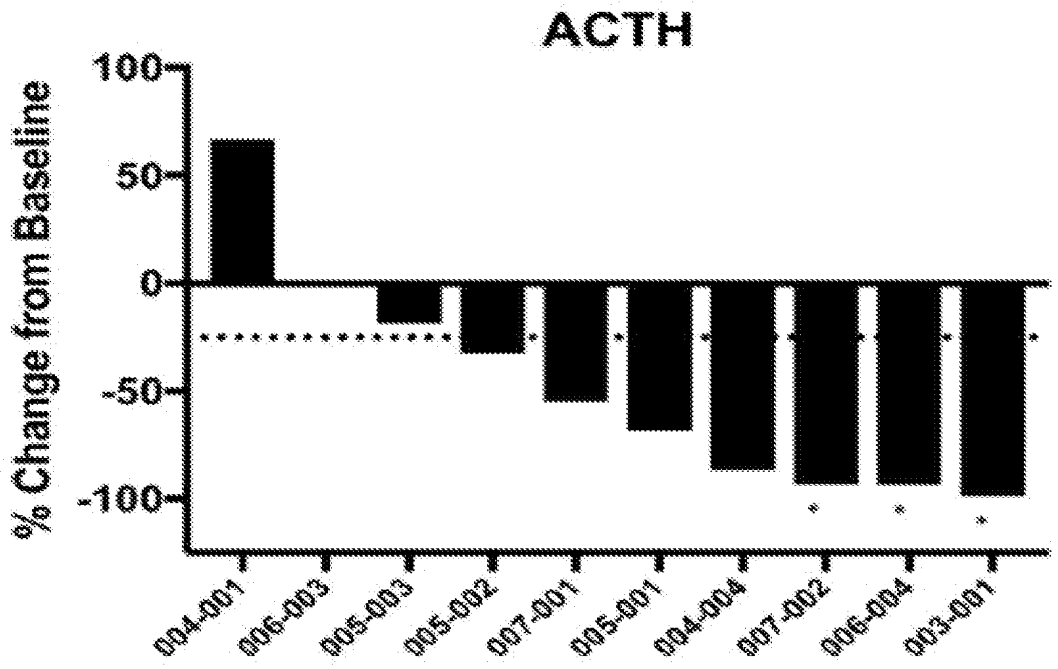

ACTH
% Change from Baseline
100
50
0
-50
-100
004-001
006-003
005-003
005-002
007-001
005-001
004-004
007-002
006-004
003-001

FIG. 4

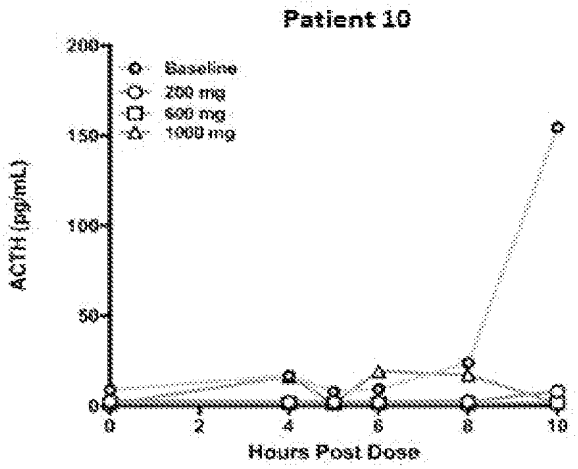


FIG. 5A

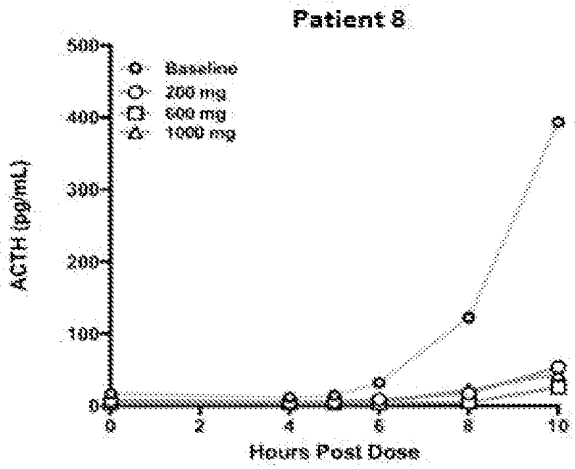


FIG. 5B

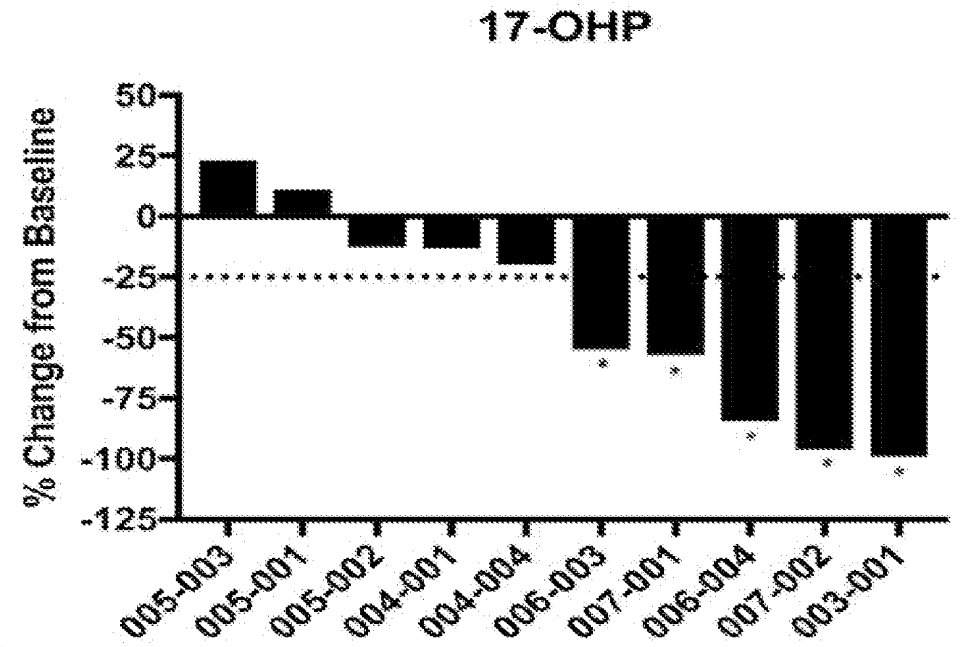


FIG. 6

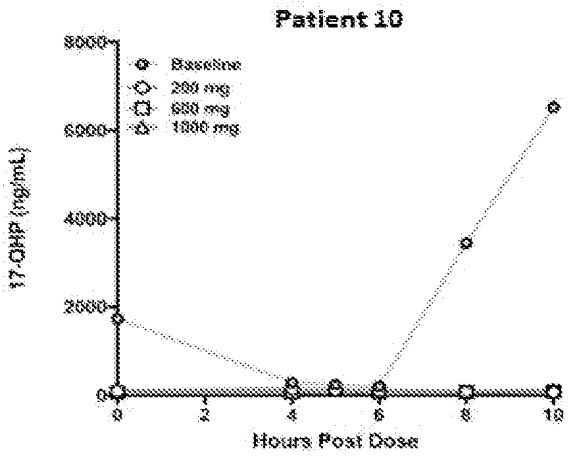


FIG. 7A

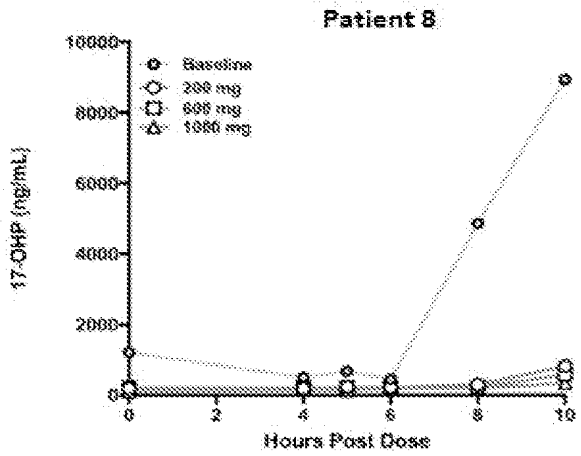


FIG. 7B

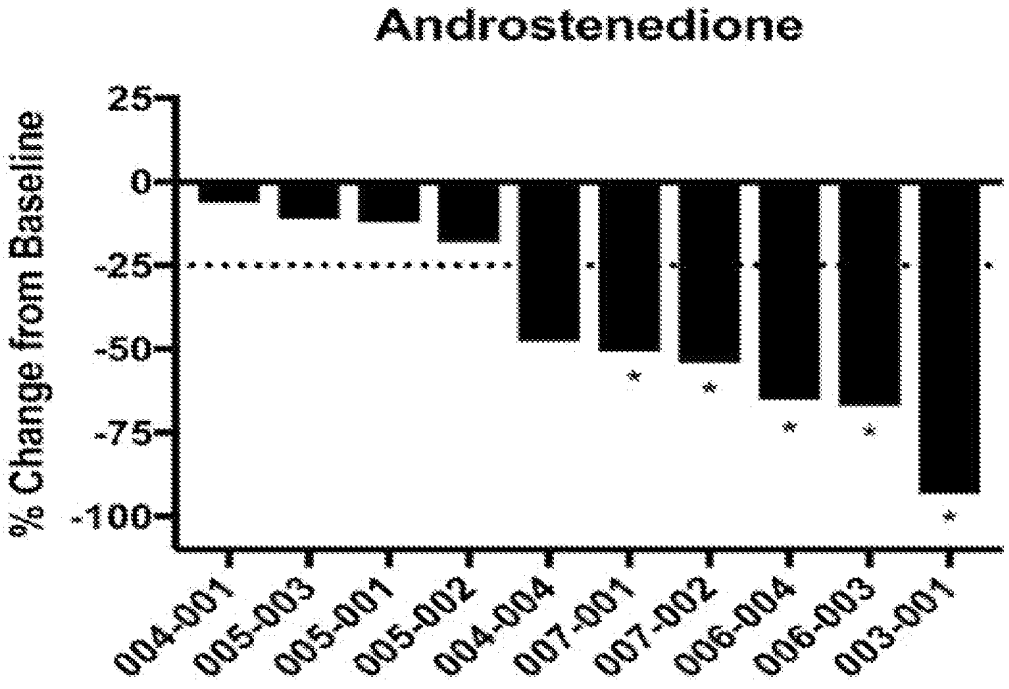


FIG. 8

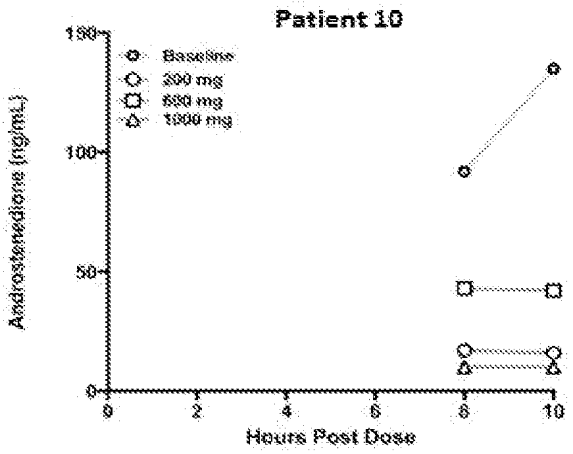


FIG. 9A

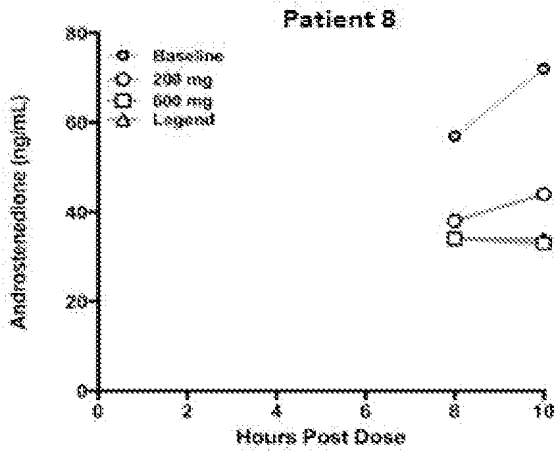


FIG. 9B

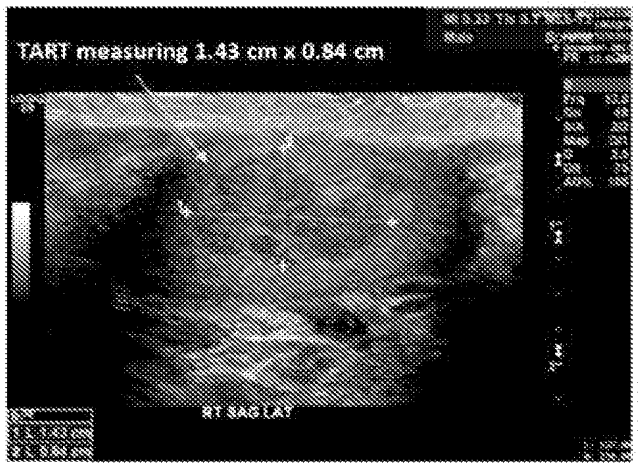


FIG. 10A

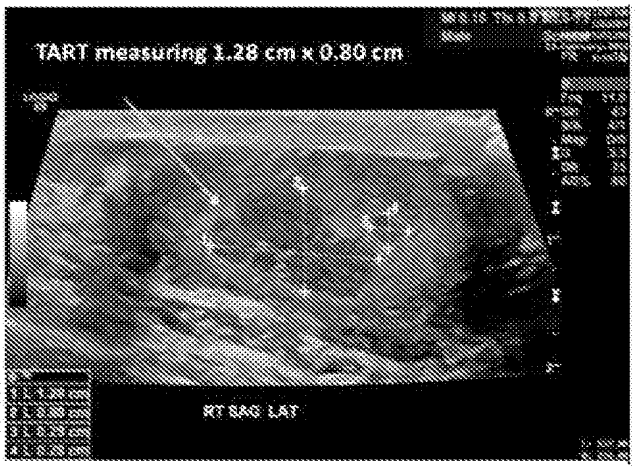


FIG. 10B

In Vitro Binding Assay

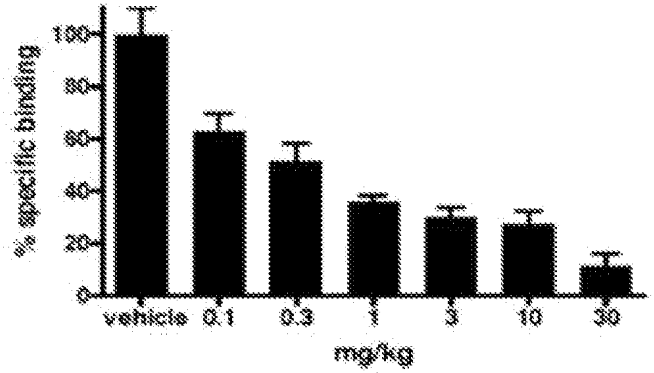


FIG. 11A

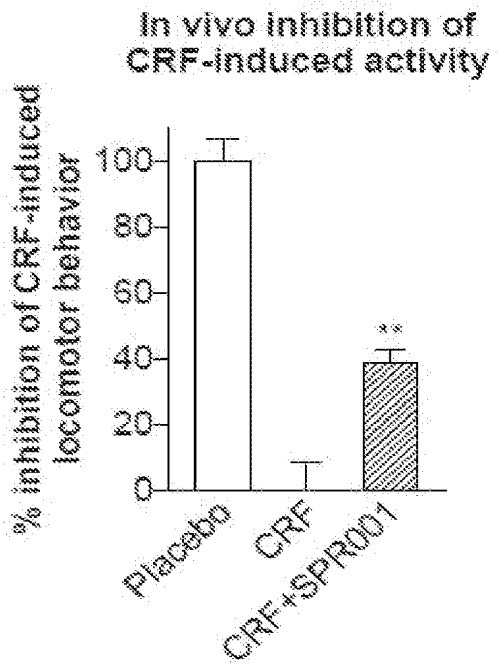


FIG. 11B

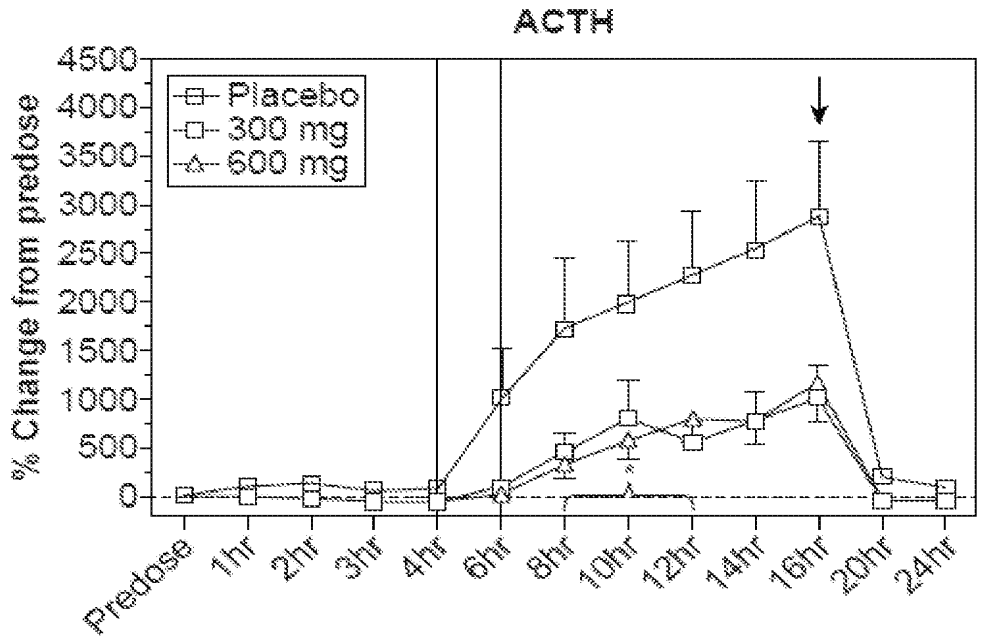


FIG. 12A

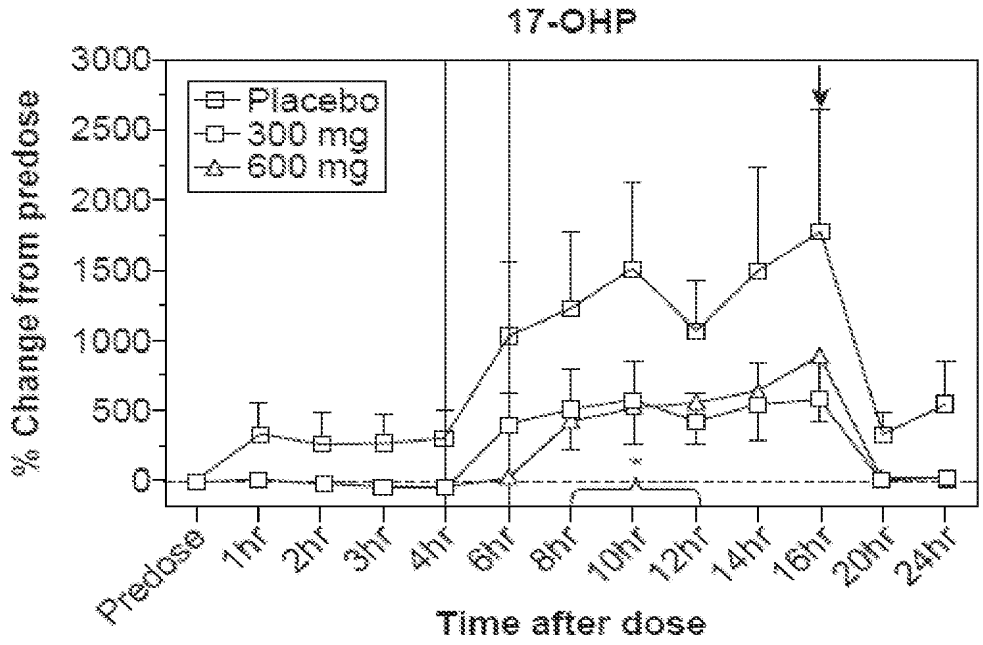


FIG. 12B

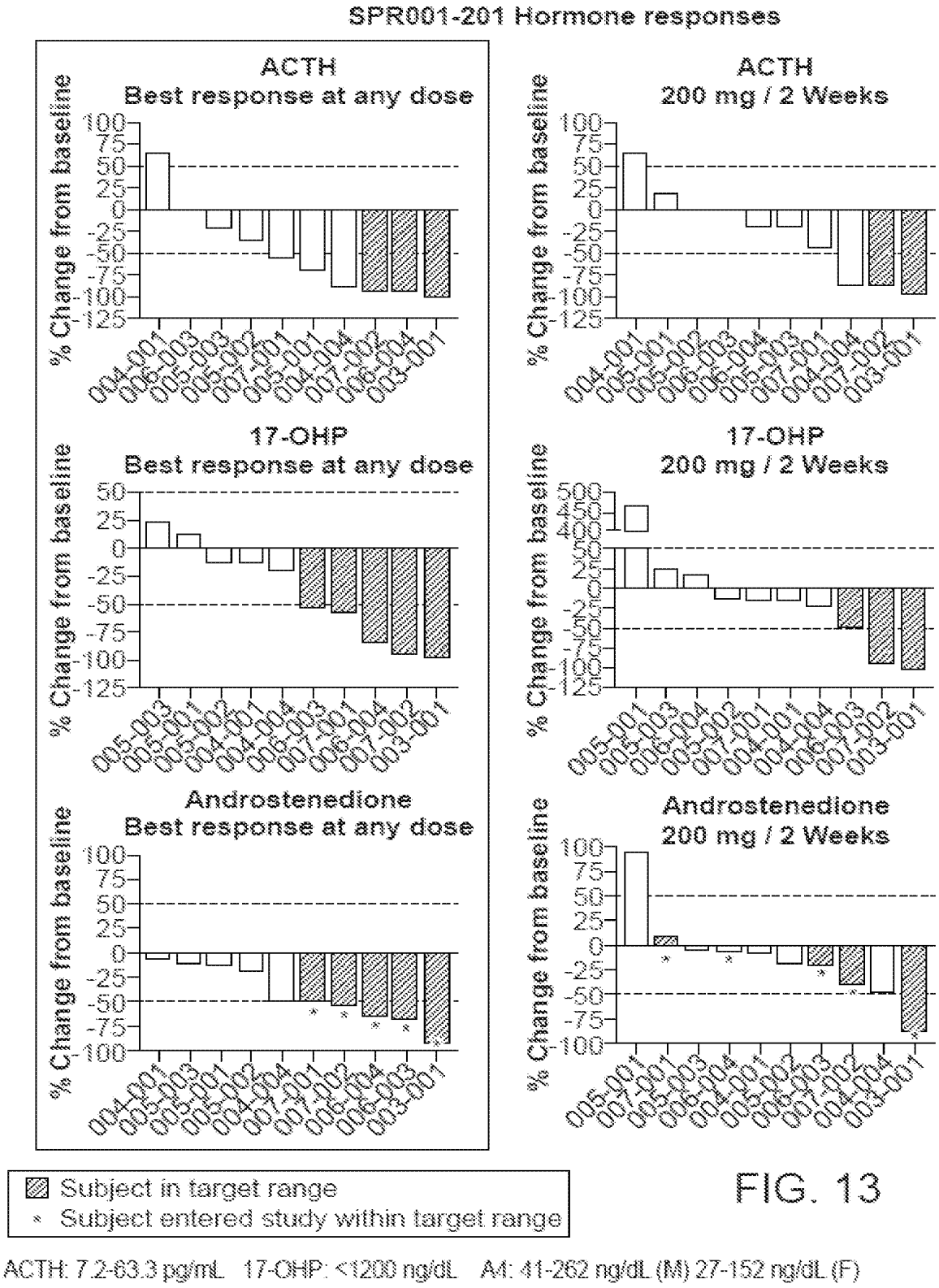


FIG. 13

ACTH: 7.2-63.3 pg/mL 17-OHP: <1200 ng/dL A4: 41-262 ng/dL (M) 27-152 ng/dL (F)

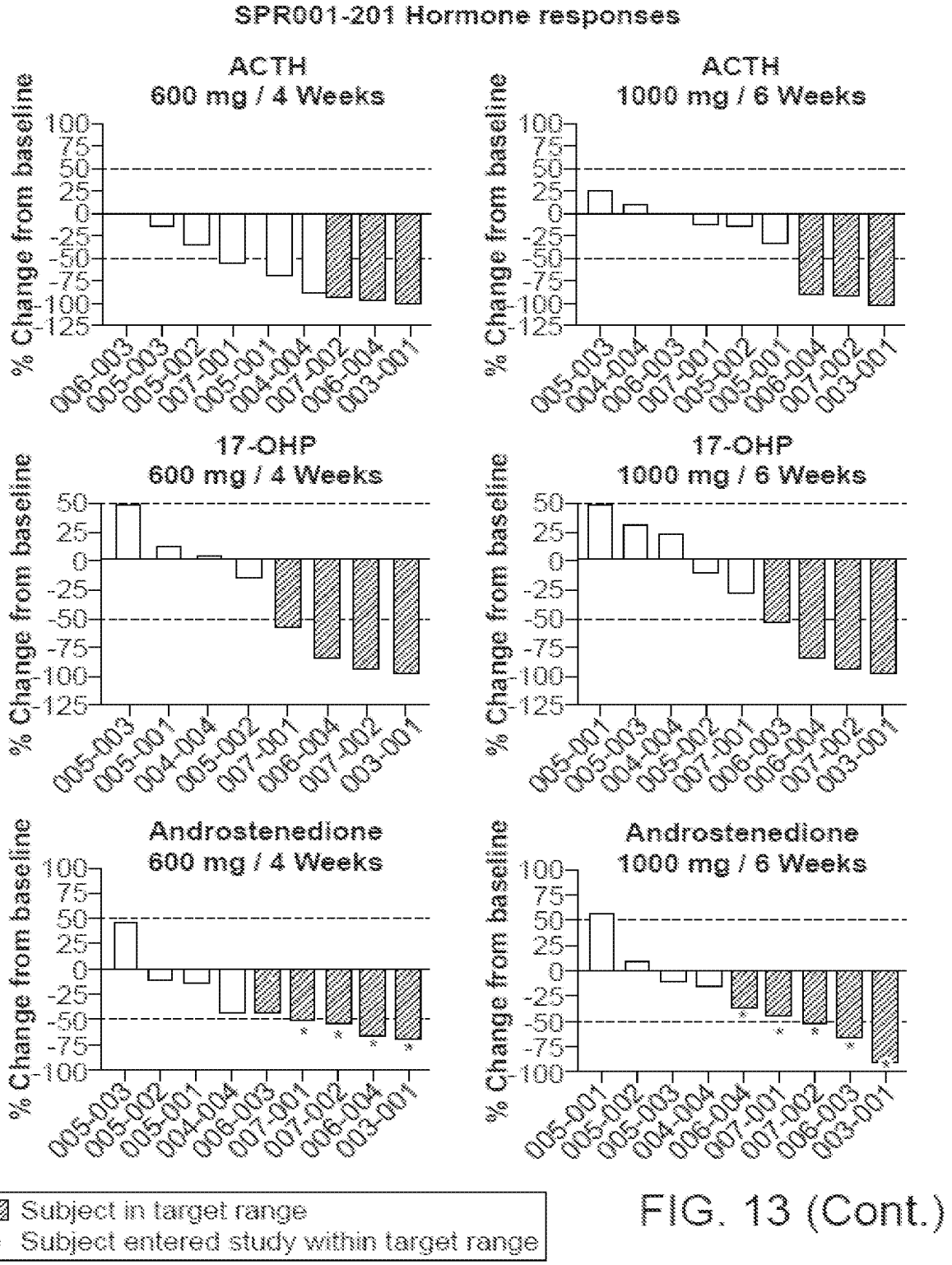


FIG. 13 (Cont.)

ACTH: 7.2-63.3 pg/mL 17-OHP: <1200 ng/dL A4: 41-262 ng/dL (M) 27-152 ng/dL (F)

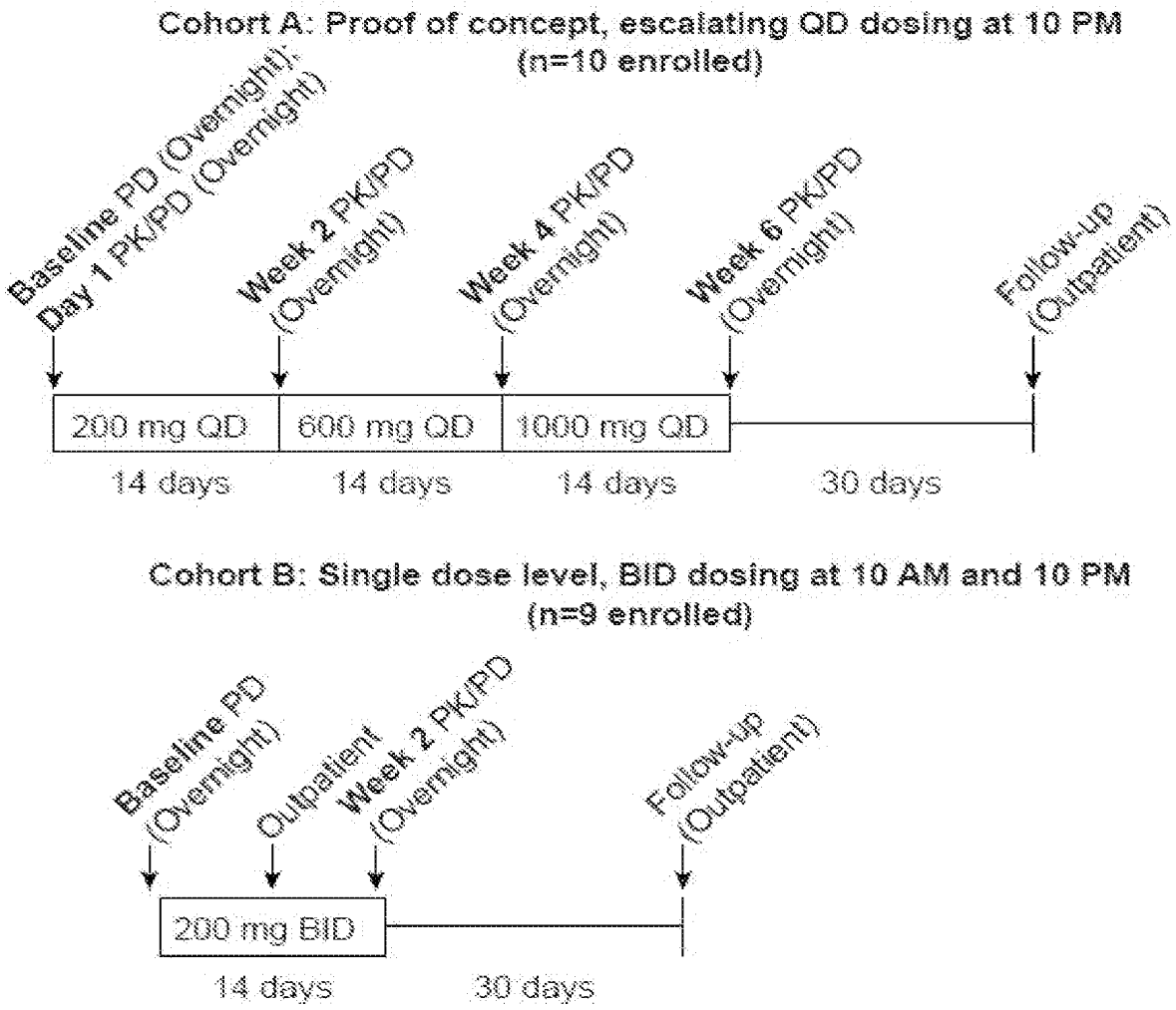

Cohort A: Proof of concept, escalating QD dosing at 10 PM
(n=10 enrolled)
Baseline PD (Overnight); Day 1 PK/PD (Overnight)
Week 2 PK/PD (Overnight)
Week 4 PK/PD (Overnight)
Week 6 PK/PD (Overnight)
Follow-up (Outpatient)
200 mg QD
600 mg QD
1000 mg QD
14 days
14 days
14 days
30 days
Cohort B: Single dose level, BID dosing at 10 AM and 10 PM
(n=9 enrolled)
Baseline PD (Overnight)
Outpatient
Week 2 PK/PD (Overnight)
Follow-up (Outpatient)
200 mg BID
14 days
30 days

FIG. 14

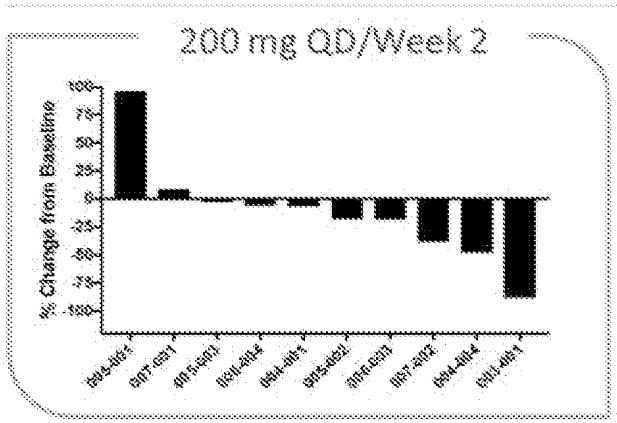


FIG. 15A

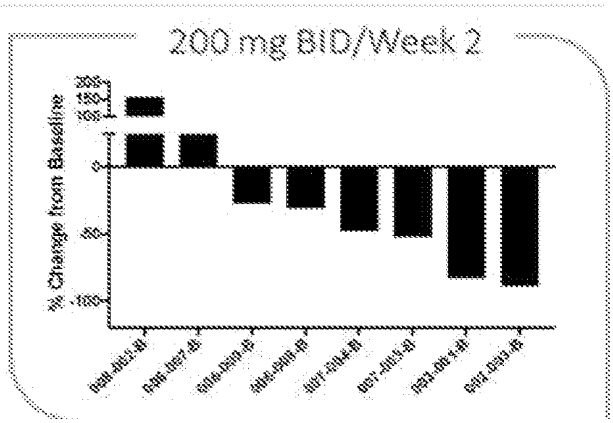


FIG. 15B

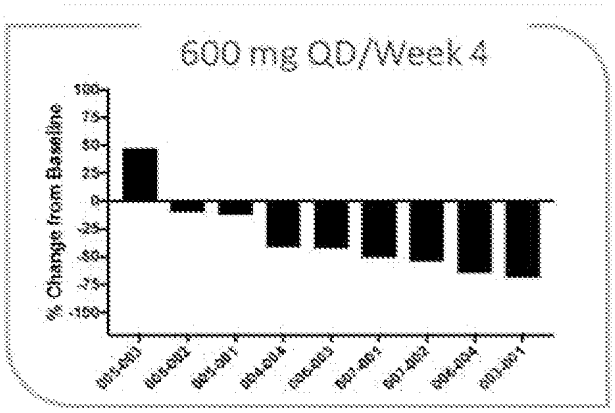


FIG. 15C

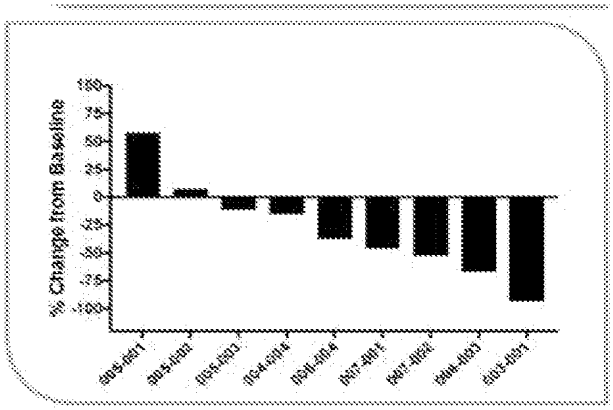


FIG. 15D

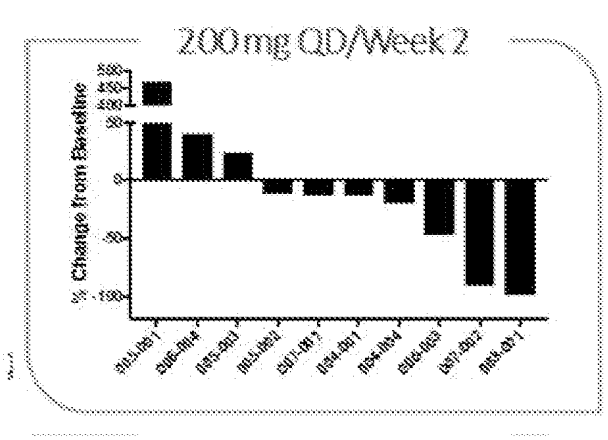


FIG. 16A

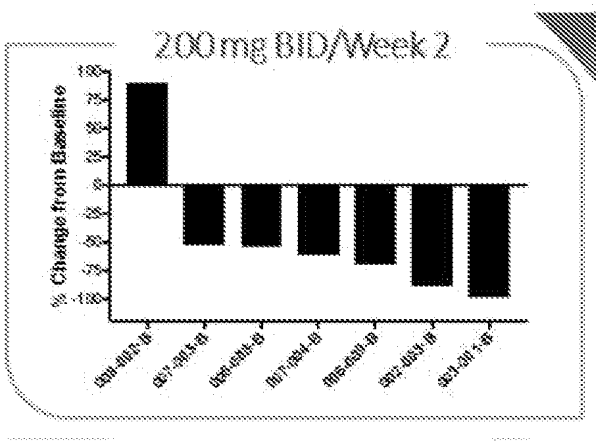


FIG. 16B

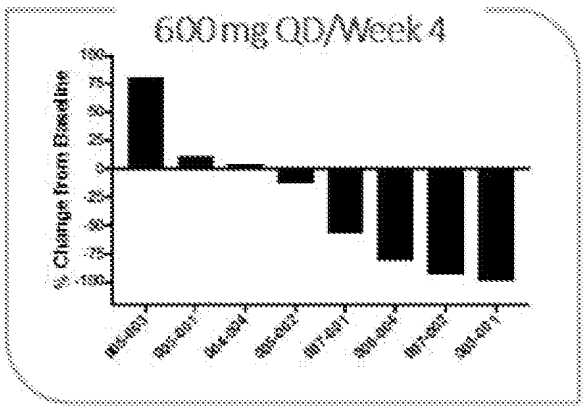


FIG. 16C

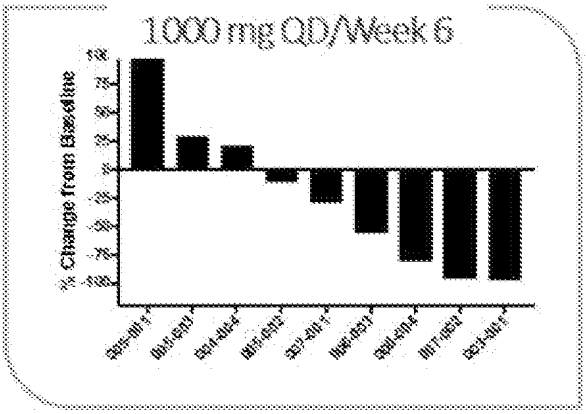


FIG. 16D

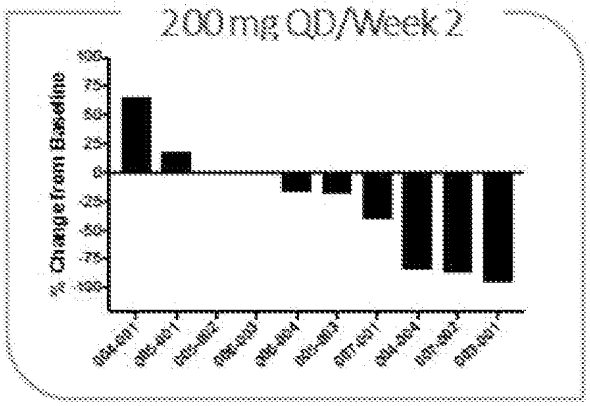


FIG. 17A

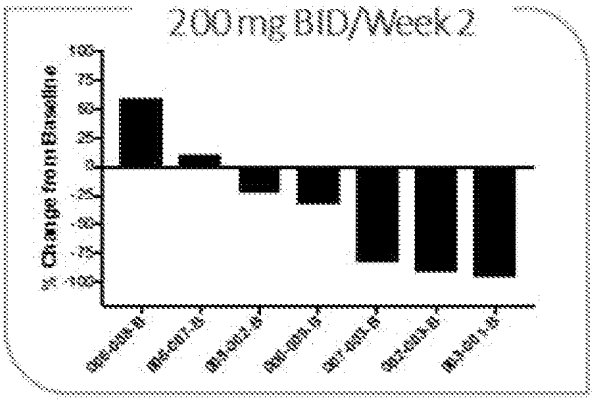


FIG. 17B

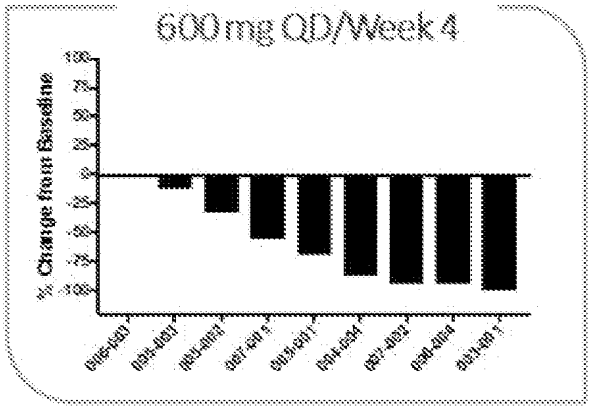


FIG. 17C

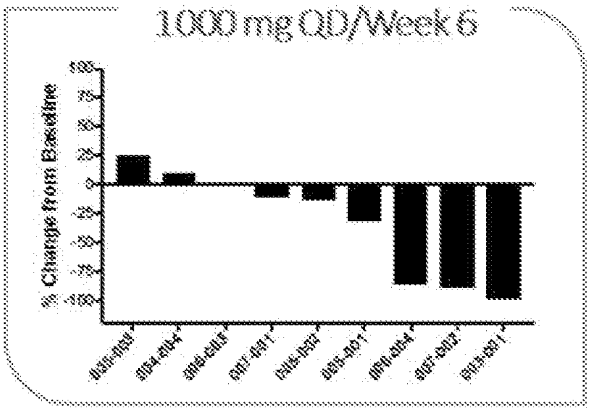


FIG. 17D

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (11-23)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Alexis HOWERTON	Nonprovisional Application Number (if known):	
Title of Invention:	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently $0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.

2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.

3. The applicable box is checked below:

I. ☑ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via USPTO patent electronic filing system.

---OR---

(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, **or** the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via USPTO patent electronic filing system.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature /Victoria Cheng/	Date 1/31/2025
Name (Print/Typed) Victoria Cheng	Practitioner Registration Number 79641

Note: *This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

☑ *Total of 1 forms are submitted.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. The United States Patent and Trademark Office (USPTO) collects the information in this record under authority of 35 U.S.C. 2. The USPTO's system of records is used to manage all applicant and owner information including name, citizenship, residence, post office address, and other information with respect to inventors and their legal representatives pertaining to the applicant's/owner's activities in connection with the invention for which a patent is sought or has been granted. The applicable Privacy Act System of Records Notice for the information collected in this form is COMMERCE/PAT-TM-7 Patent Application Files, available in the Federal Register at 78 FR 19243 (March 29, 2013). https://www.govinfo.gov/content/pkg/FR-2013-03-29/pdf/2013-07341.pdf

Routine uses of the information in this record may include disclosure to:

1) law enforcement, in the event that the system of records indicates a violation or potential violation of law;

2) a federal, state, local, or international agency, in response to its request;

3) a contractor of the USPTO having need for the information in order to perform a contract;

4) the Department of Justice for determination of whether the Freedom of Information Act (FOIA) requires disclosure of the record;

5) a Member of Congress submitting a request involving an individual to whom the record pertains, when the individual has requested the Member's assistance with respect to the subject matter of the record;

6) a court, magistrate, or administrative tribunal, in the course of presenting evidence, including disclosures to opposing counsel in the course of settlement negotiations;

7) the Administrator, General Services Administration (GSA), or their designee, during an inspection of records conducted by GSA under authority of 44 U.S.C. 2904 and 2906, in accordance with the GSA regulations and any other relevant (i.e., GSA or Commerce) directive, where such disclosure shall not be used to make determinations about individuals;

8) another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c));

9) the Office of Personnel Management (OPM) for personnel research purposes; and

10) the Office of Management and Budget (OMB) for legislative coordination and clearance.

If you do not furnish the information requested on this form, the USPTO may not be able to process and/or examine your submission, which may result in termination of proceedings, abandonment of the application, and/or expiration of the patent.

METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

CROSS-REFERENCE

[0001] This application is a continuation of U.S. Application No. 18/941,832, filed November 8, 2024, which is a continuation of U.S. Application No. 17/667,285, filed on February 8, 2022, which is a continuation of U.S. Application No. 17/081,694 filed on October 27, 2020, which issued as U.S. Pat. No. 11,304,950 on April 19, 2022, and which is a continuation of International Application No. PCT/US2019/029486 filed on April 26, 2019, which claims the benefit of U.S. Provisional Patent Application No. 62/663,951, filed April 27, 2018, and U.S. Provisional Patent Application No. 62/822,815, filed March 23, 2019, each of which is entirely incorporated herein by reference.

BACKGROUND

[0002] Corticotropes are basophilic cells in the anterior pituitary that produce melanocyte-stimulating hormone, adrenocorticotropic hormone (ACTH) and lipotropin. The cells produce pro-opiomelanocortin (POMC) which undergoes cleavage to ACTH and β-lipotropin (β-LPH). These cells respond to corticotropin-releasing factor (CRF) and make up about 20% of the cells in the anterior pituitary. CRF is a releasing hormone that belongs to the corticotropin-releasing factor family and is the primary physiological regulator of proopiomelanocortin (POMC).

[0003] CRF is produced by parvocellular neuroendocrine cells within the paraventricular nucleus of the hypothalamus and is released at the median eminence from neurosecretory terminals of these neurons into the primary capillary plexus of the hypothalamo-hypophyseal portal system. The portal system carries the CRF to the anterior lobe of the pituitary, where it stimulates corticotropes to secrete ACTH and other biologically-active substances. ACTH, in turn, stimulates the synthesis of cortisol, glucocorticoids, mineralocorticoids and DHEA.

[0004] Testicular adrenal rest tumors (TART) and ovarian adrenal rest tumors (OART) are ACTH-responsive lesions of the testes and ovaries derived from adrenal tissue interchelated within these organs during embryogenesis. In response to high ACTH, hyperplasia of this tissue occurs, resulting in single or multiple lesions that may cause pain and infertility. The identification of compounds that modulate CRF function and downstream processes is an ongoing challenge. Currently, there are no non-steroidal, non-surgical solutions to control, shrink, or reduce TART. Disclosed herein, *inter alia*, are solutions to these and other problems in the art.

SUMMARY

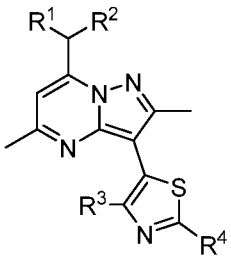
[0005] Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is a serious genetic disorder characterized by impaired adrenal synthesis of cortisol and consequent overproduction of adrenocorticotropic hormone (ACTH), 17-hydroxyprogesterone (17OHP), and adrenal androgens such as androstenedione (A4). CAH is an orphan condition with no FDA-approved therapy. To suppress androgen production, patients are often prescribed long-term supraphysiologic doses of glucocorticoid (GC), which has adverse effects and fail to suppress 17OHP and A4 in the majority of patients.

[0006] Compound 1 may be a potent, selective, nonsteroidal, oral corticotropin-releasing factor type-1 (CRF1) receptor antagonist that reduces excess ACTH, 17OHP, and A4 to improve clinical and biochemical sequelae of CAH. This may obviate the need for high dose steroids.

[0007] One aspect provided herein is a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF_1) antagonist or a pharmaceutically acceptable salt thereof.

[0008] In some embodiments, the subject has congenital adrenal hyperplasia (CAH). In some embodiments, the size and/or number of the tumors is decreased or reduced.

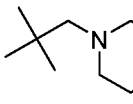
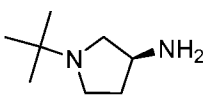
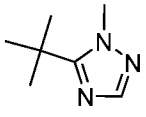
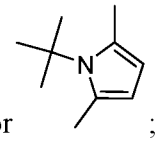
[0009] One aspect provided herein is a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a compound of structural Formula (I):


(I), or a pharmaceutically acceptable salt thereof,

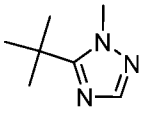
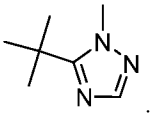
wherein:

R^1 and R^2 are independently ethyl or n-propyl;

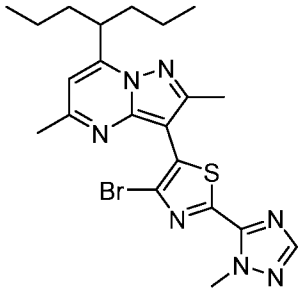
R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy; and

R^4 is hydrogen, Br, R^aR^bN–, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl,  ,  ,  , or  ;

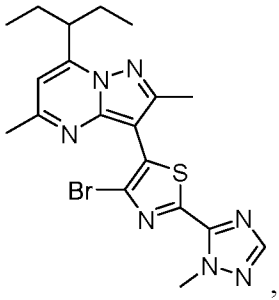
R^a and R^b are independently hydrogen, C_1-C_3alkyl, $H_2NCH_2CH_2$–, $(CH_3)_3COC(O)NHCH_2CH_2$–, or $CH_3CH_2CH_2NHCH_2CH_2$–.

[0010] In some embodiments, R^3 is Cl, Br, methyl, or trifluoromethyl. In some embodiments, R^3 is Cl, Br, or methyl. In some embodiments, R^4 is Br, R^aR^bN–, pyridin-4-yl, morpholin-4-yl, or  . In some embodiments, R^4 is morpholin-4-yl or  .

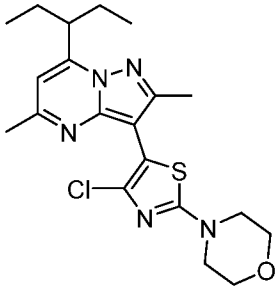
[0011] In some embodiments, R^4 is hydrogen, Br, R^aR^bN– and R^a and R^b are independently C_1-C_3alkyl. In some embodiments, the compound is

 , or a pharmaceutically acceptable salt thereof.

[0012] In some embodiments, the compound is

 , or a pharmaceutically acceptable salt thereof.

[0013] In some embodiments, the compound is

 , or a pharmaceutically acceptable salt thereof.

[0014] In some embodiments, the subject has congenital adrenal hyperplasia (CAH). In some embodiments, the size and/or number of the tumors is decreased or reduced.

[0015] In some embodiments, from about 5 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In some embodiments, from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In some embodiments, from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically

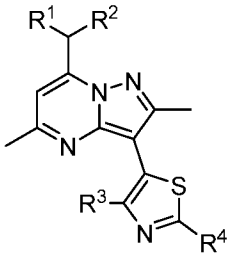
acceptable salt thereof, is administered to the subject. In some embodiments, from about 100 mg to about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject.

[0016] In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 5 mg/day to about 1000 mg/day is administered to the subject. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose from about 5 mg/day to about 25 mg/day is administered to the subject. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose from about 10 mg/day to about 400 mg/day is administered to the subject. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 100 mg/day to about 600 mg/day is administered to the subject.

[0017] In some embodiments, the compound is administered orally. In some embodiments, the compound is formulated as a capsule or tablet. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the fed state. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the fasted state.

[0018] In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at least once per day. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at bedtime. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 4 hours before sleep. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 2 hours before sleep. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 30 minutes before sleep. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the evening. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at about 10 pm at night.

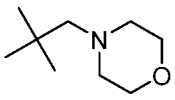
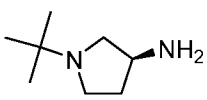
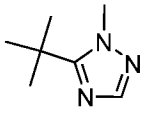
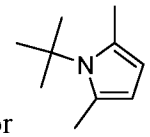
[0019] One aspect described herein is a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a pharmaceutical composition, comprising a compound of structural Formula (I) and a pharmaceutically acceptable excipient:


(I), or a pharmaceutically acceptable salt thereof,

wherein:

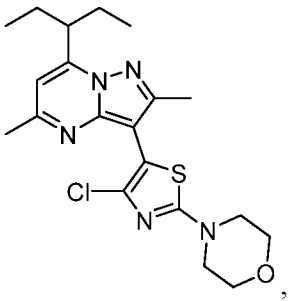
R^1 and R^2 are independently ethyl or n-propyl;

R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy; and

R^4 is hydrogen, Br, R^aR^bN–, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl,  ,  ,  , or  ;

R^a and R^b are independently hydrogen, C_1-C_3alkyl, $H_2NCH_2CH_2$–, $(CH_3)_3COC(O)NHCH_2CH_2$–, or $CH_3CH_2CH_2NHCH_2CH_2$–.

[0020] In some embodiments, the compound of Formula (I) has the structure:


, or a pharmaceutically acceptable salt thereof.

[0021] In some embodiments, the pharmaceutical composition comprises from about 5 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the pharmaceutical composition comprises from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the pharmaceutical composition comprises from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the pharmaceutical composition comprises from about 100 mg to about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[0022] In some embodiments, the pharmaceutical composition comprises about 5 mg, about 10 mg, about 15 mg, about 20 mg, 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265

mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg about 500 mg, or about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

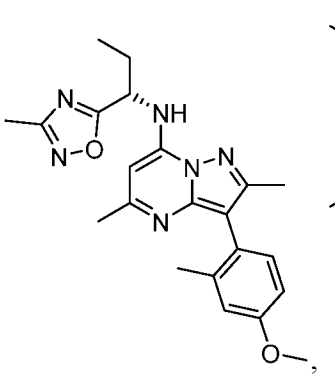
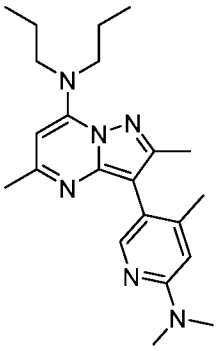
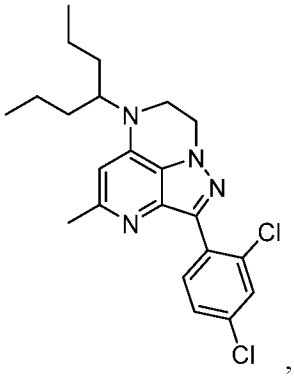
[0023] In some embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg/day to about 1000 mg/day. In some embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg/day to about 25 mg/day. In some embodiments, the pharmaceutical composition is administered at a dose of from about 10 mg/day to about 400 mg/day. In some embodiments, the pharmaceutical composition is administered at a dose of from about 100 mg/day to about 600 mg/day.

[0024] In some embodiments, the pharmaceutical composition is administered orally. In some embodiments, the pharmaceutical composition is formulated as capsule or a tablet. In some embodiments, the pharmaceutical composition is administered in the fed state. In some embodiments, the pharmaceutical composition is administered in the fasted state. In some embodiments, the pharmaceutical composition is administered at least once per day.

[0025] In some embodiments, the pharmaceutical composition is administered at bedtime. In some embodiments, the pharmaceutical composition is administered less than about 4 hours before sleep. In some embodiments, the pharmaceutical composition is administered less than about 2 hours before sleep. In some embodiments, the pharmaceutical composition is administered less than about 30 minutes before sleep. In some embodiments, the pharmaceutical composition is administered in the evening. In some embodiments, the pharmaceutical composition is administered at about 10 pm at night.

[0026] In some embodiments, the method further comprises administering to the subject an additional chemotherapeutic agent. In some embodiments, the additional chemotherapeutic agent is a glucocorticoid, a mineralocorticoid, an ACAT1 inhibitor, or an anti-androgen. In some embodiments, the glucocorticoid is beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone. In some embodiments, the mineralocorticoid is fludrocortisone.

[0027] In some embodiments, the additional chemotherapeutic agent is another CRF_1 antagonist. In some embodiments, the CRF_1 antagonist is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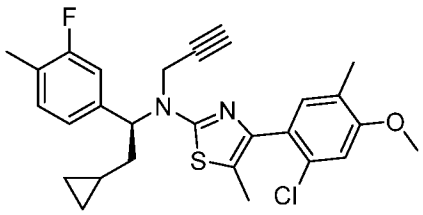
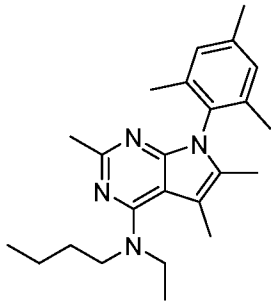
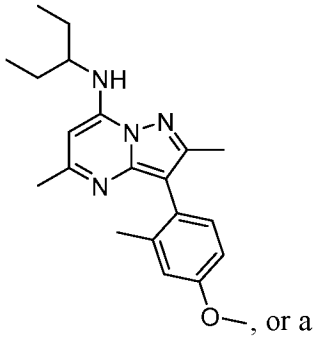
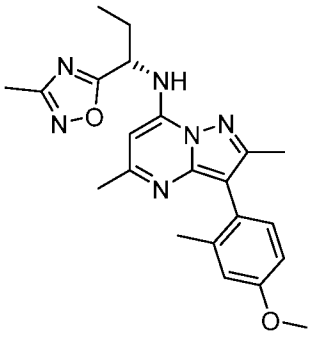


, or

, or a pharmaceutically acceptable salt thereof.

[0028] In some embodiments, the CRF_1 antagonist is


, or a pharmaceutically acceptable salt thereof.

[0029] In some embodiments, the method further comprises an additional treatment selected from surgical resection of the tumors and radiation therapy, or a combination thereof.

[0030] In some embodiments, the additional therapy is surgical resection and the surgical resection is prior to, after, and/or concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition, or any combination thereof.

[0031] In some embodiments, the surgical resection is prior to administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In some embodiments, the surgical resection is after administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In some embodiments, the surgical resection is concurrent with

administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

[0032] In some embodiments, the additional treatment is radiation therapy and the radiation therapy is prior to, after, and/or concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition, or any combination thereof. In some embodiments, the radiation therapy is prior to administration of the CRF_1 antagonist of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

[0033] In some embodiments, the radiation therapy is after administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In some embodiments, the radiation therapy is concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

[0034] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0035] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also "Figure" and "FIG." herein), of which:

[0037] FIG. 1A-1B. A schematic representation of intra-patient dose escalation study to evaluate safety, pharmacokinetics, and pharmacodynamics **(A).** A schematic representation of inhibition of CRF_1 with a CRF_1 antagonist (Compound 1) **(B)**.

[0038] FIG. 2. Graphic representation of pharmacokinetics (dose-proportional, adequate exposure at safe doses) in patients with CAH following 14-days of once daily dosing at each dose level with a CRF_1 antagonist (Compound 1).

[0039] FIG. 3. Graphic representation of pharmacokinetics (dose-proportional, adequate exposure at safe doses) in patients with CAH following 14-days of once daily dosing at each dose level with a CRF_1 antagonist (Compound 1).

[0040] FIG. 4. Graphic representation of patient-level response (measuring ACTH levels) to a CRF_1 antagonist (Compound 1).

[0041] FIG. 5A-5B. Graphic representation of patient-level response of sample responders (measuring ACTH levels) to a CRF_1 antagonist [Subject 003001 **(A)**; Subject 007002 **(B)**].

[0042] FIG. 6. Graphic representation of patient-level response (measuring 17-OHP levels) to a CRF_1 antagonist (Compound 1).

[0043] FIG. 7A-7B. Graphic representation of patient-level response of sample responders (measuring 17-OHP levels) to a CRF_1 antagonist [Subject 003001 **(A)**; Subject 007002 **(B)**].

[0044] FIG. 8. Graphic representation of patient-level response (measuring androstenedione levels) to a CRF_1 antagonist (Compound 1).

[0045] FIG. 9A-9B. Graphic representation of patient-level response of sample responders (measuring androstenedione levels) to a CRF_1 antagonist (Compound 1) [Subject 003001 **(A)**; Subject 007002 **(B)**].

[0046] FIG. 10A-10B. Images of a patient with TART pre-treatment with a CRF_1 antagonist (Compound 1) **(A)** and 6-weeks post-treatment with a CRF_1 antagonist (Compound 1) **(B)**.

[0047] FIG. 11A-11B. Graphic representation of an *in vitro* binding assay of a CRF_1 antagonist (Compound 1); ^{125}I-sauvagine binding to rat cerebellar membranes one hour after oral administration of increasing Compound 1 doses **(A)** and *in vivo* inhibition of CRF-induced activity after treatment with a CRF_1 antagonist; Compound 1 (oral, 10 mg/kg) **(B)**.

[0048] FIG. 12A-12B. Graphic representation of ACTH levels **(A)** and 17-OHP levels **(B)** after treatment with a CRF_1 antagonist (Compound 1).

[0049] FIG. 13. Graphic representation of hormone responses after treatment with a CRF_1 antagonist (Compound 1).

[0050] FIG. 14 depicts the proof of concept and escalating QD dosing at 10 pm.

[0051] FIG. 15A-15D are graphic representations of changes in androstenedione seen in participants in Cohort A and Cohort B.

[0052] FIG. 16A-16D are graphic representations of changes in 17-hydroxyprogesterone seen in participants in Cohort A and Cohort B.

[0053] FIG. 17A-17D are graphic representations of changes in adrenocorticotropic hormone seen in participants in Cohort A and Cohort B.

DETAILED DESCRIPTION

[0054] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

I. Definitions

[0055] The abbreviations used herein have their conventional meaning within the chemical and biological arts. The chemical structures and formulae set forth herein are constructed according to the standard rules of chemical valency known in the chemical arts.

[0056] Where substituent groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, e.g., $-CH_2O-$ is equivalent to $-OCH_2-$.

[0057] The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a straight (i.e., unbranched) or branched carbon chain (or carbon), or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include mono-, di- and multivalent radicals, having the number of carbon atoms designated (i.e., C_1-C_{10} means one to ten carbons). Alkyl is an uncyclized chain. Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, (cyclohexyl)methyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butynyl, and the higher homologs and isomers. An alkoxy is an alkyl attached to the remainder of the molecule via an oxygen linker (-O-).

[0058] The term "alkylene," by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from an alkyl, as exemplified, but not limited by, $-CH_2CH_2CH_2CH_2-$. Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred herein. A "lower alkyl" or "lower alkylene" is a shorter chain alkyl or alkylene group, generally having eight or fewer

carbon atoms. The term "alkenylene," by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from an alkene.

[0059] The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or combinations thereof, including at least one carbon atom and at least one heteroatom (e.g., O, N, P, Si, and S), and wherein the nitrogen and sulfur atoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) (e.g., N, S, Si, or P) may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. Heteroalkyl is an uncyclized chain. Examples include, but are not limited to: $-CH_2-CH_2-O-CH_3$, $-CH_2-CH_2-NH-CH_3$, $-CH_2-CH_2-N(CH_3)-CH_3$, $-CH_2-S-CH_2-CH_3$, $-CH_2-CH_2$, $-S(O)-CH_3$, $-CH_2-CH_2-S(O)_2-CH_3$, $-CH=CH-O-CH_3$, $-Si(CH_3)_3$, $-CH_2-CH=N-OCH_3$, $-CH=CH-N(CH_3)-CH_3$, $-O-CH_3$, $-O-CH_2-CH_3$, and -CN. Up to two or three heteroatoms may be consecutive, such as, for example, $-CH_2-NH-OCH_3$ and $-CH_2-O-Si(CH_3)_3$. A heteroalkyl moiety may include one heteroatom (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include two optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include three optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include four optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include five optionally different heteroatoms (e.g., O, N, S, Si, or P). A heteroalkyl moiety may include up to 8 optionally different heteroatoms (e.g., O, N, S, Si, or P).

[0060] Similarly, the term "heteroalkylene," by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from heteroalkyl, as exemplified, but not limited by, $-CH_2-CH_2-S-CH_2-CH_2-$ and $-CH_2-S-CH_2-CH_2-NH-CH_2-$. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (e.g., alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula $-C(O)_2R'-$ represents both $-C(O)_2R'-$ and $-R'C(O)_2-$. As described above, heteroalkyl groups, as used herein, include those groups that are attached to the remainder of the molecule through a heteroatom, such as -C(O)R', -C(O)NR', -NR'R", -OR', -SR', and/or $-SO_2R'$. Where "heteroalkyl" is recited, followed by recitations of specific heteroalkyl groups, such as -NR'R" or the like, it will be understood that the terms heteroalkyl and -NR'R" are not redundant or mutually exclusive. Rather, the specific heteroalkyl groups are recited to add clarity. Thus, the term "heteroalkyl" should not be interpreted herein as excluding specific heteroalkyl groups, such as -NR'R" or the like.

[0061] The terms "cycloalkyl" and "heterocycloalkyl," by themselves or in combination with other terms, mean, unless otherwise stated, cyclic versions of "alkyl" and "heteroalkyl,"

respectively. Cycloalkyl and heterocycloalkyl are not aromatic. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like. A "cycloalkylene" and a "heterocycloalkylene," alone or as part of another substituent, means a divalent radical derived from a cycloalkyl and heterocycloalkyl, respectively. "Cycloalkyl" is also meant to refer to bicyclic and polycyclic hydrocarbon rings such as, for example, bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane, etc.

[0062] The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as "haloalkyl" are meant to include monohaloalkyl and polyhaloalkyl. For example, the term "halo(C_1-C_4)alkyl" includes, but is not limited to, fluoromethyl, difluoromethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

[0063] The term "acyl" means, unless otherwise stated, -C(O)R where R is a substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

[0064] The term "aryl" means, unless otherwise stated, a polyunsaturated, aromatic, hydrocarbon substituent, which can be a single ring or multiple rings (preferably from 1 to 3 rings) that are fused together (i.e., a fused ring aryl) or linked covalently. A fused ring aryl refers to multiple rings fused together wherein at least one of the fused rings is an aryl ring. The term "heteroaryl" refers to aryl groups (or rings) that contain at least one heteroatom such as N, O, or S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. Thus, the term "heteroaryl" includes fused ring heteroaryl groups (i.e., multiple rings fused together wherein at least one of the fused rings is a heteroaromatic ring). A 5,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 5 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. Likewise, a 6,6-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 6 members, and wherein at least one ring is a heteroaryl ring. And a 6,5-fused ring heteroarylene refers to two rings fused together, wherein one ring has 6 members and the other ring has 5 members, and wherein at least one ring is a heteroaryl ring. A heteroaryl group can be attached to the remainder of the molecule through a carbon or heteroatom. Non-

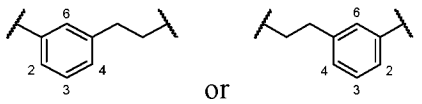
limiting examples of aryl and heteroaryl groups include phenyl, naphthyl, pyrrolyl, pyrazolyl, pyridazinyl, triazinyl, pyrimidinyl, imidazolyl, pyrazinyl, purinyl, oxazolyl, isoxazolyl, thiazolyl, furyl, thienyl, pyridyl, pyrimidyl, benzothiazolyl, benzoxazoyl benzimidazolyl, benzofuran, isobenzofuranyl, indolyl, isoindolyl, benzothiophenyl, isoquinolyl, quinoxalinyl, quinolyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalinyl, 5-quinoxalinyl, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below. An "arylene" and a "heteroarylene," alone or as part of another substituent, mean a divalent radical derived from an aryl and heteroaryl, respectively. A heteroaryl group substituent may be -O- bonded to a ring heteroatom nitrogen.

[0065] Spirocyclic rings are two or more rings wherein adjacent rings are attached through a single atom. The individual rings within spirocyclic rings may be identical or different. Individual rings in spirocyclic rings may be substituted or unsubstituted and may have different substituents from other individual rings within a set of spirocyclic rings. Possible substituents for individual rings within spirocyclic rings are the possible substituents for the same ring when not part of spirocyclic rings (e.g. substituents for cycloalkyl or heterocycloalkyl rings). Spirocylic rings may be substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkyl or substituted or unsubstituted heterocycloalkylene and individual rings within a spirocyclic ring group may be any of the immediately previous list, including having all rings of one type (e.g. all rings being substituted heterocycloalkylene wherein each ring may be the same or different substituted heterocycloalkylene). When referring to a spirocyclic ring system, heterocyclic spirocyclic rings means a spirocyclic rings wherein at least one ring is a heterocyclic ring and wherein each ring may be a different ring. When referring to a spirocyclic ring system, substituted spirocyclic rings means that at least one ring is substituted and each substituent may optionally be different.

[0066] The symbol " " denotes the point of attachment of a chemical moiety to the remainder of a molecule or chemical formula.

[0067] The term "oxo," as used herein, means an oxygen that is double bonded to a carbon atom.

[0068] The term "alkylarylene" as an arylene moiety covalently bonded to an alkylene moiety (also referred to herein as an alkylene linker). In embodiments, the alkylarylene group has the formula:



[0069] An alkylarylene moiety may be substituted (e.g. with a substituent group) on the alkylene moiety or the arylene linker (e.g. at carbons 2, 3, 4, or 6) with halogen, oxo, $-N_3$, $-CF_3$, $-CCl_3$, $-CBr_3$, $-CI_3$, -CN, -CHO, -OH, $-NH_2$, -COOH, $-CONH_2$, $-NO_2$, -SH, $-SO_2CH_3$ $-SO_3H$, $-OSO_3H$, $-SO_2NH_2$, $-NHNH_2$, $-ONH_2$, $-NHC(O)NHNH_2$, substituted or unsubstituted C_1-C_5 alkyl or substituted or unsubstituted 2 to 5 membered heteroalkyl). In embodiments, the alkylarylene is unsubstituted.

[0070] Each of the above terms (e.g., "alkyl," "heteroalkyl," "cycloalkyl," "heterocycloalkyl," "aryl," and "heteroaryl") includes both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

[0071] Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be one or more of a variety of groups selected from, but not limited to, -OR', =O, =NR', =N-OR', -NR'R", -SR', -halogen, -SiR'R"R'", -OC(O)R', -C(O)R', $-CO_2R'$, -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R'", $-NR"C(O)_2R'$, -NR-C(NR'R"R'")=NR"", -NR-C(NR'R")=NR'", -S(O)R', $-S(O)_2R'$, $-S(O)_2NR'R"$, $-NRSO_2R'$, –NR'NR"R'", –ONR'R", –NR'C(O)NR"NR'"R"", -CN, $-NO_2$, $-NR'SO_2R"$, -NR'C(O)R", -NR'C(O)-OR", -NR'OR", in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R, R', R", R'", and R"" each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl (e.g., aryl substituted with 1-3 halogens), substituted or unsubstituted heteroaryl, substituted or unsubstituted alkyl, alkoxy, or thioalkoxy groups, or arylalkyl groups. When a compound described herein includes more than one R group, for example, each of the R groups is independently selected as are each R', R", R'", and R"" group when more than one of these groups is present. When R' and R" are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 4-, 5-, 6-, or 7-membered ring. For example, -NR'R" includes, but is not limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (e.g., $-CF_3$ and $-CH_2CF_3$) and acyl (e.g., $-C(O)CH_3$, $-C(O)CF_3$, $-C(O)CH_2OCH_3$, and the like).

[0072] Similar to the substituents described for the alkyl radical, substituents for the aryl and heteroaryl groups are varied and are selected from, for example: -OR', -NR'R", -SR', -halogen, -

SiR'R"R'", -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R'", -NR"C(O)$_2$R', -NR-C(NR'R"R'")=NR"", -NR-C(NR'R")=NR'", -S(O)R', -S(O)$_2$R', -S(O)$_2$NR'R", -NRSO$_2$R', –NR'NR"R'", –ONR'R", –NR'C(O)NR"NR'"R"", -CN, -NO_2, -R', -N_3, -CH(Ph)$_2$, fluoro(C_1-C_4)alkoxy, and fluoro(C_1-C_4)alkyl, -NR'SO$_2$R", -NR'C(O)R", -NR'C(O)-OR", -NR'OR", in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R', R", R'", and R"" are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl. When a compound described herein includes more than one R group, for example, each of the R groups is independently selected as are each R', R", R'", and R"" groups when more than one of these groups is present.

[0073] Substituents for rings (e.g. cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene) may be depicted as substituents on the ring rather than on a specific atom of a ring (commonly referred to as a floating substituent). In such a case, the substituent may be attached to any of the ring atoms (obeying the rules of chemical valency) and in the case of fused rings or spirocyclic rings, a substituent depicted as associated with one member of the fused rings or spirocyclic rings (a floating substituent on a single ring), may be a substituent on any of the fused rings or spirocyclic rings (a floating substituent on multiple rings). When a substituent is attached to a ring, but not a specific atom (a floating substituent), and a subscript for the substituent is an integer greater than one, the multiple substituents may be on the same atom, same ring, different atoms, different fused rings, different spirocyclic rings, and each substituent may optionally be different. Where a point of attachment of a ring to the remainder of a molecule is not limited to a single atom (a floating substituent), the attachment point may be any atom of the ring and in the case of a fused ring or spirocyclic ring, any atom of any of the fused rings or spirocyclic rings while obeying the rules of chemical valency. Where a ring, fused rings, or spirocyclic rings contain one or more ring heteroatoms and the ring, fused rings, or spirocyclic rings are shown with one more floating substituents (including, but not limited to, points of attachment to the remainder of the molecule), the floating substituents may be bonded to the heteroatoms. Where the ring heteroatoms are shown bound to one or more hydrogens (e.g. a ring nitrogen with two bonds to ring atoms and a third bond to a hydrogen) in the structure or formula with the floating substituent, when the heteroatom is bonded to the floating substituent, the substituent will be understood to replace the hydrogen, while obeying the rules of chemical valency.

[0074] Two or more substituents may optionally be joined to form aryl, heteroaryl, cycloalkyl, or heterocycloalkyl groups. Such so-called ring-forming substituents are typically, though not

necessarily, found attached to a cyclic base structure. In one embodiment, the ring-forming substituents are attached to adjacent members of the base structure. For example, two ring-forming substituents attached to adjacent members of a cyclic base structure create a fused ring structure. In another embodiment, the ring-forming substituents are attached to a single member of the base structure. For example, two ring-forming substituents attached to a single member of a cyclic base structure create a spirocyclic structure. In yet another embodiment, the ring-forming substituents are attached to non-adjacent members of the base structure.

[0075] Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally form a ring of the formula -T-C(O)-$(CRR')_q$-U-, wherein T and U are independently -NR-, -O-, -CRR'-, or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -A-$(CH_2)_r$-B-, wherein A and B are independently -CRR'-, -O-, -NR-, -S-, -S(O) -, -$S(O)_2$-, -$S(O)_2$NR'-, or a single bond, and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -$(CRR')_s$-X'- $(C''R''R''')_d$-, where s and d are independently integers of from 0 to 3, and X' is -O-, -NR'-, -S-, -S(O)-, -$S(O)_2$-, or -$S(O)_2$NR'-. The substituents R, R', R'', and R''' are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

[0076] As used herein, the terms "heteroatom" or "ring heteroatom" are meant to include oxygen (O), nitrogen (N), sulfur (S), phosphorus (P), and silicon (Si).

[0077] A "substituent group," as used herein, means a group selected from the following moieties:

(A) oxo, halogen, -CF_3, -CN, -OH, -NH_2, -COOH, -$CONH_2$, -NO_2, -SH, -SO_3H, -SO_4H, -SO_2NH_2, –$NHNH_2$, –ONH_2, –NHC=(O)$NHNH_2$, –NHC=(O) NH_2, -$NHSO_2H$, -NHC= (O)H, -NHC(O)-OH, -NHOH, -OCF_3, -$OCHF_2$, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(B) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from:

(i) oxo, halogen, -CF_3, -CN, -OH, -NH_2, -COOH, -$CONH_2$, -NO_2, -SH, -SO_3H, -SO_4H, -SO_2NH_2, –$NHNH_2$, –ONH_2, –NHC=(O)$NHNH_2$, –NHC=(O) NH_2, -$NHSO_2H$, -NHC= (O)H, -NHC(O)-OH, -NHOH, -OCF_3, -$OCHF_2$, unsubstituted alkyl, unsubstituted heteroalkyl,

unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(ii) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from:

(a) oxo, halogen, $-CF_3$, -CN, -OH, $-NH_2$, -COOH, $-CONH_2$, $-NO_2$, -SH, $-SO_3H$, $-SO_4H$, $-SO_2NH_2$, $-NHNH_2$, $-ONH_2$, $-NHC(=O)NHNH_2$, $-NHC(=O)NH_2$, $-NHSO_2H$, -NHC=(O)H, -NHC(O)-OH, -NHOH, $-OCF_3$, $-OCHF_2$, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(b) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted with at least one substituent selected from: oxo, halogen, $-CF_3$, -CN, -OH, $-NH_2$, -COOH, $-CONH_2$, $-NO_2$, -SH, $-SO_3H$, $-SO_4H$, $-SO_2NH_2$, $-NHNH_2$, $-ONH_2$, $-NHC(=O)NHNH_2$, $-NHC(=O)NH_2$, $-NHSO_2H$, -NHC=(O)H, -NHC(O)-OH, -NHOH, $-OCF_3$, $-OCHF_2$, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl.

[0078] A "size-limited substituent" or " size-limited substituent group," as used herein, means a group selected from all of the substituents described above for a "substituent group," wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C_1-C_{20} alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_8 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 8 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 10 membered heteroaryl.

[0079] A "lower substituent" or " lower substituent group," as used herein, means a group selected from all of the substituents described above for a "substituent group," wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C_1-C_8 alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_7 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 7 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 9 membered heteroaryl.

[0080] In some embodiments, each substituted group described in the compounds herein is substituted with at least one substituent group. More specifically, in some embodiments, each substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene described in the compounds herein are substituted with at least one substituent group. In other embodiments, at least one or all of these groups are substituted with at least one size-limited substituent group. In other embodiments, at least one or all of these groups are substituted with at least one lower substituent group.

[0081] In other embodiments of the compounds herein, each substituted or unsubstituted alkyl may be a substituted or unsubstituted C_1-C_{20} alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_8 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 8 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and/or each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 10 membered heteroaryl. In some embodiments of the compounds herein, each substituted or unsubstituted alkylene is a substituted or unsubstituted C_1-C_{20} alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 20 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C_3-C_8 cycloalkylene, each substituted or unsubstituted heterocycloalkylene is a substituted or unsubstituted 3 to 8 membered heterocycloalkylene, each substituted or unsubstituted arylene is a substituted or unsubstituted C_6-C_{10} arylene, and/or each substituted or unsubstituted heteroarylene is a substituted or unsubstituted 5 to 10 membered heteroarylene.

[0082] In some embodiments, each substituted or unsubstituted alkyl is a substituted or unsubstituted C_1-C_8 alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C_3-C_7 cycloalkyl, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 7 membered heterocycloalkyl, each substituted or unsubstituted aryl is a substituted or unsubstituted C_6-C_{10} aryl, and/or each substituted or unsubstituted heteroaryl is a substituted or unsubstituted 5 to 9 membered heteroaryl. In some embodiments, each substituted or unsubstituted alkylene is a substituted or unsubstituted C_1-C_8 alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 8 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C_3-C_7 cycloalkylene, each substituted or unsubstituted heterocycloalkylene is a

substituted or unsubstituted 3 to 7 membered heterocycloalkylene, each substituted or unsubstituted arylene is a substituted or unsubstituted C_6-C_{10} arylene, and/or each substituted or unsubstituted heteroarylene is a substituted or unsubstituted 5 to 9 membered heteroarylene. In some embodiments, the compound is a chemical species set forth in the Examples section, figures, or tables below.

[0083] Certain compounds of the present invention possess asymmetric carbon atoms (optical or chiral centers) or double bonds; the enantiomers, racemates, diastereomers, tautomers, geometric isomers, stereoisometric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)- or, as (D)- or (L)- for amino acids, and individual isomers are encompassed within the scope of the present invention. The compounds of the present invention do not include those that are known in art to be too unstable to synthesize and/or isolate. The present invention is meant to include compounds in racemic and optically pure forms. Optically active (R)- and (S)-, or (D)- and (L)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers.

[0084] As used herein, the term "isomers" refers to compounds having the same number and kind of atoms, and hence the same molecular weight, but differing in respect to the structural arrangement or configuration of the atoms.

[0085] The term "tautomer," as used herein, refers to one of two or more structural isomers which exist in equilibrium and which are readily converted from one isomeric form to another.

[0086] It will be apparent to one skilled in the art that certain compounds of this invention may exist in tautomeric forms, all such tautomeric forms of the compounds being within the scope of the invention.

[0087] Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of the invention.

[0088] Unless otherwise stated, structures depicted herein are also meant to include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon by ^{13}C- or ^{14}C-enriched carbon are within the scope of this invention.

[0089] The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the

compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^{3}H), iodine-125 (^{125}I), or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are encompassed within the scope of the present invention.

[0090] It should be noted that throughout the application that alternatives are written in Markush groups, for example, each amino acid position that contains more than one possible amino acid. It is specifically contemplated that each member of the Markush group should be considered separately, thereby comprising another embodiment, and the Markush group is not to be read as a single unit.

[0091] "Analog" or "analogue" is used in accordance with its plain ordinary meaning within Chemistry and Biology and refers to a chemical compound that is structurally similar to another compound (i.e., a so-called "reference" compound) but differs in composition, e.g., in the replacement of one atom by an atom of a different element, or in the presence of a particular functional group, or the replacement of one functional group by another functional group, or the absolute stereochemistry of one or more chiral centers of the reference compound. Accordingly, an analog is a compound that is similar or comparable in function and appearance but not in structure or origin to a reference compound.

[0092] The terms "a" or "an," as used in herein means one or more. In addition, the phrase "substituted with a[n]," as used herein, means the specified group may be substituted with one or more of any or all of the named substituents. For example, where a group, such as an alkyl or heteroaryl group, is "substituted with an unsubstituted C_1-C_{20} alkyl, or unsubstituted 2 to 20 membered heteroalkyl," the group may contain one or more unsubstituted C_1-C_{20} alkyls, and/or one or more unsubstituted 2 to 20 membered heteroalkyls.

[0093] Moreover, where a moiety is substituted with an R substituent, the group may be referred to as "R-substituted." Where a moiety is R-substituted, the moiety is substituted with at least one R substituent and each R substituent is optionally different. Where a particular R group is present in the description of a chemical genus (such as Formula (I)), a Roman alphabetic symbol may be used to distinguish each appearance of that particular R group. For example, where multiple R^{13} substituents are present, each R^{13} substituent may be distinguished as R^{13A}, R^{13B}, R^{13C}, R^{13D}, etc., wherein each of R^{13A}, R^{13B}, R^{13C}, R^{13D}, etc. is defined within the scope of the definition of R^{13} and optionally differently.

[0094] A "detectable moiety" as used herein refers to a moiety that can be covalently or noncovalently attached to a compound or biomolecule that can be detected for instance, using techniques known in the art. In embodiments, the detectable moiety is covalently attached. The detectable moiety may provide for imaging of the attached compound or biomolecule. The detectable moiety may indicate the contacting between two compounds. Exemplary detectable

moieties are fluorophores, antibodies, reactive dies, radio-labeled moieties, magnetic contrast agents, and quantum dots. Exemplary fluorophores include fluorescein, rhodamine, GFP, coumarin, FITC, Alexa fluor, Cy3, Cy5, BODIPY, and cyanine dyes. Exemplary radionuclides include Fluorine-18, Gallium-68, and Copper-64. Exemplary magnetic contrast agents include gadolinium, iron oxide and iron platinum, and manganese.

[0095] Descriptions of compounds of the present invention are limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to give compounds which are not inherently unstable and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions, such as aqueous, neutral, and several known physiological conditions. For example, a heterocycloalkyl or heteroaryl is attached to the remainder of the molecule via a ring heteroatom in compliance with principles of chemical bonding known to those skilled in the art thereby avoiding inherently unstable compounds.

[0096] The term "pharmaceutically acceptable salts" is meant to include salts of the active compounds that are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, oxalic, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (*see*, for example, Berge *et al.*, "Pharmaceutical Salts", *Journal of Pharmaceutical Science*, **1977**, *66*, 1-19). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

[0097] Thus, the compounds of the present invention may exist as salts, such as with pharmaceutically acceptable acids. The present invention includes such salts. Non-limiting examples of such salts include hydrochlorides, hydrobromides, phosphates, sulfates, methanesulfonates, nitrates, maleates, acetates, citrates, fumarates, proprionates, tartrates (e.g., (+)-tartrates, (-)-tartrates, or mixtures thereof including racemic mixtures), succinates, benzoates, and salts with amino acids such as glutamic acid, and quaternary ammonium salts (e.g. methyl iodide, ethyl iodide, and the like). These salts may be prepared by methods known to those skilled in the art.

[0098] The neutral forms of the compounds are preferably regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound may differ from the various salt forms in certain physical properties, such as solubility in polar solvents. In embodiments, compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts. The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in a conventional manner. The parent form of the compounds differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but, unless specifically indicated, the salts disclosed herein are equivalent to the parent form of the compound for the purposes of the present invention.

[0099] In addition to salt forms, the present invention provides compounds, which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present invention. Prodrugs of the compounds described herein may be converted *in vivo* after administration. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an *ex vivo* environment, such as, for example, when contacted with a suitable enzyme or chemical reagent.

[0100] Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

[0101] "Pharmaceutically acceptable excipient" and "pharmaceutically acceptable carrier" refer to a substance that aids the administration of a compound to and absorption by a subject and can be included in the compositions of the present invention without causing a significant adverse toxicological effect on the patient. Non-limiting examples of pharmaceutically acceptable

excipients include water, NaCl, normal saline solutions, lactated Ringer's, normal sucrose, normal glucose, binders, fillers, disintegrants, lubricants, coatings, sweeteners, flavors, salt solutions (such as Ringer's solution), alcohols, oils, gelatins, carbohydrates such as lactose, amylose or starch, fatty acid esters, hydroxymethycellulose, polyvinyl pyrrolidine, and colors, and the like. Such preparations can be sterilized and, if desired, mixed with auxiliary agents such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, and/or aromatic substances and the like that do not deleteriously react with the compounds of the invention. One of skill in the art will recognize that other pharmaceutical excipients are useful in the present invention.

[0102] The term "preparation" is intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component with or without other carriers, is surrounded by a carrier, which is thus in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be used as solid dosage forms suitable for oral administration.

[0103] A "CRF1 inhibitor" refers to a compound (e.g., compounds described herein) that reduces the activity of CRF1 when compared to a control, such as absence of the compound or a compound with known inactivity.

[0104] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues, wherein the polymer may optionally be conjugated to a moiety that does not consist of amino acids. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer. In embodiments, the terms "polypeptide," "peptide," and "protein", used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include genetically coded and non-genetically coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified polypeptide backbones. The terms include fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence; fusion proteins with heterologous and homologous leader sequences, with or without N-terminus methionine residues; immunologically tagged proteins; and the like.

[0105] A polypeptide, or a cell is "recombinant" when it is artificial or engineered, or derived from or contains an artificial or engineered protein or nucleic acid (*e.g.* non-natural or not wild type). For example, a polynucleotide that is inserted into a vector or any other heterologous location, *e.g.*, in a genome of a recombinant organism, such that it is not associated with nucleotide sequences that normally flank the polynucleotide as it is found in nature is a

recombinant polynucleotide. A protein expressed *in vitro* or *in vivo* from a recombinant polynucleotide is an example of a recombinant polypeptide. Likewise, a polynucleotide sequence that does not appear in nature, for example a variant of a naturally occurring gene, is recombinant.

[0106] "Contacting" is used in accordance with its plain ordinary meaning and refers to the process of allowing at least two distinct species (e.g. chemical compounds including biomolecules or cells) to become sufficiently proximal to react, interact or physically touch. It should be appreciated; however, the resulting reaction product can be produced directly from a reaction between the added reagents or from an intermediate from one or more of the added reagents that can be produced in the reaction mixture.

[0107] The term "contacting" may include allowing two species to react, interact, or physically touch, wherein the two species may be a compound as described herein and a protein or enzyme. In some embodiments contacting includes allowing a compound described herein to interact with a protein or enzyme that is involved in a signaling pathway (e.g., MAP kinase pathway).

[0108] As defined herein, the term "activation", "activate", "activating" and the like in reference to a protein refers to conversion of a protein into a biologically active derivative from an initial inactive or deactivated state. The terms reference activation, or activating, sensitizing, or up-regulating signal transduction or enzymatic activity or the amount of a protein decreased in a disease.

[0109] The terms "agonist," "activator," "upregulator," etc. refer to a substance capable of detectably increasing the expression or activity of a given gene or protein. The agonist can increase expression or activity 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in comparison to a control in the absence of the agonist. In certain instances, expression or activity is 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or higher than the expression or activity in the absence of the agonist. In embodiments, an agonist is a molecule that interacts with a target to cause or promote an increase in the activation of the target. In embodiments, activators are molecules that increase, activate, facilitate, enhance activation, sensitize, or up-regulate, e.g., a gene, protein, ligand, receptor, or cell.

[0110] As defined herein, the term "inhibition", "inhibit", "inhibiting" and the like in reference to a protein-inhibitor interaction means negatively affecting (e.g. decreasing) the activity or function of the protein relative to the activity or function of the protein in the absence of the inhibitor. In embodiments inhibition means negatively affecting (e.g. decreasing) the concentration or levels of the protein relative to the concentration or level of the protein in the absence of the inhibitor. In embodiments inhibition refers to reduction of a disease or symptoms of disease. In embodiments, inhibition refers to a reduction in the activity of a particular protein

target. Thus, inhibition includes, at least in part, partially or totally blocking stimulation, decreasing, preventing, or delaying activation, or inactivating, desensitizing, or down-regulating signal transduction or enzymatic activity or the amount of a protein. In embodiments, inhibition refers to a reduction of activity of a target protein resulting from a direct interaction (e.g. an inhibitor binds to the target protein). In embodiments, inhibition refers to a reduction of activity of a target protein from an indirect interaction (e.g. an inhibitor binds to a protein that activates the target protein, thereby preventing target protein activation).

[0111] The terms "inhibitor," "repressor" or "antagonist" or "downregulator" interchangeably refer to a substance capable of detectably decreasing the expression or activity of a given gene or protein. The antagonist can decrease expression or activity 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in comparison to a control in the absence of the antagonist. In certain instances, expression or activity is 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or lower than the expression or activity in the absence of the antagonist. An antagonist prevents, reduces, inhibits, or neutralizes the activity of an agonist, and an antagonist can also prevent, inhibit, or reduce constitutive activity of a target, e.g., a target receptor, even where there is no identified agonist. In embodiments, inhibitors are molecules that decrease, block, prevent, delay activation, inactivate, desensitize, or down-regulate, e.g., a gene, protein, ligand, receptor, or cell. An inhibitor may also be defined as a molecule that reduces, blocks, or inactivates a constitutive activity. An "antagonist" is a molecule that opposes the action(s) of an agonist.

[0112] The terms "corticotropin-releasing factor type-1," "CRF_1," "corticotropin-releasing hormone receptor 1," and "CHHR1" refer to a protein (including homologs, isoforms, and functional fragments thereof) and is a receptor for corticotropin releasing-hormone. The terms include any recombinant or naturally-occurring form of CRF_1 variants thereof that maintain CRF_1 activity (e.g. within at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% activity compared to wildtype CRF1). The terms include any mutant form of CRF_1 variants (e.g., frameshift mutations) thereof that maintain CRF_1 activity (e.g. within at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% activity compared to wildtype CRF_1). In embodiments, the CRF_1 protein encoded by the *CRHR1* gene has the amino acid sequence set forth in or corresponding to Entrez 1233, UniProt P34998.

[0113] The term "expression" includes any step involved in the production of the polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion. Expression can be detected using conventional techniques for detecting protein (*e.g.*, ELISA, Western blotting, flow cytometry, immunofluorescence, immunohistochemistry, *etc.*).

[0114] The terms "disease" or "condition" refer to a state of being or health status of a patient or subject capable of being treated with the compounds or methods provided herein. The disease may be a malignant or benign tumor (e.g., testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART)). The disease may be cancer. In some further instances, "cancer" refers to human cancers and carcinomas, sarcomas, adenocarcinomas, lymphomas, leukemias, etc., including solid and lymphoid cancers, kidney, breast, lung, bladder, colon, ovarian, prostate, pancreas, adrenal, stomach, brain, head and neck, skin, uterine, testicular, glioma, esophagus, and liver cancer, including hepatocarcinoma, lymphoma, including B-acute lymphoblastic lymphoma, non-Hodgkin's lymphomas (*e.g.*, Burkitt's, Small Cell, and Large Cell lymphomas), Hodgkin's lymphoma, leukemia (including MDS, AML, ALL, ATLL and CML), or multiple myeloma.

[0115] As used herein, the term "cancer" refers to all types of cancer, neoplasm or malignant tumors found in mammals (e.g. humans), including leukemia, carcinomas and sarcomas. Exemplary cancers that may be treated with a compound or method provided herein include adrenal cancer and tumors (e.g., testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART)) brain cancer, glioma, glioblastoma, neuroblastoma, prostate cancer, colorectal cancer, pancreatic cancer, cervical cancer, gastric cancer, ovarian cancer, lung cancer, and cancer of the head. Exemplary cancers that may be treated with a compound or method provided herein include cancer of the thyroid, endocrine system, brain, breast, cervix, colon, head & neck, liver, kidney, lung, non-small cell lung, melanoma, mesothelioma, ovary, sarcoma, stomach, uterus, Medulloblastoma, colorectal cancer, pancreatic cancer. Additional examples include, thyroid carcinoma, cholangiocarcinoma, pancreatic adenocarcinoma, skin cutaneous melanoma, colon adenocarcinoma, rectum adenocarcinoma, stomach adenocarcinoma, esophageal carcinoma, head and neck squamous cell carcinoma, breast invasive carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, glioma, glioblastoma multiforme, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine or exocrine pancreas, medullary thyroid cancer, medullary thyroid carcinoma, melanoma, colorectal cancer, papillary thyroid cancer, hepatocellular carcinoma, or prostate cancer.

[0116] The term "congenital adrenal hyperplasia" or "CAH" are any of several autosomal recessive diseases resulting from mutations of genes for enzymes mediating the biochemical

steps of production of mineralocorticoids, glucocorticoids or sex steroids from cholesterol by the adrenal glands (steroidogenesis). These conditions involve excessive or deficient production of sex steroids and can alter development of primary or secondary sex characteristics in some affected infants, children, or adults.

[0117] "Adrenal rest tumor" refers to a neoplasm of the testis (or ovaries) consisting of adrenal tissue. It may be secondary to hyperplasia of an adrenal gland.

[0118] "Testicular adrenal rest tumor(s)" or "TART" and/or ovarian adrenal rest tumor(s) are a complication of congenital adrenal hyperplasia (CAH), which may develop from ectopic remnants of intra-testicular adrenal tissue stimulated by adrenocorticotropic hormone (ACTH) hypersecretion. TART lesions are typically located within the rete testis and are bilateral, synchronous, nodular and multiple. TART can lead to testicular structural damage, spermatogenesis disorders, infertility and mass-forming lesions.

[0119] The term "leukemia" refers broadly to progressive, malignant diseases of the blood-forming organs and is generally characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. Leukemia is generally clinically classified on the basis of (1) the duration and character of the disease-acute or chronic; (2) the type of cell involved; myeloid (myelogenous), lymphoid (lymphogenous), or monocytic; and (3) the increase or non-increase in the number abnormal cells in the blood-leukemic or aleukemic (subleukemic). Exemplary leukemias that may be treated with a compound or method provided herein include, for example, acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemic leukemia, basophylic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, multiple myeloma, plasmacytic leukemia, promyelocytic leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, or undifferentiated cell leukemia.

[0120] The term "sarcoma" generally refers to a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Sarcomas that may be treated with a compound or method

provided herein include a chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, or telangiectaltic sarcoma.

[0121] The term "melanoma" is taken to mean a tumor arising from the melanocytic system of the skin and other organs. Melanomas that may be treated with a compound or method provided herein include, for example, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, nodular melanoma, subungal melanoma, or superficial spreading melanoma.

[0122] The term "carcinoma" refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas that may be treated with a compound or method provided herein include, for example, thyroid carcinoma, cholangiocarcinoma, pancreatic adenocarcinoma, skin cutaneous melanoma, colon adenocarcinoma, rectum adenocarcinoma, stomach adenocarcinoma, esophageal carcinoma, head and neck squamous cell carcinoma, breast invasive carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, medullary thyroid carcinoma, familial medullary thyroid carcinoma, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniforni carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypernephroid carcinoma,

infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, or carcinoma villosum.

[0123] The terms "treating" or "treatment" refer to any indicia of success in the therapy or amelioration of an injury, disease, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to the patient; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving a patient's physical or mental well-being. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of a physical examination, neuropsychiatric exams, and/or a psychiatric evaluation. The term "treating" and conjugations thereof, may include prevention of an injury, pathology, condition, or disease. In embodiments, treating is preventing. In embodiments, treating does not include preventing.

[0124] "Treating" or "treatment" as used herein (and as well-understood in the art) also broadly includes any approach for obtaining beneficial or desired results in a subject's condition, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of the extent of a disease, stabilizing (*i.e.*, not worsening) the state of disease, prevention of a disease's transmission or spread, delay or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission, whether partial or total and whether detectable or undetectable. In other words, "treatment" as used herein includes any cure, amelioration, or prevention of a disease. Treatment may prevent the disease from occurring; inhibit the disease's spread; relieve the disease's symptoms (*e.g.*, ocular pain, seeing

halos around lights, red eye, very high intraocular pressure), fully or partially remove the disease's underlying cause, shorten a disease's duration, or do a combination of these things.

[0125] "Treating" and "treatment" as used herein include prophylactic treatment. Treatment methods include administering to a subject a therapeutically effective amount of a compound described herein. The administering step may consist of a single administration or may include a series of administrations. The length of the treatment period depends on a variety of factors, such as the severity of the condition, the age of the patient, the concentration of the compound, the activity of the compositions used in the treatment, or a combination thereof. It will also be appreciated that the effective dosage of an agent used for the treatment or prophylaxis may increase or decrease over the course of a particular treatment or prophylaxis regime. Changes in dosage may result and become apparent by standard diagnostic assays known in the art. In some instances, chronic administration may be required. For example, the compositions are administered to the subject in an amount and for a duration sufficient to treat the patient.

[0126] The term "prevent" refers to a decrease in the occurrence of disease symptoms in a patient. As indicated above, the prevention may be complete (no detectable symptoms) or partial, such that fewer symptoms are observed than would likely occur absent treatment. In embodiments, prevent refers to slowing the progression of the disease, disorder or condition or inhibiting progression thereof to a harmful or otherwise undesired state.

[0127] "Patient" or "subject in need thereof" refers to a living organism suffering from or prone to a disease or condition that can be treated by administration of a pharmaceutical composition as provided herein. Non-limiting examples include humans, other mammals, bovines, rats, mice, dogs, monkeys, goat, sheep, cows, deer, and other non-mammalian animals. In some embodiments, a patient is human.

[0128] An "effective amount" is an amount sufficient for a compound to accomplish a stated purpose relative to the absence of the compound (e.g., achieve the effect for which it is administered, treat a disease, reduce enzyme activity, increase enzyme activity, reduce a signaling pathway, or reduce one or more symptoms of a disease or condition). An example of an "effective amount" is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which could also be referred to as a "therapeutically effective amount." A "reduction" of a symptom or symptoms (and grammatical equivalents of this phrase) means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). A "prophylactically effective amount" of a drug is an amount of a drug that, when administered to a subject, will have the intended prophylactic effect, e.g., preventing or delaying the onset (or reoccurrence) of an injury, disease, pathology or condition, or reducing the likelihood of the onset (or reoccurrence) of an injury, disease, pathology, or

condition, or their symptoms. The full prophylactic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a prophylactically effective amount may be administered in one or more administrations. An "activity decreasing amount," as used herein, refers to an amount of antagonist required to decrease the activity of an enzyme relative to the absence of the antagonist. A "function disrupting amount," as used herein, refers to the amount of antagonist required to disrupt the function of an enzyme or protein relative to the absence of the antagonist. The exact amounts will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*see*, *e.g.*, Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); Pickar, *Dosage Calculations* (1999); and *Remington: The Science and Practice of Pharmacy*, 20th Edition, 2003, Gennaro, Ed., Lippincott, Williams & Wilkins). The therapeutically effective amount can be ascertained by measuring relevant physiological effects, and it can be adjusted in connection with the dosing regimen and diagnostic analysis of the subject's condition, and the like. By way of example, measurement of the serum level of a CRF1 inhibitor (or, e.g., a metabolite thereof) at a particular time post-administration may be indicative of whether a therapeutically effective amount has been administered.

[0129] For any compound described herein, the therapeutically effective amount can be initially determined from cell culture assays. Target concentrations will be those concentrations of active compound(s) that are capable of achieving the methods described herein, as measured using the methods described herein or known in the art.

[0130] Therapeutically effective amounts for use in humans can also be determined from animal models. For example, a dose for humans can be formulated to achieve a concentration that has been found to be effective in animals. The dosage in humans can be adjusted by monitoring compounds effectiveness and adjusting the dosage upwards or downwards, as described above. Adjusting the dose to achieve maximal efficacy in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan. Adjusting the dose to achieve maximal therapeutic window efficacy or toxicity in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan.

[0131] The term "therapeutically effective amount," as used herein, refers to that amount of the therapeutic agent sufficient to ameliorate the disorder, as described above. For example, for the given parameter, a therapeutically effective amount will show an increase or decrease of at least 5%, 10%, 15%, 20%, 25%, 40%, 50%, 60%, 75%, 80%, 90%, or at least 100%. Therapeutic efficacy can also be expressed as "-fold" increase or decrease. For example, a therapeutically

effective amount can have at least a 1.2-fold, 1.5-fold, 2-fold, 5-fold, or more effect over a control.

[0132] The terms "QD," "qd," and "q.d." refer to once a day, i.e., a drug or therapeutic agent is administered once a day for the duration of the therapeutic regimen.

[0133] The terms "BD," "bd," and "b.i.d." refer to twice a day, i.e., a drug or therapeutic agent is administered two times a day for the duration of the therapeutic regimen.

[0134] The terms "QID," "qid," and "q.i.d." refer to three times a day, i.e., a drug or therapeutic agent is administered three times a day for the duration of the therapeutic regimen.

[0135] Dosages may be varied depending upon the requirements of the patient and the compound being employed. The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial therapeutic response in the patient over time. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects. Determination of the proper dosage for a particular situation is within the skill of the practitioner. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under circumstances is reached. Dosage amounts and intervals can be adjusted individually to provide levels of the administered compound effective for the particular clinical indication being treated. This will provide a therapeutic regimen that is commensurate with the severity of the individual's disease state.

[0136] As used herein, the term "administering" means oral administration, administration as a suppository, topical contact, intravenous, parenteral, intraperitoneal, intramuscular, intralesional, intrathecal, intracranial, intranasal or subcutaneous administration, or the implantation of a slow-release device, *e.g.,* a mini-osmotic pump, to a subject. Administration is by any route, including parenteral and transmucosal (*e.g.,* buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, *e.g.,* intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, *etc.* By "co-administer" it is meant that a composition described herein is administered at the same time, just prior to, or just after the administration of one or more additional therapies (e.g. anti-cancer agent, chemotherapeutic, or treatment for a neurodegenerative disease). The compound of the invention can be administered alone or can be coadministered to the patient. Coadministration is meant to include simultaneous or sequential administration of the compound individually or in combination (more than one compound or agent). Thus, the preparations can also be combined, when desired, with other active substances (e.g. to reduce metabolic degradation). The compositions of the present invention can be

delivered by transdermally, by a topical route, formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols. Oral preparations include tablets, pills, powder, dragees, capsules, liquids, lozenges, cachets, gels, syrups, slurries, suspensions, etc., suitable for ingestion by the patient. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water/propylene glycol solutions. The compositions of the present invention may additionally include components to provide sustained release and/or comfort. Such components include high molecular weight, anionic mucomimetic polymers, gelling polysaccharides and finely-divided drug carrier substrates. These components are discussed in greater detail in U.S. Pat. Nos. 4,911,920; 5,403,841; 5,212,162; and 4,861,760. The entire contents of these patents are incorporated herein by reference in their entirety for all purposes. The compositions of the present invention can also be delivered as microspheres for slow release in the body. For example, microspheres can be administered via intradermal injection of drug-containing microspheres, which slowly release subcutaneously (see Rao, *J. Biomater Sci. Polym. Ed.* 7:623-645, 1995; as biodegradable and injectable gel formulations (see, e.g., Gao *Pharm. Res.* 12:857-863, 1995); or, as microspheres for oral administration (see, e.g., Eyles, *J. Pharm. Pharmacol.* 49:669-674, 1997). In another embodiment, the formulations of the compositions of the present invention can be delivered by the use of liposomes which fuse with the cellular membrane or are endocytosed, *i.e.,* by employing receptor ligands attached to the liposome, that bind to surface membrane protein receptors of the cell resulting in endocytosis. By using liposomes, particularly where the liposome surface carries receptor ligands specific for target cells, or are otherwise preferentially directed to a specific organ, one can focus the delivery of the compositions of the present invention into the target cells in vivo. (See, *e.g.*, Al-Muhammed, *J. Microencapsul.* 13:293-306, 1996; Chonn, *Curr. Opin. Biotechnol.* 6:698-708, 1995; Ostro, *Am. J. Hosp. Pharm.* 46:1576-1587, 1989). The compositions of the present invention can also be delivered as nanoparticles.

[0137] By "co-administer" it is meant that a composition described herein is administered at the same time, just prior to, or just after the administration of one or more additional therapies. The compounds of the invention can be administered alone or can be coadministered to the patient. Coadministration is meant to include simultaneous or sequential administration of the compounds individually or in combination (more than one compound). The compositions of the present invention can be delivered transdermally, by a topical route, or formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols.

[0138] For any compound described herein, the therapeutically effective amount can be initially determined from cell culture assays. Target concentrations will be those concentrations of active compound(s) that are capable of achieving the methods described herein, as measured using the methods described herein or known in the art.

[0139] Utilizing the teachings provided herein, an effective prophylactic or therapeutic treatment regimen can be planned that does not cause substantial toxicity and yet is effective to treat the clinical symptoms demonstrated by the particular patient. This planning should involve the careful choice of active compound by considering factors such as compound potency, relative bioavailability, patient body weight, presence and severity of adverse side effects, preferred mode of administration and the toxicity profile of the selected agent.

[0140] The compounds described herein can be used in combination with one another, with other active agents known to be useful in treating cancer (e.g. colon cancer), cardiovascular disease, metabolic disease, immune or inflammatory disease or disorder.

[0141] In some embodiments, co-administration includes administering one active agent within 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24 hours, 2 days, 4 days, 1 week or 1 month of a second active agent. Co-administration includes administering two active agents simultaneously, approximately simultaneously (e.g., within about 1, 5, 10, 15, 20, or 30 minutes of each other), or sequentially in any order. In some embodiments, co-administration can be accomplished by co-formulation, i.e., preparing a single pharmaceutical composition including both active agents. In other embodiments, the active agents can be formulated separately. In another embodiment, the active and/or adjunctive agents may be linked or conjugated to one another. In some embodiments, the compounds described herein may be combined with treatments for cancer (e.g. colon cancer), cardiovascular disease, metabolic disease, immune or inflammatory disease or disorder.

[0142] The compounds described herein can be administered to treat a fertility disease or disorder and/or cancer. In this regard, the compounds disclosed herein may be administered either alone to treat such diseases or disorders or may be co-administered with another therapeutic agent to treat such diseases or disorders.

[0143] The compounds disclosed herein may be co-administered with an agonist or antagonist of CRF_1 function. The compounds described herein can be co-administered with conventional anti-cancer/chemotherapeutic.

[0144] "Anti-cancer agent" is used in accordance with its plain ordinary meaning and refers to a composition (e.g. compound, drug, antagonist, inhibitor, modulator) having antineoplastic properties or the ability to inhibit the growth or proliferation of cells. In some embodiments, an anti-cancer agent is a chemotherapeutic. In some embodiments, an anti-cancer agent is an agent

identified herein having utility in methods of treating cancer. In some embodiments, an anti-cancer agent is an agent approved by the FDA or similar regulatory agency of a country other than the USA, for treating cancer. Examples of anti-cancer agents include, but are not limited to, MEK (e.g. MEK1, MEK2, or MEK1 and MEK2) inhibitors (e.g. XL518, CI-1040, PD035901, selumetinib/ AZD6244, GSK1120212/ trametinib, GDC-0973, ARRY-162, ARRY-300, AZD8330, PD0325901, U0126, PD98059, TAK-733, PD318088, AS703026, BAY 869766), alkylating agents (*e.g.,* cyclophosphamide, ifosfamide, chlorambucil, busulfan, melphalan, mechlorethamine, uramustine, thiotepa, nitrosoureas, nitrogen mustards (e.g., mechloroethamine, cyclophosphamide, chlorambucil, meiphalan), ethylenimine and methylmelamines (e.g., hexamethlymelamine, thiotepa), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine, lomusitne, semustine, streptozocin), triazenes (decarbazine)), anti-metabolites (*e.g.,* 5-azathioprine, leucovorin, capecitabine, fludarabine, gemcitabine, pemetrexed, raltitrexed, folic acid analog (e.g., methotrexate), or pyrimidine analogs (e.g., fluorouracil, floxouridine, Cytarabine), purine analogs (e.g., mercaptopurine, thioguanine, pentostatin), *etc.*), plant alkaloids (*e.g.,* vincristine, vinblastine, vinorelbine, vindesine, podophyllotoxin, paclitaxel, docetaxel, *etc.*), topoisomerase inhibitors (*e.g.,* irinotecan, topotecan, amsacrine, etoposide (VP16), etoposide phosphate, teniposide, *etc.*), antitumor antibiotics (*e.g.,* doxorubicin, adriamycin, daunorubicin, epirubicin, actinomycin, bleomycin, mitomycin, mitoxantrone, plicamycin, *etc.*), platinum-based compounds (*e.g.* cisplatin, oxaloplatin, carboplatin), anthracenedione (e.g., mitoxantrone), substituted urea (e.g., hydroxyurea), methyl hydrazine derivative (e.g., procarbazine), adrenocortical suppressant (e.g., mitotane, aminoglutethimide), epipodophyllotoxins (e.g., etoposide), antibiotics (e.g., daunorubicin, doxorubicin, bleomycin), enzymes (e.g., L-asparaginase), inhibitors of mitogen-activated protein kinase signaling (e.g. U0126, PD98059, PD184352, PD0325901, ARRY-142886, SB239063, SP600125, BAY 43-9006, wortmannin, or LY294002, Syk inhibitors, mTOR inhibitors, antibodies (e.g., rituxan), gossyphol, genasense, polyphenol E, Chlorofusin, all trans-retinoic acid (ATRA), bryostatin, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), 5-aza-2'-deoxycytidine, all trans retinoic acid, doxorubicin, vincristine, etoposide, gemcitabine, imatinib (Gleevec.RTM.), geldanamycin, 17-N-Allylamino-17-Demethoxygeldanamycin (17-AAG), flavopiridol, LY294002, bortezomib, trastuzumab, BAY 11-7082, PKC412, PD184352, 20-epi-1, 25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense

oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; 9-dioxamycin; diphenyl spiromustine; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone;

miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylerie conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen-binding protein; sizofuran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem

cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; zinostatin stimalamer, Adriamycin, Dactinomycin, Bleomycin, Vinblastine, Cisplatin, acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; fluorocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; iimofosine; interleukin I1 (including recombinant interleukin II, or rlL.sub.2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-1a; interferon gamma-1b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazoie; nogalamycin; ormaplatin; oxisuran; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone

hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride, agents that arrest cells in the G2-M phases and/or modulate the formation or stability of microtubules, (e.g. Taxol.TM (i.e. paclitaxel), Taxotere.TM, compounds comprising the taxane skeleton, Erbulozole (i.e. R-55104), Dolastatin 10 (i.e. DLS-10 and NSC-376128), Mivobulin isethionate (i.e. as CI-980), Vincristine, NSC-639829, Discodermolide (i.e. as NVP-XX-A-296), ABT-751 (Abbott, i.e. E-7010), Altorhyrtins (e.g. Altorhyrtin A and Altorhyrtin C), Spongistatins (e.g. Spongistatin 1, Spongistatin 2, Spongistatin 3, Spongistatin 4, Spongistatin 5, Spongistatin 6, Spongistatin 7, Spongistatin 8, and Spongistatin 9), Cemadotin hydrochloride (i.e. LU-103793 and NSC-D-669356), Epothilones (e.g. Epothilone A, Epothilone B, Epothilone C (i.e. desoxyepothilone A or dEpoA), Epothilone D (i.e. KOS-862, dEpoB, and desoxyepothilone B), Epothilone E, Epothilone F, Epothilone B N-oxide, Epothilone A N-oxide, 16-aza-epothilone B, 21-aminoepothilone B (i.e. BMS-310705), 21-hydroxyepothilone D (i.e. Desoxyepothilone F and dEpoF), 26-fluoroepothilone, Auristatin PE (i.e. NSC-654663), Soblidotin (i.e. TZT-1027), LS-4559-P (Pharmacia, i.e. LS-4577), LS-4578 (Pharmacia, i.e. LS-477-P), LS-4477 (Pharmacia), LS-4559 (Pharmacia), RPR-112378 (Aventis), Vincristine sulfate, DZ-3358 (Daiichi), FR-182877 (Fujisawa, i.e. WS-9885B), GS-164 (Takeda), GS-198 (Takeda), KAR-2 (Hungarian Academy of Sciences), BSF-223651 (BASF, i.e. ILX-651 and LU-223651), SAH-49960 (Lilly/Novartis), SDZ-268970 (Lilly/Novartis), AM-97 (Armad/Kyowa Hakko), AM-132 (Armad), AM-138 (Armad/Kyowa Hakko), IDN-5005 (Indena), Cryptophycin 52 (i.e. LY-355703), AC-7739 (Ajinomoto, i.e. AVE-8063A and CS-39.HCl), AC-7700 (Ajinomoto, i.e. AVE-8062, AVE-8062A, CS-39-L-Ser.HCl, and RPR-258062A), Vitilevuamide, Tubulysin A, Canadensol, Centaureidin (i.e. NSC-106969), T-138067 (Tularik, i.e. T-67, TL-138067 and TI-138067), COBRA-1 (Parker Hughes Institute, i.e. DDE-261 and WHI-261), H10 (Kansas State University), H16 (Kansas State University), Oncocidin A1 (i.e. BTO-956 and DIME), DDE-313 (Parker Hughes Institute), Fijianolide B, Laulimalide, SPA-2 (Parker Hughes Institute), SPA-1 (Parker Hughes Institute, i.e. SPIKET-P), 3-IAABU (Cytoskeleton/Mt. Sinai School of Medicine, i.e. MF-569), Narcosine (also known as NSC-5366), Nascapine, D-24851 (Asta Medica), A-105972 (Abbott), Hemiasterlin, 3-BAABU (Cytoskeleton/Mt. Sinai School of Medicine, i.e. MF-191), TMPN (Arizona State University), Vanadocene acetylacetonate, T-138026 (Tularik), Monsatrol, lnanocine (i.e. NSC-698666), 3-IAABE (Cytoskeleton/Mt. Sinai

School of Medicine), A-204197 (Abbott), T-607 (Tuiarik, i.e. T-900607), RPR-115781 (Aventis), Eleutherobins (such as Desmethyleleutherobin, Desaetyleleutherobin, lsoeleutherobin A, and Z-Eleutherobin), Caribaeoside, Caribaeolin, Halichondrin B, D-64131 (Asta Medica), D-68144 (Asta Medica), Diazonamide A, A-293620 (Abbott), NPI-2350 (Nereus), Taccalonolide A, TUB-245 (Aventis), A-259754 (Abbott), Diozostatin, (-)-Phenylahistin (i.e. NSCL-96F037), D-68838 (Asta Medica), D-68836 (Asta Medica), Myoseverin B, D-43411 (Zentaris, i.e. D-81862), A-289099 (Abbott), A-318315 (Abbott), HTI-286 (i.e. SPA-110, trifluoroacetate salt) (Wyeth), D-82317 (Zentaris), D-82318 (Zentaris), SC-12983 (NCI), Resverastatin phosphate sodium, BPR-OY-007 (National Health Research Institutes), and SSR-250411 (Sanofi)), steroids (*e.g.,* dexamethasone), finasteride, aromatase inhibitors, gonadotropin-releasing hormone agonists (GnRH) such as goserelin or leuprolide, adrenocorticosteroids (e.g., prednisone), progestins (e.g., hydroxyprogesterone caproate, megestrol acetate, medroxyprogesterone acetate), estrogens (e.g., diethlystilbestrol, ethinyl estradiol), antiestrogen (e.g., tamoxifen), androgens (e.g., testosterone propionate, fluoxymesterone), antiandrogen (e.g., flutamide), immunostimulants (*e.g.,* Bacillus Calmette-Guérin (BCG), levamisole, interleukin-2, alpha-interferon, *etc.*), monoclonal antibodies (*e.g.,* anti-CD20, anti-HER2, anti-CD52, anti-HLA-DR, and anti-VEGF monoclonal antibodies), immunotoxins (*e.g.,* anti-CD33 monoclonal antibody-calicheamicin conjugate, anti-CD22 monoclonal antibody-pseudomonas exotoxin conjugate, *etc.*), radioimmunotherapy (*e.g.,* anti-CD20 monoclonal antibody conjugated to ^{111}In, ^{90}Y, or ^{131}I, *etc.*), triptolide, homoharringtonine, dactinomycin, doxorubicin, epirubicin, topotecan, itraconazole, vindesine, cerivastatin, vincristine, deoxyadenosine, sertraline, pitavastatin, irinotecan, clofazimine, 5-nonyloxytryptamine, vemurafenib, dabrafenib, erlotinib, gefitinib, EGFR inhibitors, epidermal growth factor receptor (EGFR)-targeted therapy or therapeutic (e.g. gefitinib (Iressa ™), erlotinib (Tarceva ™), cetuximab (Erbitux™), lapatinib (Tykerb™), panitumumab (Vectibix™), vandetanib (Caprelsa™), afatinib/BIBW2992, CI-1033/canertinib, neratinib/HKI-272, CP-724714, TAK-285, AST-1306, ARRY334543, ARRY-380, AG-1478, dacomitinib/PF299804, OSI-420/desmethyl erlotinib, AZD8931, AEE788, pelitinib/EKB-569, CUDC-101, WZ8040, WZ4002, WZ3146, AG-490, XL647, PD153035, BMS-599626), sorafenib, imatinib, sunitinib, dasatinib, or the like.

[0145] "Chemotherapeutic" or "chemotherapeutic agent" is used in accordance with its plain ordinary meaning and refers to a chemical composition or compound having antineoplastic properties or the ability to inhibit the growth or proliferation of cells.

[0146] The compounds disclosed herein may be co-administered with an antiproliferative/antineoplastic drug or a combination thereof, as used in medical oncology, such as an alkylating agent (for example cis-platin, carboplatin, cyclophosphamide, nitrogen mustard,

melphalan, chlorambucil, busulphan or a nitrosourea); an antimetabolite (for example an antifolate such as a fluoropyrimidine like 5-fluorouracil or tegafur, raltitrexed, methotrexate, cytosine arabinoside, hydroxyurea, gemcitabine or paclitaxel); an antitumour antibiotic (for example an anthracycline such as adriamycin, bleomycin, doxorubicin, daunomycin, epirubicin, idarubicin, mitomycin-C, dactinomycin or mithramycin); an antimitotic agent (for example a vinca alkaloid such as vincristine, vinblastine, vindesine or vinorelbine, or a taxoid such as taxol or taxotere); or a topoisomerase inhibitor (for example an epipodophyllotoxin such as etoposide, teniposide, amsacrine, topotecan or a camptothecin); (ii) a cytostatic agent such as an antioestrogen (for example tamoxifen, toremifene, raloxifene, droloxifene or iodoxyfene), an oestrogen receptor down regulator (for example fulvestrant), an antiandrogen (for example bicalutamide, flutamide, nilutamide or cyproterone acetate), a LHRH antagonist or LHRH agonist (for example goserelin, leuprorelin or buserelin), a progestogen (for example megestrol acetate), an aromatase inhibitor (for example as anastrozole, letrozole, vorazole or exemestane) or an inhibitor of 5.alpha.-reductase such as finasteride; (iii) an agent which inhibits cancer cell invasion (for example a metalloproteinase inhibitor like marimastat or an inhibitor of urokinase plasminogen activator receptor function); (iv) an inhibitor of growth factor function, for example: a growth factor antibody (for example the anti-erbb2 antibody trastuzumab, or the anti-erbb1 antibody cetuximab [C225]), a farnesyl transferase inhibitor, a tyrosine kinase inhibitor or a serine/threonine kinase inhibitor, an inhibitor of the epidermal growth factor family (for example an EGFR family tyrosine kinase inhibitor such as N-(3-chloro-4-fluorophenyl)-7-methoxy-6-(3-morpholinopropoxy)quinazoli- n-4-amine (gefitinib, AZD 1839), N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine (erlotinib, OSI-774) or 6-acrylamido-N-(3-chloro-4-fluorophenyl)-7-(3-morpholinopropoxy)quinazoli- n-4-amine (CI 1033)), an inhibitor of the platelet-derived growth factor family, or an inhibitor of the hepatocyte growth factor family; (v) an antiangiogenic agent such as one which inhibits the effects of vascular endothelial growth factor (for example the anti-vascular endothelial cell growth factor antibody bevacizumab, a compound disclosed in WO 97/22596, WO 97/30035, WO 97/32856 or WO 98/13354), or a compound that works by another mechanism (for example linomide, an inhibitor of integrin .alpha.v.beta.3 function or an angiostatin); (vi) a vascular damaging agent such as combretastatin A4, or a compound disclosed in WO 99/02166, WO 00/40529, WO 00/41669, WO 01/92224, WO 02/04434 or WO 02/08213; (vii) an agent used in antisense therapy, for example one directed to one of the targets listed above, such as ISIS 2503, an anti-ras antisense; (viii) an agent used in a gene therapy approach, for example approaches to replace aberrant genes such as aberrant p53 or aberrant BRCA1 or BRCA2, GDEPT (gene-directed enzyme pro-drug therapy) approaches such as those using cytosine deaminase, thymidine kinase or a

bacterial nitroreductase enzyme and approaches to increase patient tolerance to chemotherapy or radiotherapy such as multi-drug resistance gene therapy; or (ix) an agent used in an immunotherapeutic approach, for example ex-vivo and in-vivo approaches to increase the immunogenicity of patient tumour cells, such as transfection with cytokines such as interleukin 2, interleukin 4 or granulocyte-macrophage colony stimulating factor, approaches to decrease T-cell anergy, approaches using transfected immune cells such as cytokine-transfected dendritic cells, approaches using cytokine-transfected tumour cell lines and approaches using anti-idiotypic antibodies.

[0147] In embodiments, the compounds disclosed herein can be co-administered with an antibody, such as a monoclonal antibody targeting B-Lymphocytes (such as CD20 (rituximab), MRA-aIL16R and T-Lymphocytes, CTLA4-Ig, HuMax I1-15) or antibody modulating Ig function such as anti-IgE (for example omalizumab).

[0148] In embodiments, treatment of cancer includes administration of an effective amount of at least two of the following: a CRF1 inhibitor, an inhibitor of the PD-L1/PD-1 pathway, an inhibitor of CTLA-4, an agonistic antibody of CD137 (4-1BB). In some embodiments, the method may comprise the use of two or more combinations.

[0149] In a further embodiment, the compounds described herein can be co-administered with conventional radiotherapeutic agents including, but not limited to, radionuclides such as ^{47}Sc, ^{64}Cu, ^{67}Cu, ^{89}Sr, ^{86}Y, ^{87}Y, ^{90}Y, ^{105}Rh, ^{111}Ag, ^{111}In, ^{117m}Sn, ^{149}Pm, ^{153}Sm, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{211}At, and ^{212}Bi, optionally conjugated to antibodies directed against tumor antigens.

[0150] In addition, a CRF_1 inhibitor may be combined with the therapeutic administration of immune cells, sometimes referred to as adoptive cell transfer. These cells may be cells from the patient, a genetically related or unrelated donor, they may be genetically modified (e.g. CAR-T cells, NK cells, etc), cell lines, genetically modified cell lines and live or dead versions of the above. CRF1 inhibitors may also be combined with vaccines of any kind (e.g. protein/peptide, viral, bacterial, cellular) to stimulate immune responses to cancer.

[0151] The CRF_1 inhibitors/CRF_1 antagonists disclosed herein can be administered by any acceptable route, such oral, intraadiposal, intraarterial, intraarticular, intracranial, intradermal, intralesional, intramusculay, intranasal, intraocularal, intrapericardial, intraperitoneal, intrapleural, intraprostatical, intrarectal, intrathecal, intratracheal, intratumoral, intraumbilical, intravaginal, intravenousl, intravesicullar, intravitreal, liposomal, local, mucosal, parenteral, rectal, subconjunctival, subcutaneous, sublingual, topical, transbuccal, transdermal, vaginal, in cremes, in lipid compositions, via a catheter, via a lavage, via continuous infusion, via infusion, via inhalation, via injection, via local delivery, via localized perfusion, bathing target cells directly, or any combination thereof.

[0152] The CRF_1 inhibitors/CRF_1 antagonists disclosed herein can be administered by any acceptable route, such oral, intraadiposal, intraarterial, intraarticular, intracranial, intradermal, intralesional, intramusculay, intranasal, intraocularal, intrapericardial, intraperitoneal, intrapleural, intraprostatical, intrarectal, intrathecal, intratracheal, intratumoral, intraumbilical, intravaginal, intravenousl, intravesicullar, intravitreal, liposomal, local, mucosal, parenteral, rectal, subconjunctival, subcutaneous, sublingual, topical, transbuccal, transdermal, vaginal, in cremes, in lipid compositions, via a catheter, via a lavage, via continuous infusion, via infusion, via inhalation, via injection, via local delivery, via localized perfusion, bathing target cells directly, or any combination thereof.

[0153] The CRF_1 inhibitors/CRF_1 antagonists disclosed herein may be administered once daily until study reached endpoint. The CRF_1 inhibitors/CRF_1 antagonists disclosed herein may be administered at least three times but in some studies four or more times depending on the length of the study and/or the design of the study.

[0154] The methods disclosed herein may be used in combination with additional cancer therapy. In some embodiments, the distinct cancer therapy comprises surgery, radiotherapy, chemotherapy, toxin therapy, immunotherapy, cryotherapy or gene therapy. In some embodiments, the cancer is a chemotherapy-resistant or radio-resistant cancer.

[0155] A "cell" as used herein, refers to a cell carrying out metabolic or other function sufficient to preserve or replicate its genomic DNA. A cell can be identified by well-known methods in the art including, for example, presence of an intact membrane, staining by a particular dye, ability to produce progeny or, in the case of a gamete, ability to combine with a second gamete to produce a viable offspring. Cells may include prokaryotic and eukaroytic cells. Prokaryotic cells include but are not limited to bacteria. Eukaryotic cells include but are not limited to yeast cells and cells derived from plants and animals, for example mammalian, insect (*e.g.*, spodoptera) and human cells. Cells may be useful when they are naturally nonadherent or have been treated not to adhere to surfaces, for example by trypsinization.

[0156] "Control" or "control experiment" is used in accordance with its plain ordinary meaning and refers to an experiment in which the subjects or reagents of the experiment are treated as in a parallel experiment except for omission of a procedure, reagent, or variable of the experiment. In some instances, the control is used as a standard of comparison in evaluating experimental effects. In some embodiments, a control is the measurement of the activity of a protein in the absence of a compound as described herein (including embodiments and examples).

[0157] The term "modulator" refers to a composition that increases or decreases the level of a target molecule or the function of a target molecule or the physical state of the target of the molecule. In some embodiments, a CRF1 associated disease modulator is a compound that

reduces the severity of one or more symptoms of a disease associated with CRF_1 (e.g. an endocrine disorder, a cancer including but not limited to TART or OART). A CRF_1 modulator is a compound that increases or decreases the activity or function or level of activity or level of function of CRF_1. A modulator may act alone, or it may use a cofactor, e.g., a protein, metal ion, or small molecule. Examples of modulators include small molecule compounds and other bioorganic molecules. Numerous libraries of small molecule compounds (e.g., combinatorial libraries) are commercially available and can serve as a starting point for identifying a modulator. The skilled artisan is able to develop one or more assays (e.g., biochemical or cell-based assays) in which such compound libraries can be screened in order to identify one or more compounds having the desired properties; thereafter, the skilled medicinal chemist is able to optimize such one or more compounds by, for example, synthesizing and evaluating analogs and derivatives thereof. Synthetic and/or molecular modeling studies can also be utilized in the identification of an activator.

[0158] The term "modulate" is used in accordance with its plain ordinary meaning and refers to the act of changing or varying one or more properties. "Modulation" refers to the process of changing or varying one or more properties. For example, as applied to the effects of a modulator on a target protein, to modulate means to change by increasing or decreasing a property or function of the target molecule or the amount of the target molecule. In embodiments, the terms "modulate", "modulation" and the like refer to the ability of a molecule (e.g., an activator or an inhibitor) to increase or decrease the function or activity of CRF_1, either directly or indirectly, relative to the absence of the molecule.

[0159] The term "associated" or "associated with" in the context of a substance or substance activity or function associated with a disease (e.g. a protein associated disease, an endocrine disorder, a disease associated with CRF_1 activity, a CRF_1 associated disease (e.g., testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART)) means that the disease (e.g., testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART)) is caused by (in whole or in part), or a symptom of the disease is caused by (in whole or in part) the substance or substance activity or function. For example, an endocrine disorder associated with CRF_1 activity or function may be an endocrine disorder that results (entirely or partially) from aberrant CRF_1 function (e.g. enzyme activity, protein-protein interaction, signaling pathway) or an endocrine disorder wherein a particular symptom of the disease is caused (entirely or partially) by aberrant CRF_1 activity or function. As used herein, what is described as being associated with a disease, if a causative agent, could be a target for treatment of the disease. For example, an endocrine disorder associated with CRF_1 activity or function or a CRF_1 associated disease (e.g., an endocrine disorder including but not limited to TART or OART), may be treated with a

compound described herein (e.g., CRF_1 modulator, CRF_1 inhibitor, or CRF_1 antagonist), in the instance where increased CRF_1 activity or function (e.g. signaling pathway activity) causes the disease (e.g., an endocrine disorder such as TART or OART). For example, an endocrine disorder associated with CRF_1 activity or function or a CRF_1 associated endocrine disorder, may be treated with an CRF_1 modulator or CRF_1 inhibitor, in the instance where increased CRF_1 activity or function (e.g. signaling pathway activity) causes the disease.

[0160] The term "aberrant" as used herein refers to different from normal. When used to describe enzymatic activity or protein function, aberrant refers to activity or function that is greater or less than a normal control or the average of normal non-diseased control samples. Aberrant activity may refer to an amount of activity that results in a disease, wherein returning the aberrant activity to a normal or non-disease-associated amount (e.g. by administering a compound or using a method as described herein), results in reduction of the disease or one or more disease symptoms.

[0161] The term "signaling pathway" as used herein refers to a series of interactions between cellular and optionally extra-cellular components (e.g. proteins, nucleic acids, small molecules, ions, lipids) that conveys a change in one component to one or more other components, which in turn may convey a change to additional components, which is optionally propogated to other signaling pathway components. For example, binding of a CRF_1 with a compound as described herein may reduce the level of a product of the CRF_1 catalyzed reaction or the level of a downstream derivative of the product or binding may reduce the interactions between the CRF_1 or a reaction product and downstream effectors or signaling pathway components (e.g., MAP kinase pathway), resulting in changes in cell growth, proliferation, or survival.

[0162] As used herein, the terms "corticotropin-releasing factor type-1," "CRF_1," "corticotropin-releasing hormone receptor 1," and "CHHR1" and all other related art-accepted terms, many of which are set forth below, refer to a compound capable of modulating, either directly or indirectly, the CRF_1 receptor in an *in vitro* assay, an *in vivo* model, and/or other means indicative of therapeutic efficacy. The terms also refer to a compound that exhibits at least some therapeutic benefit in a human subject.

[0163] The phrase "in a sufficient amount to effect a change" means that there is a detectable difference between a level of an indicator measured before (e.g., a baseline level) and after administration of a particular therapy. Indicators include any objective parameter (e.g., serum concentration) or subjective parameter (e.g., a subject's feeling of well-being).

[0164] The "activity" of a molecule may describe or refer to the binding of the molecule to a ligand or to a receptor; to catalytic activity; to the ability to stimulate gene expression or cell signaling, differentiation, or maturation; to antigenic activity; to the modulation of activities of

other molecules; and the like. The term "proliferative activity" encompasses an activity that promotes, that is necessary for, or that is specifically associated with, for example, normal cell division, as well as cancer, tumors, dysplasia, cell transformation, metastasis, and angiogenesis.

[0165] "Substantially pure" indicates that a component makes up greater than about 50% of the total content of the composition, and typically greater than about 60% of the total polypeptide content. More typically, "substantially pure" refers to compositions in which at least 75%, at least 85%, at least 90% or more of the total composition is the component of interest. In some cases, the polypeptide will make up greater than about 90%, or greater than about 95% of the total content of the composition (percentage in a weight per weight basis).

[0166] The terms "specifically binds" and "selectively binds," when referring to a ligand/receptor, antibody/antigen, or other binding pair, indicates a binding reaction which is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated conditions, a specified ligand binds to a particular receptor and does not bind in a significant amount to other proteins present in the sample. The antibody, or binding composition derived from the antigen-binding site of an antibody, of the contemplated method binds to its antigen, or a variant or mutein thereof, with an affinity that is at least two-fold greater, at least 10-times greater, at least 20-times greater, or at least 100-times greater than the affinity with any other antibody, or binding composition derived therefrom. In embodiments, the antibody will have an affinity that is greater than about 10^9 liters/mol, as determined by, e.g., Scatchard analysis (Munsen, et al. (1980) Analyt. Biochem. 107:220-239).

[0167] The terms "DNA," "nucleic acid," "nucleic acid molecule," "polynucleotide" and the like are used interchangeably herein to refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Non-limiting examples of polynucleotides include linear and circular nucleic acids, messenger RNA (mRNA), complementary DNA (cDNA), recombinant polynucleotides, vectors, probes, primers and the like.

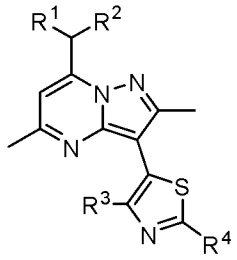
[0168] As used herein, the terms "variants" and "homologs" are used interchangeably to refer to amino acid or nucleic acid sequences that are similar to reference amino acid or nucleic acid sequences, respectively. The term encompasses naturally-occurring variants and non-naturally-occurring variants. Naturally-occurring variants include homologs (polypeptides and nucleic acids that differ in amino acid or nucleotide sequence, respectively, from one species to another), and allelic variants (polypeptides and nucleic acids that differ in amino acid or nucleotide sequence, respectively, from one individual to another within a species). Thus, variants and homologs encompass naturally occurring amino acid and nucleic acid sequences encoded thereby and their isoforms, as well as splice variants of a protein or gene. The terms also

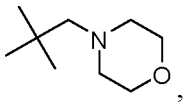
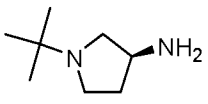
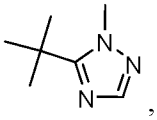
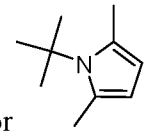
encompass nucleic acid sequences that vary in one or more bases from a naturally-occurring nucleic acid sequence but still translate into an amino acid sequence that corresponds to the naturally-occurring protein due to degeneracy of the genetic code. Non-naturally-occurring variants and homologs include polypeptides and nucleic acids that comprise a change in amino acid or nucleotide sequence, respectively, where the change in sequence is artificially introduced (e.g., muteins); for example, the change is generated in the laboratory by human intervention ("hand of man"). Therefore, non-naturally occurring variants and homologs may also refer to those that differ from the naturally-occurring sequences by one or more conservative substitutions and/or tags and/or conjugates.

[0169] The term "muteins" as used herein refers broadly to mutated recombinant proteins. These proteins usually carry single or multiple amino acid substitutions and are frequently derived from cloned genes that have been subjected to site-directed or random mutagenesis, or from completely synthetic genes.

II. Compounds

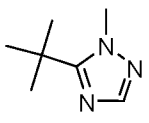
[0170] In an aspect provided herein, is a compound of Formula (I):

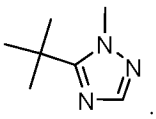


, or a pharmaceutically acceptable salt thereof. R^1 and R^2 are independently ethyl or n-propyl. R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy. R^4 is hydrogen, Br, R^aR^bN–, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl,  ,  ,  , or  . R^a and R^b are independently hydrogen, C_1-C_3alkyl, $H_2NCH_2CH_2$–, $(CH_3)_3COC(O)NHCH_2CH_2$–, or $CH_3CH_2CH_2NHCH_2CH_2$–In embodiments, the inhibitor forms an irreversible bond with the amino acid residue. In embodiments, the bond is a covalent bond.

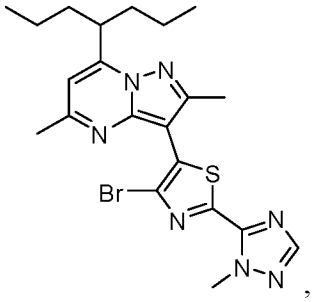
[0171] In certain embodiments, R^3 is Cl, Br, methyl, or trifluoromethyl.

[0172] In certain embodiments, R^3 is Cl, Br, or methyl.

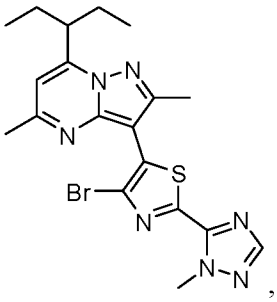
[0173] In certain embodiments, R^4 is Br, R^aR^bN–, pyridin-4-yl, morpholin-4-yl, or  .

[0174] In certain embodiments, R^4 is morpholin-4-yl or  .

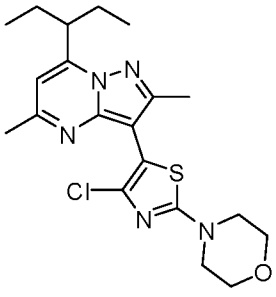
[0175] In certain embodiments, R^4 is hydrogen, Br, R^aR^bN– and R^a and R^b are independently C_1-C_3alkyl.

[0176] In certain embodiments, the compound is  , or a pharmaceutically acceptable salt thereof.

[0177] In certain embodiments, the compound is

 , or a pharmaceutically acceptable salt thereof.

[0178] In certain embodiments, the compound is

 , or a pharmaceutically acceptable salt thereof.

[0179] In one aspect described herein, there is a method of treating a subject in need thereof comprising administering an astressin or a pharmaceutically acceptable salt or solvate thereof. An astressin generally refers to a nonselective corticotropin releasing hormone antagonist that reduces the synthesis of ACTH and cortisol.

[0180] In one aspect described herein, there is a method of treating congenital adrenal hyperplasia (CAH) in a subject in need thereof, comprising administering an astressin or a variant having at least about 70% identity thereto or a pharmaceutically acceptable salt or solvate thereof. In one aspect described herein, there is a method of treating congenital adrenal hyperplasia (CAH) in a subject in need thereof, the method comprising: (i) measuring a hormone

level in the subject in need thereof; (ii) administering an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof; and repeating steps (i) and (ii) until the hormone level reaches a pre-determined range followed by a maintenance therapy of a daily dosing of said astressin.

[0181] In one aspect described herein, there is a method of improving hyperandrogenic symptoms in a subject in need thereof, comprising administering an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof. In one aspect described herein, there is a method of treating menstrual irregularity, ovulatory dysfunction or infertility, in a subject in need thereof, comprising administering an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof. In one aspect described herein, there is a method of improving metabolic symptoms in a subject in need thereof, comprising administering an astressin or a variant having at least about 70% identity thereto; or a pharmaceutically acceptable salt or solvate thereof.

[0182] In one aspect described herein, there is a method of improving quality of life in a subject in need thereof, comprising administering an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof.

III. Pharmaceutical Compositions

[0183] In an aspect, there is provided a pharmaceutical composition, including a compound as described herein, including embodiments, or the structural Formula (I), and a pharmaceutically acceptable excipient.

[0184] The compounds (e.g., CRF_1 inhibitors/antagonists) of the present disclosure may be in the form of compositions suitable for administration to a subject. In general, such compositions are "pharmaceutical compositions" comprising a compound (e.g., CRF_1 inhibitor(s)/ antagonist(s)) and one or more pharmaceutically acceptable or physiologically acceptable diluents, carriers or excipients. In certain embodiments, the compound (e.g., CRF_1 inhibitor/antagonist) are present in a therapeutically acceptable amount. The pharmaceutical compositions may be used in the methods of the present disclosure; thus, for example, the pharmaceutical compositions can be administered *ex vivo* or *in vivo* to a subject in order to practice the therapeutic and prophylactic methods and uses described herein.

[0185] In one aspect described herein, there is a pharmaceutical composition in the form of a capsule comprising an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof. In one aspect described herein, there is a pharmaceutical composition in the form of a tablet comprising an astressin or a variant having at least about 70% identity thereto, or a pharmaceutically acceptable salt or solvate thereof.

[0186] The pharmaceutical compositions of the present disclosure can be formulated to be compatible with the intended method or route of administration; exemplary routes of administration are set forth herein.

[0187] The pharmaceutical compositions containing the active ingredient (e.g., an inhibitor of CRF_1 function) may be in a form suitable for oral use, for example, as tablets, capsules, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups, solutions, microbeads or elixirs. Pharmaceutical compositions intended for oral use may be prepared for the manufacture of pharmaceutical compositions, and such compositions may contain one or more agents such as, for example, sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets, capsules and the like contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture thereof. These excipients may be, for example, diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc.

[0188] The tablets, capsules and the like suitable for oral administration may be uncoated or coated to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action. For example, a time-delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated to form osmotic therapeutic tablets for controlled release. Additional agents include biodegradable or biocompatible particles or a polymeric substance such as polyesters, polyamine acids, hydrogel, polyvinyl pyrrolidone, polyanhydrides, polyglycolic acid, ethylene-vinylacetate, methylcellulose, carboxymethylcellulose, protamine sulfate, or lactide/glycolide copolymers, polylactide/glycolide copolymers, or ethylenevinylacetate copolymers in order to control delivery of an administered composition. For example, the oral agent can be entrapped in microcapsules prepared by coacervation techniques or by interfacial polymerization, by the use of hydroxymethylcellulose or gelatin-microcapsules or poly(methylmethacrolate) microcapsules, respectively, or in a colloid drug delivery system. Colloidal dispersion systems include macromolecule complexes, nano-capsules, microspheres, microbeads, and lipid-based systems, including oil-in-water emulsions, micelles, mixed micelles, and liposomes. Methods for the preparation of the above-mentioned formulations will be apparent to those skilled in the art.

[0189] Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium

phosphate, kaolin or microcrystalline cellulose, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

[0190] Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture thereof. Such excipients can be suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxy-propylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents, for example a naturally-occurring phosphatide (e.g., lecithin), or condensation products of an alkylene oxide with fatty acids (e.g., polyoxy-ethylene stearate), or condensation products of ethylene oxide with long chain aliphatic alcohols (e.g., for heptadecaethyleneoxycetanol), or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol (e.g., polyoxyethylene sorbitol monooleate), or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides (e.g., polyethylene sorbitan monooleate). The aqueous suspensions may also contain one or more preservatives.

[0191] Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation.

[0192] Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, and optionally one or more suspending agents and/or preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified herein.

[0193] The pharmaceutical compositions of the present disclosure may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example, liquid paraffin, or mixtures of these. Suitable emulsifying agents may be naturally occurring gums, for example, gum acacia or gum tragacanth; naturally occurring phosphatides, for example, soy bean, lecithin, and esters or partial esters derived from fatty acids; hexitol anhydrides, for example, sorbitan monooleate; and condensation products of partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monooleate.

[0194] The pharmaceutical compositions typically comprise a therapeutically effective amount of a CRF_1 inhibitor/antagonist contemplated by the present disclosure and one or more pharmaceutically and physiologically acceptable formulation agents. Suitable pharmaceutically acceptable or physiologically acceptable diluents, carriers or excipients include, but are not limited to, antioxidants (e.g., ascorbic acid and sodium bisulfate), preservatives (e.g., benzyl

alcohol, methyl parabens, ethyl or n-propyl, p-hydroxybenzoate), emulsifying agents, suspending agents, dispersing agents, solvents, fillers, bulking agents, detergents, buffers, vehicles, diluents, and/or adjuvants. For example, a suitable vehicle may be physiological saline solution or citrate-buffered saline, possibly supplemented with other materials common in pharmaceutical compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Those skilled in the art will readily recognize a variety of buffers that can be used in the pharmaceutical compositions and dosage forms contemplated herein. Typical buffers include, but are not limited to, pharmaceutically acceptable weak acids, weak bases, or mixtures thereof. As an example, the buffer components can be water soluble materials such as phosphoric acid, tartaric acids, lactic acid, succinic acid, citric acid, acetic acid, ascorbic acid, aspartic acid, glutamic acid, and salts thereof. Acceptable buffering agents include, for example, a Tris buffer; N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES); 2-(N-Morpholino)ethanesulfonic acid (MES); 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES); 3-(N-Morpholino)propanesulfonic acid (MOPS); and N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS).

[0195] After a pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form, a lyophilized form requiring reconstitution prior to use, a liquid form requiring dilution prior to use, or other acceptable form. In some embodiments, the pharmaceutical composition is provided in a single-use container (e.g., a single-use vial, ampule, syringe, or autoinjector (similar to, e.g., an EpiPen®)), whereas a multi-use container (e.g., a multi-use vial) is provided in other embodiments.

[0196] Formulations can also include carriers to protect the composition against rapid degradation or elimination from the body, such as a controlled release formulation, including liposomes, hydrogels, prodrugs and microencapsulated delivery systems. For example, a time-delay material such as glyceryl monostearate or glyceryl stearate alone, or in combination with a wax, may be employed. Any drug delivery apparatus may be used to deliver a CRF_1 inhibitor/antagonist, including implants (e.g., implantable pumps) and catheter systems, slow injection pumps and devices, all of which are well known to the skilled artisan.

[0197] Depot injections, which are generally administered subcutaneously or intramuscularly, may also be utilized to release the compound (e.g., CRF_1 inhibitor/antagonist) disclosed herein over a defined period of time. Depot injections are usually either solid- or oil-based and generally comprise at least one of the formulation components set forth herein. One of ordinary skill in the art is familiar with possible formulations and uses of depot injections.

[0198] The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents mentioned herein. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Acceptable diluents, solvents and dispersion media that may be employed include water, Ringer's solution, isotonic sodium chloride solution, Cremophor® EL (BASF, Parsippany, NJ) or phosphate buffered saline (PBS), ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof. In addition, sterile fixed oils are conventionally employed as a solvent or suspending medium; for this purpose, any bland fixed oil may be employed, including synthetic mono- or diglycerides. Moreover, fatty acids, such as oleic acid, find use in the preparation of injectables. Prolonged absorption of particular injectable formulations can be achieved by including an agent that delays absorption (e.g., aluminum monostearate or gelatin).

[0199] The present disclosure contemplates the administration of the compound (e.g., CRF_1 inhibitor/antagonist) in the form of suppositories for rectal administration. The suppositories can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include, but are not limited to, cocoa butter and polyethylene glycols.

[0200] The compound (e.g., CRF_1 inhibitor/antagonist) contemplated by the present disclosure may be in the form of any other suitable pharmaceutical composition (e.g., sprays for nasal or inhalation use) currently known or developed in the future.

IV. Methods

[0201] In certain embodiments, provided herein are methods for treating or preventing a disease comprising administering an effective amount of a compound of Formula (I), or an isotopic variant thereof or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof.

[0202] In one embodiment, provided herein are methods for treating or preventing a tumor, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing a benign tumor, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing a malignant tumor, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing an adrenal

tumor, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing an adrenal rest tumor, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof.

[0203] In one embodiment, provided herein are methods for treating or preventing infertility, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing male infertility, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for improving or increasing sperm count, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for improving or increasing sperm motility, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for improving or increasing sperm morphology, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In one embodiment, provided herein are methods for treating or preventing female infertility, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof.

[0204] In one embodiment, provided herein are methods for treating or preventing a proliferative or hyperproliferative disease or disorder, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In certain embodiments, the proliferative disease is cancer. In certain embodiments, the proliferative disease is a cancer of the breast, skin, prostate, cervix, uterus, ovary, testes, bladder, lung, liver, larynx, oral cavity, colon and gastrointestinal tract (e.g., esophagus, stomach, pancreas), brain, thyroid, blood, and lymphatic system.

[0205] In certain embodiments, the cancer treatable with the methods provided herein includes, but is not limited to, (1) leukemias, including, but not limited to, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemias such as myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia leukemias and myelodysplastic syndrome or a symptom thereof (such as anemia, thrombocytopenia, neutropenia, bicytopenia or pancytopenia), refractory anemia (RA), RA with ringed sideroblasts (RARS), RA with excess blasts (RAEB),

RAEB in transformation (RAEB-T), preleukemia, and chronic myelomonocytic leukemia (CMML), (2) chronic leukemias, including, but not limited to, chronic rnyelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, and hairy cell leukemia; (3) polycythemia vera; (4) lymphomas, including, but not limited to, Hodgkin's disease and non-Hodgkin's disease; (5) multiple myelomas, including, but not limited to, smoldering multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma, and extramedullary plasmacytoma; (6) Waldenstrom's macroglobulinernia; (7) monoclonal gammopathy of undetermined significance; (8) benign monoclonal gammopathy; (9) heavy chain disease; (10) bone and connective tissue sarcomas, including, but not limited to, bone sarcoma, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, metastatic cancers, neurilemmoma, rhabdomyosarcoma, and synovial sarcoma; (11) brain tumors, including, but not limited to, glioma, astrocytoma, brain stem glioma, ependymoma, aligodendrogliorna, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma, pineocytoma, pineoblastoma, and primary brain lymphoma; (12) breast cancer, including, but not limited to, adenocarcinoma, lobular (small cell) carcinoma, intraductal carcinoma, medullary breast cancer, mutinous breast cancer, tubular breast cancer, papillary breast cancer, primary cancers, Paget's disease, and inflammatory breast cancer; (13) adrenal cancer, including, but not limited to, pheochromocytom and adrenocortical carcinoma; (14) thyroid cancer, including, but not limited to, papillary or follicular thyroid cancer, medullary thyroid cancer, and anaplastic thyroid cancer; (15) pancreatic cancer, including, but not limited to, insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, and carcinoid or islet cell tumor; (16) pituitary cancer, including, but limited to, Cushing's disease, prol actin-secreting tumor, acromegaly, and diabetes insipius; (17) eye cancer, including, but not limited, to ocular melanoma such as iris melanoma, choroidal melanoma, and cilliary body melanoma, and retinoblastoma; (18) vaginal cancer, including, but not limited to, squamous cell carcinoma, adenocarcinoma, and melanoma; (19) vulvar cancer, including, but not limited to, squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, sarcoma, and Paget's disease; (20) cervical cancers, including, but not limited to, squamous cell carcinoma, and adenocarcinoma; (21) uterine cancer, including, but not limited to, endometrial carcinoma and uterine sarcoma; (22) ovarian cancer, including, but not limited to, ovarian epithelial carcinoma, borderline tumor, germ cell tumor, and stromal tumor; (23) esophageal cancer, including, but not limited to, squamous cancer, adenocarcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, adenosquamous

carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and oat cell (small cell) carcinoma; (24) stomach cancer, including, but not limited to, adenocarcinoma, fungating (polypoid), ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma, fibrosarcoma, and carcinosarcoma; (25) colon cancer; (26) rectal cancer; (27) liver cancer, including, but not limited to, hepatocellular carcinoma and hepatoblastoma; (28) gallbladder cancer, including, but not limited to, adenocarcinoma; (29) cholangiocarcinomas, including, but not limited to, pappillary, nodular, and diffuse; (30) lung cancer, including, but not limited to, non-small cell lung cancer, squamous cell carcinoma (epidermoid carcinoma), adenocarcinoma, large-cell carcinoma, and small-cell lung cancer; (31) testicular cancer, including, but not limited to, germinal tumor, seminoma, anaplastic, classic (typical), spermatocytic, nonserninoma, embryonal carcinoma, teratoma carcinoma, and choriocarcinoma (yolk-sac tumor); (32) prostate cancer, including, but not limited to, adenocarcinoma, leiomyosarcoma, and rhabdomyosarcorna; (33) penal cancer; (34) oral cancer, including, but not limited to, squamous cell carcinoma; (35) basal cancer; (36) salivary gland cancer, including, but not limited to, adenocarcinoma, mucoepidermoid carcinoma, and adenoidcystic carcinoma; (37) pharynx cancer, including, but not limited to, squamous cell cancer and verrucous; (38) skin cancer, including, but not limited to, basal cell carcinoma, squamous cell carcinoma and melanoma, superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, and acral lentiginous melanoma; (39) kidney cancer, including, but not limited to, renal cell cancer, adenocarcinoma, hypernephroma, fibrosarcoma, and transitional cell cancer (renal pelvis and/or uterer); (40) Wilms' tumor; (41) bladder cancer, including, but not limited to, transitional cell carcinoma, squamous cell cancer, adenocarcinoma, and carcinosarcoma; and other cancer, including, not limited to, myxosarcoma, osteogenic sarcoma, endotheliosarcoma, lymphangio-endotheliosarcoma, mesothelioma, synovioma, hemangioblastoma, epithelial carcinoma, cystadenocarcinoma, bronchogenic carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, and papillary adenocarcinomas (*See* Fishman *et al.*, 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia and Murphy *et al.*, 1997, *Informed Decisions: The Complete Book of Cancer Diagnosis, Treatment, and Recovery*, Viking Penguin, Penguin Books U.S.A., Inc., United States of America).

[0206] Depending on the disorder, disease, or condition to be treated, and the subject's condition, the compounds or pharmaceutical compositions provided herein can be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracistemal injection or infusion, subcutaneous injection, or implant), inhalation, nasal, vaginal, rectal, sublingual, or topical (e.g., transdermal or local) routes of administration and can be formulated, alone or

together, in suitable dosage unit with pharmaceutically acceptable excipients, carriers, adjuvants, and vehicles appropriate for each route of administration as described elsewhere herein.

[0207] The suitability of a particular route of administration employed for a particular active agent will depend on the active agent itself (e.g., whether it can be administered orally without decomposing prior to entering the blood stream) and the disease being treated. Recommended routes of administration for the second active agents are known to those of ordinary skill in the art. See, e.g., Physicians' Desk Reference, 1755-1760 (56th ed., 2002).

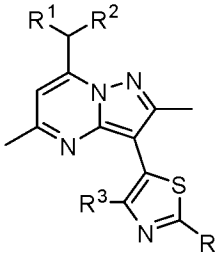
[0208] In one embodiment, provided herein are methods for treating or preventing testicular adrenal rest tumors (TART) in a subject, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof, wherein the subject has congenital adrenal hyperplasia (CAH).

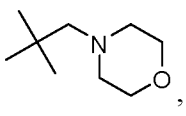
[0209] In one embodiment, provided herein are methods for treating or preventing ovarian adrenal rest tumors (OART) in a subject, comprising administering a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof, wherein the subject has congenital adrenal hyperplasia (CAH).

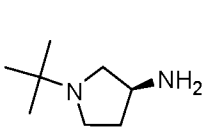
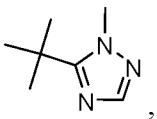
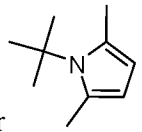
[0210] In an aspect provided herein, is a method of treating or preventing testicular adrenal rest tumors (TART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF_1) antagonist or a pharmaceutically acceptable salt thereof.

[0211] In an aspect provided herein, is a method of treating or preventing ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF_1) antagonist or a pharmaceutically acceptable salt thereof.

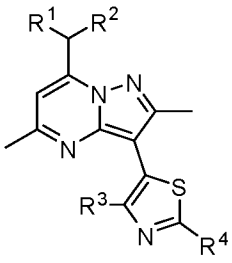
[0212] In an aspect provided herein, is a method of treating or preventing testicular adrenal rest tumors (TART), comprising administering to a subject in need thereof a compound of structural Formula (I):



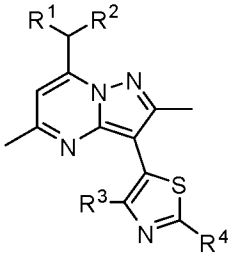
, or a pharmaceutically acceptable salt thereof. R^1 and R^2 are independently ethyl or n-propyl. R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy. R^4 is hydrogen, Br, R^aR^bN–, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl,  ,

 ,  , or  . R^a and R^b are independently hydrogen, C_1-C_3alkyl, $H_2NCH_2CH_2$–, $(CH_3)_3COC(O)NHCH_2CH_2$–, or $CH_3CH_2CH_2NHCH_2CH_2$–.

[0213] In an aspect provided herein, is a method of treating or preventing ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a compound of structural Formula (I):

 , or a pharmaceutically acceptable salt thereof. R^1, R^2, R^3 and R^4 are as described herein, including embodiments.

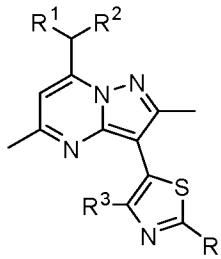
[0214] In an aspect provided herein, is a method of decreasing or reducing adrenocorticotropic hormone (ACTH) levels in a subject in need thereof, comprising administering to the subject contacting the ACTH with a compound of structural Formula (I):

 , or a pharmaceutically acceptable salt thereof. R^1, R^2, R^3 and R^4 are as described herein, including embodiments. R^1, R^2, R^3 and R^4 are as described herein, including embodiments. In certain embodiments, at least about 50% of the subjects demonstrate reduction in ACTH.In certain embodiments, at least about 60% of the subjects demonstrate reduction in ACTH.In certain embodiments, at least about 70% of the subjects demonstrate reduction in ACTH. In certain embodiments, at least about 80% of the subjects demonstrate reduction in ACTH. In embodiments, at least about 10% of subjects were in the standard or normal range for ACTH after treatment.

[0215] In embodiments, at least about 20% of subjects were in the standard or normal range for ACTH after treatment. In embodiments, at least about 30% of subjects were in the standard or normal range for ACTH after treatment. In certain embodiments, ACTH levels are decreased or reduced within about 30 minutes post dosing. In certain embodiments, ACTH levels are decreased or reduced within about 1 hour post dosing. In certain embodiments, ACTH levels are

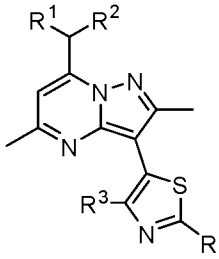
decreased or reduced within about 90 minutes post dosing. In certain embodiments, ACTH levels are decreased or reduced within about 2 hours post dosing.

[0216] In an aspect provided herein, is a method of decreasing or reducing 17α-hydroxyprogesterone (17-OHP) levels in a subject in need thereof, comprising administering to the subject a compound of structural Formula (I):



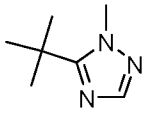
, or a pharmaceutically acceptable salt thereof. R^1, R^2, R^3 and R^4 are as described herein, including embodiments. R^1, R^2, R^3 and R^4 are as described herein, including embodiments. In certain embodiments, at least about 50% of the subjects demonstrate reduction in 17-OHP. In certain embodiments, at least about 60% of the subjects demonstrate reduction in 17-OHP. In certain embodiments, at least about 70% of the subjects demonstrate reduction in 17-OHP. In certain embodiments, at least about 80% of the subjects demonstrate reduction in 17-OHP. In embodiments, at least about 30% of subjects were in the standard or normal range of 1200 ng/dL after treatment. In embodiments, at least about 40% of subjects were in the standard or normal range of 1200 ng/dL after treatment. In embodiments, at least about 50% of subjects were in the standard or normal range of 1200 ng/dL after treatment. In embodiments, at least about 60% of subjects were in the standard or normal range of 1200 ng/dL after treatment. In embodiments, at least about 30% of subjects demonstrated an at least about 25% reduction in 17-OHP after treatment. In embodiments, at least about 40% of subjects demonstrated an at least about 25% reduction in 17-OHP after treatment. In embodiments, at least about 50% of subjects demonstrated an at least about 25% reduction in 17-OHP after treatment. In embodiments, at least about 60% of subjects demonstrated an at least about 25% reduction in 17-OHP after treatment.

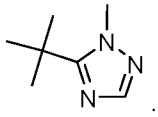
[0217] In an aspect provided herein, is a method of decreasing or reducing androstenedione levels in a subject in need thereof, comprising administering to the subject a compound of structural Formula (I):

 , or a pharmaceutically acceptable salt thereof. R^1, R^2, R^3 and R^4 are as described herein, including embodiments. R^1, R^2, R^3 and R^4 are as described herein, including embodiments. In certain embodiments, at least about 50% of the subjects demonstrate reduction in androstenedione.In certain embodiments, at least about 60% of the subjects demonstrate reduction in androstenedione.In certain embodiments, at least about 70% of the subjects demonstrate reduction in androstenedione. In certain embodiments, at least about 80% of the subjects demonstrate reduction in androstenedione. In certain embodiments, at least about 90% of the subjects demonstrate reduction in androstenedione. In certain embodiments, at least 100% of the subjects demonstrate reduction in androstenedione. In embodiments, at least about 30% of subjects demonstrated an at least about 25% reduction in androstenedione after treatment. In embodiments, at least about 40% of subjects demonstrated an at least about 25% reduction in androstenedione after treatment. In embodiments, at least about 50% of subjects demonstrated an at least about 25% reduction in androstenedione after treatment. In embodiments, at least 60% of subjects demonstrated an at least about 25% reduction in androstenedione after treatment. In embodiments, at least about 30% of subjects were in the standard or normal range for androstenedione after treatment. In embodiments, at least about 40% of subjects were in the standard or normal range for androstenedione after treatment. In embodiments, at least about 50% of subjects were in the standard or normal range for androstenedione after treatment.

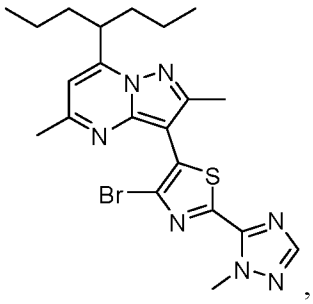
[0218] In certain embodiments, R^3 is Cl, Br, methyl, or trifluoromethyl.

[0219] In certain embodiments, R^3 is Cl, Br, or methyl.

[0220] In certain embodiments, R^4 is Br, R^aR^bN–, pyridin-4-yl, morpholin-4-yl, or  .

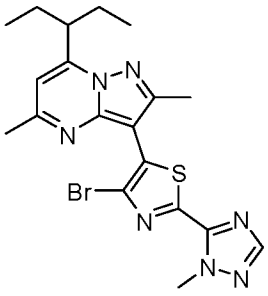
[0221] In certain embodiments, R^4 is morpholin-4-yl or  .

[0222] In certain embodiments, R^4 is hydrogen, Br, R^aR^bN– and R^a and R^b are independently C_1-C_3alkyl.



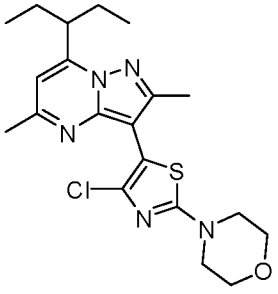
[0223] In certain embodiments, the compound is , or a pharmaceutically acceptable salt thereof.

[0224] In certain embodiments, the compound is



, or a pharmaceutically acceptable salt thereof.

[0225] In certain embodiments, the compound is



, or a pharmaceutically acceptable salt thereof.

[0226] In certain embodiments, a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is cyclically administered to a patient. Cycling therapy involves the administration of an active agent or a combination of active agents for a period of time, followed by a rest for a period of time, and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improves the efficacy of the treatment.

[0227] In certain embodiments, in the treatment, prevention, or amelioration of one or more symptoms of the disorders, diseases, or conditions described herein (e.g., treatment or prevention of testicular adrenal rest tumors (TART) or treatment or prevention of ovarian adrenal rest tumors (OART)), an appropriate dosage level of a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof generally is ranging from about 1 mg to 3000 mg, from about 1 mg to 2000 mg, from about 1 mg to 1000 mg, from about 1 mg to about 500 mg, from about 5 mg to about 500 mg,

from about 5 mg to about 400 mg, from about 5 mg to about 300 mg, from about 5 mg to about 250 mg, from about 5 mg to about 200 mg, from about 50 mg to about 200 mg, from about 10 mg to about 400 mg, from about 5 mg to about 25 mg, from about 10 mg to about 25 mg, or from about 25 to about 200 mg, which can be administered in single or multiple doses. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1 mg, about 5 mg, about 10 mg mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 105 mg, about 110 mg, about 115 mg, about 120 mg, about 125 mg, about 130 mg, about 135 mg, about 140 mg, about 145 mg, about 150 mg, about 155 mg, about 160 mg, about 165 mg, about 170 mg, about 175 mg, about 180 mg, about 185 mg, about 190 mg, about 195 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 450 mg, about 500 mg, about 600 mg, about 800 mg, or about 1000 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1 mg, about 5 mg, about 10 mg mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 105 mg, about 110 mg, about 115 mg, about 120 mg, about 125 mg, about 130 mg, about 135 mg, about 140 mg, about 145 mg, about 150 mg, about 155 mg, about 160 mg, about 165 mg, about 170 mg, about 175 mg, about 180 mg, about 185 mg, about 190 mg, about 195 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 450 mg, about 500 mg, about 600 mg, about 800 mg, or about 1000 mg/day.

[0228] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 5 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an

amount of about 270 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 280 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is

administered in an amount of about 600 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg.

[0229] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 105 mg, about 110 mg, about 115 mg, about 120 mg, about 125 mg, about 130 mg, about 135 mg, about 140 mg, about 145 mg, about 150 mg, about 155 mg, about 160 mg, about 165 mg, about 170 mg, about 175 mg, about 180 mg, about 185 mg, about 190 mg, about 195 mg, about 200 mg, or about 1000 mg.

[0230] For oral administration, the pharmaceutical compositions provided herein can be formulated in the form of tablets or capsules containing from about 1.0 mg to about 1,000 mg of a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In certain embodiments, for oral administration, the pharmaceutical compositions provided herein can be formulated in the form of tablets or capsules containing about 1 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 105 mg, about 110 mg, about 115 mg, about 120 mg, about 125 mg, about 130 mg, about 135 mg, about 140 mg, about 145 mg, about 150 mg, about 155 mg, about 160 mg, about 165 mg, about 170 mg, about 175 mg, about 180 mg, about 185 mg, about 190 mg, about 195 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about

350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 405 mg, about 410 mg, about 415 mg, about 420 mg, about 425 mg, about 430 mg, about 435 mg, about 440 mg, about 445 mg, about 450 mg, about 455 mg, about 460 mg, about 465 mg, about 470 mg, about 475 mg, about 480 mg, about 485 mg, about 490 mg, about 495 mg, about 500 mg, about 505 mg, about 510 mg, about 515 mg, about 520 mg, about 525 mg, about 530 mg, about 535 mg, about 540 mg, about 545 mg, about 550 mg, about 555 mg, about 560 mg, about 565 mg, about 570 mg, about 575 mg, about 580 mg, about 585 mg, about 590 mg, about 595 mg, about 600 mg, about 605 mg, about 610 mg, about 615 mg, about 620 mg, about 625 mg, about 630 mg, about 635 mg, about 640 mg, about 645 mg, about 650 mg, about 655 mg, about 660 mg, about 665 mg, about 670 mg, about 675 mg, about 680 mg, about 685 mg, about 690 mg, about 695 mg, about 700 mg, about 705 mg, about 710 mg, about 715 mg, about 720 mg, about 725 mg, about 730 mg, about 735 mg, about 740 mg, about 745 mg, about 750 mg, about 755 mg, about 760 mg, about 765 mg, about 770 mg, about 775 mg, about 780 mg, about 785 mg, about 790 mg, about 795 mg, about 800 mg, about 810 mg, about 820 mg, about 830 mg, about 840 mg, about 850 mg, about 860 mg, about 870 mg, about 880 mg, about 890 mg, about 900 mg, about 910 mg, about 920 mg, about 930 mg, about 940 mg, about 950 mg, about 960 mg, about 970 mg, about 990 mg, about 990 mg, or about 1000 mg of a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof for the symptomatic adjustment of the dosage to the patient to be treated. In certain embodiments, for oral administration, the pharmaceutical compositions provided herein can be formulated in the form of tablets or capsules containing about 50 mg of a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. In certain embodiments, for oral administration, the pharmaceutical compositions provided herein can be formulated in the form of tablets or capsules containing about 200 mg of a compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof.The pharmaceutical compositions can be administered on a regimen of one (1) to four (4) times per day, including once, twice, three times, and four times per day. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered once per day. In some embodiments, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about

310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 405 mg, about 410 mg, about 415 mg, about 420 mg, about 425 mg, about 430 mg, about 435 mg, about 440 mg, about 445 mg, about 450 mg, about 455 mg, about 460 mg, about 465 mg, about 470 mg, about 475 mg, about 480 mg, about 485 mg, about 490 mg, about 495 mg, about 500 mg, about 505 mg, about 510 mg, about 515 mg, about 520 mg, about 525 mg, about 530 mg, about 535 mg, about 540 mg, about 545 mg, about 550 mg, about 555 mg, about 560 mg, about 565 mg, about 570 mg, about 575 mg, about 580 mg, about 585 mg, about 590 mg, about 595 mg, about 600 mg, about 605 mg, about 610 mg, about 615 mg, about 620 mg, about 625 mg, about 630 mg, about 635 mg, about 640 mg, about 645 mg, about 650 mg, about 655 mg, about 660 mg, about 665 mg, about 670 mg, about 675 mg, about 680 mg, about 685 mg, about 690 mg, about 695 mg, about 700 mg, about 705 mg, about 710 mg, about 715 mg, about 720 mg, about 725 mg, about 730 mg, about 735 mg, about 740 mg, about 745 mg, about 750 mg, about 755 mg, about 760 mg, about 765 mg, about 770 mg, about 775 mg, about 780 mg, about 785 mg, about 790 mg, about 795 mg, about 800 mg, about 810 mg, about 820 mg, about 830 mg, about 840 mg, about 850 mg, about 860 mg, about 870 mg, about 880 mg, about 890 mg, about 900 mg, about 910 mg, about 920 mg, about 930 mg, about 940 mg, about 950 mg, about 960 mg, about 970 mg, about 990 mg, about 990 mg, or about 1000 mg of the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered once per day. In some embodiments, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 405 mg, about 410 mg, about 415 mg, about 420 mg, about 425 mg, about 430 mg, about 435 mg, about 440 mg, about 445 mg, about 450 mg, about 455 mg, about 460 mg, about 465 mg, about 470 mg, about 475 mg, about 480 mg, about 485 mg, about 490 mg, about 495 mg, about 500 mg, about 505 mg, about 510 mg, about 515 mg, about 520 mg, about 525 mg, about 530 mg, about 535 mg, about 540 mg, about 545 mg, about 550 mg, about 555 mg, about 560 mg, about 565 mg, about 570 mg, about 575 mg, about 580 mg, about 585 mg, about 590 mg, about 595 mg, about 600 mg, about 605 mg,

about 610 mg, about 615 mg, about 620 mg, about 625 mg, about 630 mg, about 635 mg, about 640 mg, about 645 mg, about 650 mg, about 655 mg, about 660 mg, about 665 mg, about 670 mg, about 675 mg, about 680 mg, about 685 mg, about 690 mg, about 695 mg, about 700 mg, about 705 mg, about 710 mg, about 715 mg, about 720 mg, about 725 mg, about 730 mg, about 735 mg, about 740 mg, about 745 mg, about 750 mg, about 755 mg, about 760 mg, about 765 mg, about 770 mg, about 775 mg, about 780 mg, about 785 mg, about 790 mg, about 795 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, or about 1000 mg of the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered twice per day. In some embodiments, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 405 mg, about 410 mg, about 415 mg, about 420 mg, about 425 mg, about 430 mg, about 435 mg, about 440 mg, about 445 mg, about 450 mg, about 455 mg, about 460 mg, about 465 mg, about 470 mg, about 475 mg, about 480 mg, about 485 mg, about 490 mg, about 495 mg, about 500 mg, about 505 mg, about 510 mg, about 515 mg, about 520 mg, about 525 mg, about 530 mg, about 535 mg, about 540 mg, about 545 mg, about 550 mg, about 555 mg, about 560 mg, about 565 mg, about 570 mg, about 575 mg, about 580 mg, about 585 mg, about 590 mg, about 595 mg, about 600 mg, about 605 mg, about 610 mg, about 615 mg, about 620 mg, about 625 mg, about 630 mg, about 635 mg, about 640 mg, about 645 mg, about 650 mg, about 655 mg, about 660 mg, about 665 mg, about 670 mg, about 675 mg, about 680 mg, about 685 mg, about 690 mg, about 695 mg, about 700 mg, about 705 mg, about 710 mg, about 715 mg, about 720 mg, about 725 mg, about 730 mg, about 735 mg, about 740 mg, about 745 mg, about 750 mg, about 755 mg, about 760 mg, about 765 mg, about 770 mg, about 775 mg, about 780 mg, about 785 mg, about 790 mg, about 795 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, or about 1000 mg of the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered three times per day.

{0231} In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered for a period of one day. In some embodiments, the compound of Formula (I), or an isotopic variant

thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of two days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of three days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of four days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of five days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of six days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of seven days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of eight days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of nine days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of ten days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 11 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 12 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 13 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 14 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered for a period of 15 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 16 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 17 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically

acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 18 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 19 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 20 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 21 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 22 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 23 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 24 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 25 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 26 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 27 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 28 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 29 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered for a period of 30 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 31 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 32 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 33 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate,

hydrate, or prodrug thereof is administered over a period of 34 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 35 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 36 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 37 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 38 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 39 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 40 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 41 days. In some embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered over a period of 42 days or more.

[0232] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg once daily for a period of up to at least about 14 days.

[0233] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically

acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 140 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg once daily for a period of up to at least

about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically

acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg once daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg once daily for a period of up to at least about 14 days.

[0234] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 5 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg twice daily

for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 140 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a

pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg twice daily

for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg twice daily for a period of up to at least about 14 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg twice daily for a period of up to at least about 14 days.

[0235] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 5 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant

thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 280 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330

mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant

thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg once daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg once daily for a period of up to at least about 28 days.

[0236] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 5 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an

amount of about 220 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 280 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula

(I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg twice daily for a period of up to at least about 28 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a

pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg twice daily for a period of up to at least about 28 days.

[0237] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 255 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230

mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 420 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant

thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 370 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg once daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg once daily for a period of up to at least about 42 days.

[0238] In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an

amount of about 5 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 10 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 15 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 20 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 25 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 50 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 100 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 150 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 200 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 210 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 220 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 230 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 240 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula

(I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 250 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 260 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 270 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 420 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 290 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 300 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 310 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 320 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 330 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 340 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 350 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 360 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an

amount of about 370 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 380 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 390 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 400 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 450 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 500 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 600 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 700 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 800 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 900 mg twice daily for a period of up to at least about 42 days. In certain embodiments, the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered in an amount of about 1000 mg twice daily for a period of up to at least about 42 days.

[0239] In some embodiments, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 75 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, or about 400 mg of the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered twice per day. In some embodiments, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 75 mg, about 100 mg, about 150 mg, about 200

mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, or about 800 mg of the compound of Formula (I), or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof is administered at least once per day.

[0240] In certain embodiments, from about 5 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, from about 25 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, from about 100 mg to about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject.

[0241] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered orally.

[0242] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is formulated as a capsule or tablet.

[0243] In certain embodiments, from about 25 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject.

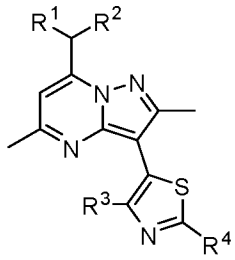
[0244] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 5 mg/day to about 1000 mg/day is administered to the subject. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 25 mg/day to about 1000 mg/day is administered to the subject. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 5 mg/day to about 25 mg/day is administered to the subject. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 10 mg/day to about 400 mg/day is administered to the subject.In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at a dose of from about 100 mg/day to about 600 mg/day.

[0245] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the fed state. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the fasted state. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at least once per day.

[0246] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at bedtime. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 6 hours before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 5 hours before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 4 hours before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 3 hours before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 2 hours before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 1 hour before sleep. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered less than about 30 minutes before sleep.

[0247] In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in the evening. In certain embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at about 10 pm at night.

[0248] In another aspect is provided a method of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART), comprising administering to a subject in need thereof a pharmaceutical composition, comprising a compound of structural Formula (I): and a pharmaceutically acceptable excipientcomprising with a compound as described herein, including embodiments, or the structural Formulae (I) and a pharmaceutically acceptable excipient:



, or a pharmaceutically acceptable salt thereof. R^1, R^2, R^3, and R^4 are as described herein, including embodiments.

[0249] In certain embodiments, the pharmaceutical composition comprises from about 25 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments, the pharmaceutical composition comprises from about 25 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments, the pharmaceutical composition comprises from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments,

the pharmaceutical composition comprises from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments, the pharmaceutical composition comprises from about 100 mg to about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[0250] In certain embodiments, the pharmaceutical composition comprises about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg about 500 mg, or about 600 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[0251] In certain embodiments, the pharmaceutical composition is administered orally.

[0252] In certain embodiments, the pharmaceutical composition is formulated as a capsule or tablet. In certain embodiments, the capsule or tablet comprises about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 205 mg, about 210 mg, about 215 mg, about 220 mg, about 225 mg, about 230 mg, about 235 mg, about 240 mg, about 245 mg, about 250 mg, about 255 mg, about 260 mg, about 265 mg, about 270 mg, about 275 mg, about 280 mg, about 285 mg, about 290 mg, about 295 mg, about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, or about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments, the capsule or tablet comprises about 50 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In certain embodiments, the capsule or tablet comprises about 200 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[0253] In certain embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 25 mg to about 1000 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg to about 25 mg of a compound of Formula (I), or a pharmaceutically

acceptable salt thereof, is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 10 mg to about 400 mg of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject.

[0254] In certain embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg/day to about 1000 mg/day is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 25 mg/day to about 1000 mg/day is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 5 mg/day to about 25 mg/day is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 10 mg/day to about 400 mg/day is administered to the subject. In certain embodiments, the pharmaceutical composition is administered at a dose of from about 100 mg/day to about 600 mg/day.

[0255] In certain embodiments, the pharmaceutical composition is administered in the fed state. In certain embodiments, the pharmaceutical composition is administered in the fasted state. In certain embodiments, the pharmaceutical composition is administered at least once per day.

[0256] In certain embodiments, the pharmaceutical composition is administered at bedtime. In certain embodiments, the pharmaceutical composition is administered less than about 6 hours before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 5 hours before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 4 hours before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 3 hours before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 2 hours before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 1 hour before sleep. In certain embodiments, the pharmaceutical composition is administered less than about 30 minutes before sleep.

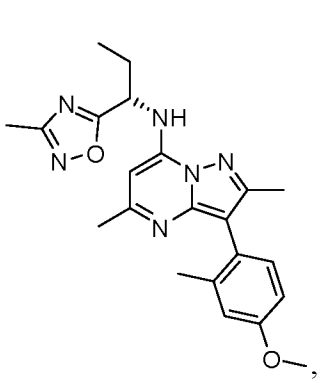
[0257] In certain embodiments, the pharmaceutical composition is administered in the evening. In certain embodiments, the pharmaceutical composition is administered at about 10 pm at night.

[0258] In certain embodiments, the subject has congenital adrenal hyperplasia (CAH).

[0259] In certain embodiments, the size and/or number of the tumors is decreased or reduced. In certain embodiments, the size of the tumors is decreased or reduced. In certain embodiments, the size of the tumors is decreased or reduced.

[0260] In certain embodiments of the methods disclosed herein, the method further comprises administering to the subject an additional chemotherapeutic agent.

[0261] In certain embodiments, the the additional chemotherapeutic agent is a glucocorticoid, a mineralocorticoid, an ACAT1 inhibitor, or an anti-androgen. In certain embodiments, the glucocorticoid is beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone. In certain embodiments, the mineralocorticoid is fludrocortisone. In certain embodiments, the additional chemotherapeutic agent is another CRF_1 antagonist. In certain embodiments, the CRF_1 antagonist is: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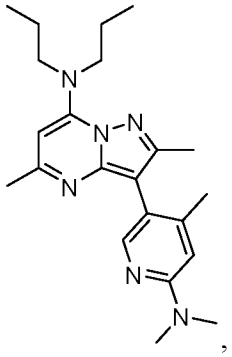
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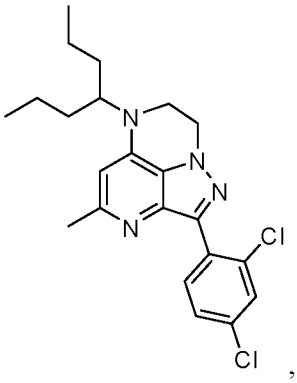
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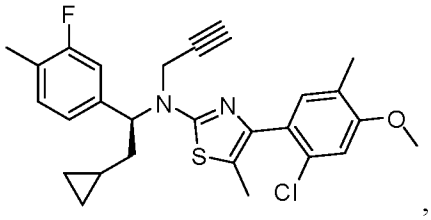
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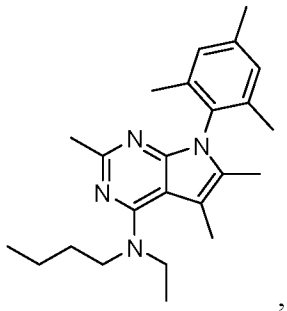
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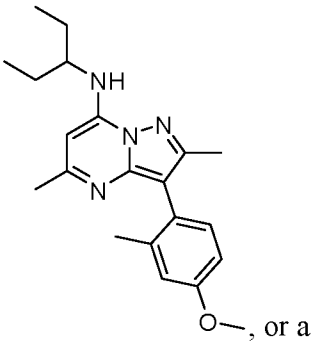
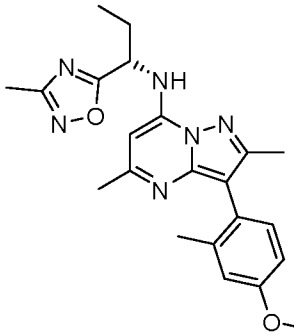

, or a pharmaceutically acceptable salt thereof. In certain embodiments, the CRF_1 antagonist is


, or a pharmaceutically acceptable salt thereof.

[0262] In certain embodiments of the methods disclosed herein, the method further comprises an additional treatment selected from surgical resection of the tumors and radiation therapy, or a combination thereof. In certain embodiments, the additional therapy is surgical resection and the surgical resection is prior to, after, and/or concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition, or any combination thereof. In certain embodiments, the surgical resection is prior

to administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In certain embodiments, the surgical resection is after administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In certain embodiments, the surgical resection is concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

[0263] In certain embodiments, the additional treatment is radiation therapy and the radiation therapy is prior to, after, and/or concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition, or any combination thereof. In certain embodiments, the radiation therapy is prior to administration of the CRF_1 antagonist of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In certain embodiments, the radiation therapy is after administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition. In certain embodiments, the radiation therapy is concurrent with administration of the compound of structural Formula (I), or a pharmaceutically acceptable salt thereof or the pharmaceutical composition.

[0264] In one embodiment, the pharmaceutical compositions are provided in a dosage form for oral administration, which comprise a compound provided herein, and one or more pharmaceutically acceptable excipients or carriers. The pharmaceutical compositions provided herein that are formulated for oral administration may be in tablet, capsule, powder, or liquid form. In some embodiments, a tablet comprises a solid carrier or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil, or synthetic oil. Physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol, or polyethylene glycol may be included. In some embodiments, a capsule comprises a solid carrier such as gelatin.

[0265] In another embodiment, the pharmaceutical compositions are provided in a dosage form for parenteral administration, which comprise a compound provided herein, and one or more pharmaceutically acceptable excipients or carriers. Where pharmaceutical compositions may be formulated for intravenous, cutaneous or subcutaneous injection, the active ingredient will be in the form of a parenterally acceptable aqueous solution, which is pyrogen-free and has a suitable pH, isotonicity, and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles, such as Sodium Chloride injection, Ringer's injection, or Lactated Ringer's injection. In some embodiments, preservatives, stabilisers, buffers, antioxidants, and/or other additives are included as required.

[0266] In yet another embodiment, the pharmaceutical compositions are provided in a dosage form for topical administration, which comprise a compound provided herein, and one or more pharmaceutically acceptable excipients or carriers.

[0267] The pharmaceutical compositions can also be formulated as modified release dosage forms, including delayed-, extended-, prolonged-, sustained-, pulsatile-, controlled-, accelerated-, fast-, targeted-, and programmed-release, and gastric retention dosage forms. These dosage forms can be prepared according to conventional methods and techniques known to those skilled in the art (*see, Remington: The Science and Practice of Pharmacy*, supra; *Modified-Release Drug Delivery Technology*, 2nd Edition, Rathbone *et al.*, Eds., Marcel Dekker, Inc.: New York, NY, 2008).

[0268] The pharmaceutical compositions provided herein can be provided in a unit-dosage form or multiple-dosage form. A unit-dosage form, as used herein, refers to physically discrete a unit suitable for administration to a human and animal subject, and packaged individually as is known in the art. Each unit-dose contains a predetermined quantity of an active ingredient(s) sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carriers or excipients. Examples of a unit-dosage form include an ampoule, syringe, and individually packaged tablet and capsule. A unit-dosage form may be administered in fractions or multiples thereof. A multiple-dosage form is a plurality of identical unit-dosage forms packaged in a single container to be administered in segregated unit-dosage form. Examples of a multiple-dosage form include a vial, bottle of tablets or capsules, or bottle of pints or gallons.

[0269] The pharmaceutical compositions provided herein can be administered at once, or multiple times at intervals of time. It is understood that the precise dosage and duration of treatment may vary with the age, weight, and condition of the patient being treated, and may be determined empirically using known testing protocols or by extrapolation from *in vivo* or *in vitro* test or diagnostic data. It is further understood that for any particular individual, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the formulations.

[0270] In certain embodiments, the pharmaceutical compositions provided herein further comprise one or more chemotherapeutic agents as defined herein.

A. Oral Administration

[0271] The pharmaceutical compositions provided herein for oral administration can be provided in solid, semisolid, or liquid dosage forms for oral administration. As used herein, oral administration also includes buccal, lingual, and sublingual administration. Suitable oral dosage

forms include, but are not limited to, tablets, fastmelts, chewable tablets, capsules, pills, strips, troches, lozenges, pastilles, cachets, pellets, medicated chewing gum, bulk powders, effervescent or non-effervescent powders or granules, oral mists, solutions, emulsions, suspensions, wafers, sprinkles, elixirs, and syrups. In addition to the active ingredient(s), the pharmaceutical compositions can contain one or more pharmaceutically acceptable carriers or excipients, including, but not limited to, binders, fillers, diluents, disintegrants, wetting agents, lubricants, glidants, coloring agents, dye-migration inhibitors, sweetening agents, flavoring agents, emulsifying agents, suspending and dispersing agents, preservatives, solvents, non-aqueous liquids, organic acids, and sources of carbon dioxide.

[0272] Binders or granulators impart cohesiveness to a tablet to ensure the tablet remaining intact after compression. Suitable binders or granulators include, but are not limited to, starches, such as corn starch, potato starch, and pre-gelatinized starch (e.g., STARCH 1500); gelatin; sugars, such as sucrose, glucose, dextrose, molasses, and lactose; natural and synthetic gums, such as acacia, alginic acid, alginates, extract of Irish moss, panwar gum, ghatti gum, mucilage of isabgol husks, carboxymethylcellulose, methylcellulose, polyvinylpyrrolidone (PVP), Veegum, larch arabogalactan, powdered tragacanth, and guar gum; celluloses, such as ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose, methyl cellulose, hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC), hydroxypropyl methyl cellulose (HPMC); microcrystalline celluloses, such as AVICEL-PH-101, AVICEL-PH-103, AVICEL RC-581, AVICEL-PH-105 (FMC Corp., Marcus Hook, PA); and mixtures thereof. Suitable fillers include, but are not limited to, talc, calcium carbonate, microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The amount of a binder or filler in the pharmaceutical compositions provided herein varies upon the type of formulation, and is readily discernible to those of ordinary skill in the art. The binder or filler may be present from about 50 to about 99% by weight in the pharmaceutical compositions provided herein.

[0273] Suitable diluents include, but are not limited to, dicalcium phosphate, calcium sulfate, lactose, sorbitol, sucrose, inositol, cellulose, kaolin, mannitol, sodium chloride, dry starch, and powdered sugar. Certain diluents, such as mannitol, lactose, sorbitol, sucrose, and inositol, when present in sufficient quantity, can impart properties to some compressed tablets that permit disintegration in the mouth by chewing. Such compressed tablets can be used as chewable tablets. The amount of a diluent in the pharmaceutical compositions provided herein varies upon the type of formulation, and is readily discernible to those of ordinary skill in the art.

[0274] Suitable disintegrants include, but are not limited to, agar; bentonite; celluloses, such as methylcellulose and carboxymethylcellulose; wood products; natural sponge; cation-exchange

resins; alginic acid; gums, such as guar gum and Veegum HV; citrus pulp; cross-linked celluloses, such as croscarmellose; cross-linked polymers, such as crospovidone; cross-linked starches; calcium carbonate; microcrystalline cellulose, such as sodium starch glycolate; polacrilin potassium; starches, such as corn starch, potato starch, tapioca starch, and pre-gelatinized starch; clays; aligns; and mixtures thereof. The amount of a disintegrant in the pharmaceutical compositions provided herein varies upon the type of formulation, and is readily discernible to those of ordinary skill in the art. The amount of a disintegrant in the pharmaceutical compositions provided herein varies upon the type of formulation, and is readily discernible to those of ordinary skill in the art. The pharmaceutical compositions provided herein may contain from about 0.5 to about 15% or from about 1 to about 5% by weight of a disintegrant.

[0275] Suitable lubricants include, but are not limited to, calcium stearate; magnesium stearate; mineral oil; light mineral oil; glycerin; sorbitol; mannitol; glycols, such as glycerol behenate and polyethylene glycol (PEG); stearic acid; sodium lauryl sulfate; talc; hydrogenated vegetable oil, including peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil; zinc stearate; ethyl oleate; ethyl laureate; agar; starch; lycopodium; silica or silica gels, such as AEROSIL® 200 (W.R. Grace Co., Baltimore, MD) and CAB-O-SIL® (Cabot Co. of Boston, MA); and mixtures thereof. The pharmaceutical compositions provided herein may contain about 0.1 to about 5% by weight of a lubricant.

[0276] Suitable glidants include, but are not limited to, colloidal silicon dioxide, CAB-O-SIL® (Cabot Co. of Boston, MA), and asbestos-free talc. Suitable coloring agents include, but are not limited to, any of the approved, certified, water soluble FD&C dyes, and water insoluble FD&C dyes suspended on alumina hydrate, and color lakes and mixtures thereof. A color lake is the combination by adsorption of a water-soluble dye to a hydrous oxide of a heavy metal, resulting in an insoluble form of the dye. Suitable flavoring agents include, but are not limited to, natural flavors extracted from plants, such as fruits, and synthetic blends of compounds which produce a pleasant taste sensation, such as peppermint and methyl salicylate. Suitable sweetening agents include, but are not limited to, sucrose, lactose, mannitol, syrups, glycerin, and artificial sweeteners, such as saccharin and aspartame. Suitable emulsifying agents include, but are not limited to, gelatin, acacia, tragacanth, bentonite, and surfactants, such as polyoxyethylene sorbitan monooleate (TWEEN® 20), polyoxyethylene sorbitan monooleate 80 (TWEEN® 80), and triethanolamine oleate. Suitable suspending and dispersing agents include, but are not limited to, sodium carboxymethylcellulose, pectin, tragacanth, Veegum, acacia, sodium carbomethylcellulose, hydroxypropyl methylcellulose, and polyvinylpyrrolidone. Suitable preservatives include, but are not limited to, glycerin, methyl and propylparaben, benzoic add,

sodium benzoate and alcohol. Suitable wetting agents include, but are not limited to, propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate, and polyoxyethylene lauryl ether. Suitable solvents include, but are not limited to, glycerin, sorbitol, ethyl alcohol, and syrup. Suitable non-aqueous liquids utilized in emulsions include, but are not limited to, mineral oil and cottonseed oil. Suitable organic acids include, but are not limited to, citric and tartaric acid. Suitable sources of carbon dioxide include, but are not limited to, sodium bicarbonate and sodium carbonate.

[0277] It should be understood that many carriers and excipients may serve several functions, even within the same formulation.

[0278] The pharmaceutical compositions provided herein for oral administration can be provided as compressed tablets, tablet triturates, chewable lozenges, rapidly dissolving tablets, multiple compressed tablets, or enteric-coating tablets, sugar-coated, or film-coated tablets. Enteric-coated tablets are compressed tablets coated with substances that resist the action of stomach acid but dissolve or disintegrate in the intestine, thus protecting the active ingredients from the acidic environment of the stomach. Enteric-coatings include, but are not limited to, fatty acids, fats, phenyl salicylate, waxes, shellac, ammoniated shellac, and cellulose acetate phthalates. Sugar-coated tablets are compressed tablets surrounded by a sugar coating, which may be beneficial in covering up objectionable tastes or odors and in protecting the tablets from oxidation. Film-coated tablets are compressed tablets that are covered with a thin layer or film of a water-soluble material. Film coatings include, but are not limited to, hydroxyethylcellulose, sodium carboxymethylcellulose, polyethylene glycol 4000, and cellulose acetate phthalate. Film coating imparts the same general characteristics as sugar coating. Multiple compressed tablets are compressed tablets made by more than one compression cycle, including layered tablets, and press-coated or dry-coated tablets.

[0279] The tablet dosage forms can be prepared from the active ingredient in powdered, crystalline, or granular forms, alone or in combination with one or more carriers or excipients described herein, including binders, disintegrants, controlled-release polymers, lubricants, diluents, and/or colorants. Flavoring and sweetening agents are especially useful in the formation of chewable tablets and lozenges.

[0280] The pharmaceutical compositions provided herein for oral administration can be provided as soft or hard capsules, which can be made from gelatin, methylcellulose, starch, or calcium alginate. The hard gelatin capsule, also known as the dry-filled capsule (DFC), consists of two sections, one slipping over the other, thus completely enclosing the active ingredient. The soft elastic capsule (SEC) is a soft, globular shell, such as a gelatin shell, which is plasticized by the addition of glycerin, sorbitol, or a similar polyol. The soft gelatin shells may contain a

preservative to prevent the growth of microorganisms. Suitable preservatives are those as described herein, including methyl- and propyl-parabens, and sorbic acid. The liquid, semisolid, and solid dosage forms provided herein may be encapsulated in a capsule. Suitable liquid and semisolid dosage forms include solutions and suspensions in propylene carbonate, vegetable oils, or triglycerides. Capsules containing such solutions can be prepared as described in U.S. Pat. Nos. 4,328,245; 4,409,239; and 4,410,545. The capsules may also be coated as known by those of skill in the art in order to modify or sustain dissolution of the active ingredient.

[0281] Coloring and flavoring agents can be used in all of the above dosage forms.

[0282] The pharmaceutical compositions provided herein for oral administration can be formulated as immediate or modified release dosage forms, including delayed-, sustained, pulsed-, controlled, targeted-, and programmed-release forms.

B. Parenteral Administration

[0283] The pharmaceutical compositions provided herein can be administered parenterally by injection, infusion, or implantation, for local or systemic administration. Parenteral administration, as used herein, include intravenous, intraarterial, intraperitoneal, intrathecal, intraventricular, intraurethral, intrasternal, intracranial, intramuscular, intrasynovial, intravesical, and subcutaneous administration.

[0284] The pharmaceutical compositions provided herein for parenteral administration can be formulated in any dosage forms that are suitable for parenteral administration, including solutions, suspensions, emulsions, micelles, liposomes, microspheres, nanosystems, and solid forms suitable for solutions or suspensions in liquid prior to injection. Such dosage forms can be prepared according to conventional methods known to those skilled in the art of pharmaceutical science (*see, Remington: The Science and Practice of Pharmacy*, supra).

[0285] The pharmaceutical compositions intended for parenteral administration can include one or more pharmaceutically acceptable carriers and excipients, including, but not limited to, aqueous vehicles, water-miscible vehicles, non-aqueous vehicles, antimicrobial agents or preservatives against the growth of microorganisms, stabilizers, solubility enhancers, isotonic agents, buffering agents, antioxidants, local anesthetics, suspending and dispersing agents, wetting or emulsifying agents, complexing agents, sequestering or chelating agents, cryoprotectants, lyoprotectants, thickening agents, pH adjusting agents, and inert gases.

[0286] Suitable aqueous vehicles include, but are not limited to, water, saline, physiological saline or phosphate buffered saline (PBS), sodium chloride injection, Ringers injection, isotonic dextrose injection, sterile water injection, dextrose and lactated Ringers injection. Suitable non-aqueous vehicles include, but are not limited to, fixed oils of vegetable origin, castor oil, corn oil, cottonseed oil, olive oil, peanut oil, peppermint oil, safflower oil, sesame oil, soybean oil,

hydrogenated vegetable oils, hydrogenated soybean oil, and medium-chain triglycerides of coconut oil, and palm seed oil. Suitable water-miscible vehicles include, but are not limited to, ethanol, 1,3-butanediol, liquid polyethylene glycol (e.g., polyethylene glycol 300 and polyethylene glycol 400), propylene glycol, glycerin, *N*-methyl-2-pyrrolidone, *N,N*-dimethylacetamide, and dimethyl sulfoxide.

[0287] Suitable antimicrobial agents or preservatives include, but are not limited to, phenols, cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoates, thimerosal, benzalkonium chloride (e.g., benzethonium chloride), methyl-and propyl-parabens, and sorbic acid. Suitable isotonic agents include, but are not limited to, sodium chloride, glycerin, and dextrose. Suitable buffering agents include, but are not limited to, phosphate and citrate. Suitable antioxidants are those as described herein, including bisulfite and sodium metabisulfite. Suitable local anesthetics include, but are not limited to, procaine hydrochloride. Suitable suspending and dispersing agents are those as described herein, including sodium carboxymethylcelluose, hydroxypropyl methylcellulose, and polyvinylpyrrolidone. Suitable emulsifying agents are those described herein, including polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate 80, and triethanolamine oleate. Suitable sequestering or chelating agents include, but are not limited to EDTA. Suitable pH adjusting agents include, but are not limited to, sodium hydroxide, hydrochloric acid, citric acid, and lactic acid. Suitable complexing agents include, but are not limited to, cyclodextrins, including α-cyclodextrin, β-cyclodextrin, hydroxypropyl-β-cyclodextrin, sulfobutylether-β-cyclodextrin, and sulfobutylether 7-β-cyclodextrin (CAPTISOL®, CyDex, Lenexa, KS).

[0288] When the pharmaceutical compositions provided herein are formulated for multiple dosage administration, the multiple dosage parenteral formulations must contain an antimicrobial agent at bacteriostatic or fungi static concentrations. All parenteral formulations must be sterile, as known and practiced in the art.

[0289] In one embodiment, the pharmaceutical compositions for parenteral administration are provided as ready-to-use sterile solutions. In another embodiment, the pharmaceutical compositions are provided as sterile dry soluble products, including lyophilized powders and hypodermic tablets, to be reconstituted with a vehicle prior to use. In yet another embodiment, the pharmaceutical compositions are provided as ready-to-use sterile suspensions. In yet another embodiment, the pharmaceutical compositions are provided as sterile dry insoluble products to be reconstituted with a vehicle prior to use. In still another embodiment, the pharmaceutical compositions are provided as ready-to-use sterile emulsions.

[0290] The pharmaceutical compositions provided herein for parenteral administration can be formulated as immediate or modified release dosage forms, including delayed-, sustained, pulsed-, controlled, targeted-, and programmed-release forms.

[0291] The pharmaceutical compositions provided herein for parenteral administration can be formulated as a suspension, solid, semi-solid, or thixotropic liquid, for administration as an implanted depot. In one embodiment, the pharmaceutical compositions provided herein are dispersed in a solid inner matrix, which is surrounded by an outer polymeric membrane that is insoluble in body fluids but allows the active ingredient in the pharmaceutical compositions diffuse through.

[0292] Suitable inner matrixes include, but are not limited to, polymethylmethacrylate, polybutyl-methacrylate, plasticized or unplasticized polyvinylchloride, plasticized nylon, plasticized polyethylene terephthalate, natural rubber, polyisoprene, polyisobutylene, polybutadiene, polyethylene, ethylene-vinyl acetate copolymers, silicone rubbers, polydimethylsiloxanes, silicone carbonate copolymers, hydrophilic polymers, such as hydrogels of esters of acrylic and metliacrylic acid, collagen, cross-linked polyvinyl alcohol, and cross-linked partially hydrolyzed polyvinyl acetate.

[0293] Suitable outer polymeric membranes include but are not limited to, polyethylene, polypropylene, ethylene/propylene copolymers, ethylene/ethyl acrylate copolymers, ethylene/vinyl acetate copolymers, silicone rubbers, polydimethyl siloxanes, neoprene rubber, chlorinated polyethylene, polyvinylchloride, vinyl chloride copolymers with vinyl acetate, vinylidene chloride, ethylene and propylene, ionomer polyethylene terephthalate, butyl rubber epichlorohydrin rubbers, ethylene/vinyl alcohol copolymer, ethylene/vinyl acetate/vinyl alcohol terpolymer, and ethylene/vinyloxyethanol copolymer.

V. Examples

[0294] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

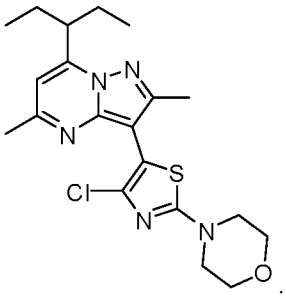
Example 1. A Phase 2, Open-Label Study to Evaluate the Efficacy and Safety of Compound 1 in Subjects with Testicular Adrenal Rest Tumors

[0295] Testicular adrenal rest tumors (TART) is a common complication in congenital adrenal hyperplasia (CAH) that is caused by adrenocorticotropin hormone (ACTH)–driven

overstimulation of aberrant adrenal cells within the testes and results in impaired spermatogenesis and infertility. TARTS can be found anywhere on the testes, though a typical (very common) location is within the mediastinum. This is a particularly problematic location. Because of the central location of TARTs being in the mediastinum, compression of the nearby seminiferous tubules is common and can lead to obstructive azoospermia, irreversible damage to surrounding tissue, and permanent infertility in males.

[0296] Current treatment for TART consists of chronic high-dose glucocorticoids, a problematic therapy with significant side effects and a narrow therapeutic window. Given the serious nature of TART and the limitations and risks of chronic steroid therapy, new treatment modalities are needed for patients with TART. Compound 1, a high-affinity and selective small-molecule antagonist of the corticotropin-releasing factor type-1 (CRF_1) receptor, may be effective in blocking ACTH synthesis, thereby reducing the size of testicular lesions in TART and reducing the need for chronic supraphysiologic doses of glucocorticoids. This mechanism of action has been validated in a previous proof-of-concept study using a similar small-molecule CRF_1 receptor antagonist, which produced dose-dependent reductions in ACTH and 17-hydroxyprogesterone (17-OHP) in subjects with CAH. Compound 1 has been shown to have an acceptable safety and tolerability profile in nonclinical toxicology studies and in 2 completed Phase 1 clinical studies in healthy volunteers. These promising results provide a rationale for investigating the use of Compound 1 in the treatment of TART, in combination with replacement glucocorticoids and mineralocorticoids.

[0297] The structure of Compound 1 is:



[0298] Building upon the 2 completed Phase 1 studies of Compound 1 in healthy volunteers and the ongoing Phase 2 proof-of-concept study of Compound 1 in subjects with CAH, this Phase 2 will study Compound 1 specifically in male CAH subjects with TART for the first time. A sample size of approximately 12 subjects was selected to provide sufficient data for an initial evaluation of efficacy and safety in subjects with TART while maintaining feasible subject recruitment goals for this rare condition. Given the rarity of the disease and thus the small sample size that can be recruited, this study will be open label, and all enrolled subjects will

receive active study drug. Subjects will serve as their own controls in that their outcome measures after 12 weeks of study drug dosing will be compared to their baseline measures.

[0299] The schedule of study visits (every 2 weeks through Week 4, then every 4 weeks for the remainder of the study) provides an appropriate balance between minimizing subject burden and closely monitoring subject safety and response to therapy. The 4-week post-treatment washout period will allow for adequate safety follow-up and analysis of any HPA axis rebound after the last dose of study drug.

[0300] The specification of study drug dosing at 10pm and after consumption of a snack is based on information from the Phase 1 studies in healthy volunteers. The plasma C_{max} (maximum concentration) for Compound 1 in the Phase 1 studies occurred approximately 5 hours after dosing. Therefore, Compound 1 will be dosed daily at 10pm in this study in order to achieve peak plasma study drug concentration at the time of day when most adrenal androgens are produced. Subjects will consume a standardized snack before taking study drug because the Phase 1 studies showed minimal study drug absorption when subjects were dosed in a fasted state. Specific dose reduction criteria, strict individual treatment stopping criteria, and appropriate laboratory and medical assessments have been established to ensure subject safety. (**FIGS. 1A-1B**).

Objectives	Endpoints
Primary	
• To assess the efficacy of Compound 1 in subjects with TART	• Reduction in the size/volume of TART on testicular ultrasound from screening to Week 12
Secondary	
• To evaluate the safety of Compound 1 in subjects with TART	• Adverse events (AEs), serious adverse events (SAEs), AEs leading to withdrawal, AEs of special interest (AESIs) • Physical examination findings • Vital signs • Changes in electrocardiogram (ECG) • Changes in clinical laboratory values
• To assess the pharmacodynamic (PD) biomarker effects of Compound 1	• Changes in plasma concentrations of key PD biomarkers, including ACTH, 17-OHP, testosterone, androstenedione, luteinizing hormone (LH),

	follicle-stimulating hormone (FSH), inhibin B, sex hormone–binding globulin (SHBG), renin, and aldosterone from baseline to Week 12
• To assess changes on semen analysis	• Changes in sperm count, morphology, and motility from baseline to Week 12
• To assess changes in metabolism	• Changes in metabolic parameters, including fasting glucose, HbA1c, insulin sensitivity, and lipid profile (including LDL cholesterol, HDL cholesterol, and triglycerides), from baseline to Week 12 • Changes in body weight, body mass index (BMI), waist circumference, and blood pressure
• To evaluate changes in quality of life (QoL) and mood	• Changes in QoL measured using the Short Form 36 (SF-36) • Changes in mood measured using the Hospital Anxiety and Depression Scale (HADS)

Study Population

[0301] Approximately 12 male subjects with TART will be enrolled at approximately 6 investigative sites in the United States, United Kingdom, and possibly other countries. Up to 12 additional subjects may be added to further evaluate specific safety, PD, or efficacy findings.

Eligibility Criteria

Inclusion Criteria

- Adult male subjects aged 18 to 30, inclusive
- Documented historical diagnosis of CAH based on documented genetic mutation or historical documentation of elevated 17-OHP
- Diagnosis of TART via testicular ultrasound and/or magnetic resonance imaging (MRI) at screening with ≥1 lesion(s) measuring ≥2 cm in diameter
- On a stable regimen of GC replacement for ≥30 days before baseline
- Agree to follow contraception guidelines and refrain from donating sperm throughout the treatment period and for 90 days after the last dose of study drug
- Subject is able to understand all study procedures and risks involved and provides written informed consent indicating willingness to comply with all aspects of the protocol

Exclusion Criteria

- Any clinically significant unstable medical condition, medically significant illness, or chronic disease within 30 days of screening, including but not limited to:

  - A malignancy or <3 years of remission history from any malignancy, other than successfully treated localized skin cancer
  - Presence of clinically significant renal disease, as evidenced by an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73m^2
  - Current or chronic history of liver disease or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones)
  - Confirmed positive test at screening for active hepatitis B, hepatitis C, or human immunodeficiency virus (HIV)
- Any clinically significant psychiatric disorders, including any current major depressive episode, bipolar disorder, schizophrenia, schizoaffective disorder, major depressive disorder with psychotic features, or any other psychotic disorder within the preceding 6 months
- HADS score >12 for either depression or anxiety
- At increased risk of suicide on the basis of the Investigator's judgment or the results of the Columbia-Suicide Severity Rating Scale (C-SSRS) conducted at screening and baseline (eg, C-SSRS Type 3, 4, or 5 ideation during the preceding 6 months or any suicidal behavior within the past 12 months)
- Clinically significant and abnormal clinical or laboratory assessments must be discussed with the Medical Monitor to determine eligibility for this study. Abnormal assessments that must be reviewed to determine eligibility include but are not limited to:
  - Clinically meaningful abnormal ECG results, in the opinion of the Investigator
  - Fridericia-corrected QT interval (QTcF) >450 msec
  - Alanine aminotransferase (ALT) >2x upper limit of normal (ULN)
  - Bilirubin >1.5x ULN (isolated bilirubin >1.5x ULN is acceptable if bilirubin is fractionated and direct bilirubin is <35%)
- A history that includes bilateral adrenalectomy or hypopituitarism
- Use of any other investigational drug within 30 days or 5 half-lives, whichever is longer, before initial screening
- Require use of prohibited concomitant medications, including strong inhibitors and/or inducers of CYP3A4 (with the exception of glucocorticoids, hydrocortisone, and dexamethasone) within 30 days or 5 half-lives (whichever is longer) of first dose of study drug. Medications that are sensitive substrates or substrates with narrow therapeutic ranges (metabolized by CYP3A4, 2C8, 2C9, or 2C19) should be discussed on a case-by-case basis with the Medical Monitor to determine whether the medication should be discontinued or may be continued with caution. If washout is feasible, then the medication should be

withdrawn at least 30 days or 5 half-lives (whichever is longer) before the first dose of study drug.

- Donation of blood within 60 days before first dose of study drug, or donation of platelets, white blood cells, or plasma within 15 days before first dose of study drug.

Study Duration

[0302] The expected duration of study participation for each subject is up to approximately 20 weeks. This includes a screening period of ≤4 weeks, a treatment period of 12 weeks, and a safety follow-up period of 4 weeks.

Study Design

[0303] This is a Phase 2, open-label efficacy and safety study of Compound 1 for the treatment of TART. All subjects will receive a daily oral dose of Compound 1 for 12 weeks.In-clinic study visits will occur for screening and at Day 1 (baseline) and Weeks 2, 4, 8, 12 (end of treatment), and 16 (follow-up).

Dose, Route, Regimen

[0304] All subjects will receive a daily oral dose of Compound 1 for 12 weeks. Subjects will receive 600 mg/day during the first 2 weeks and 1000 mg/day during the remaining 10 weeks of the treatment period. Study drug will be taken daily at 10pm (or bedtime, if earlier), 5 to 15 minutes after consumption of a standardized snack.

[0305] On mornings of study visits with 8am blood draws for laboratory assessments, subjects should take their morning glucocorticoid replacement after the blood draws. For all other days during the study, subjects may take their morning glucocorticoid replacement at their usual time (generally between 6am and 8am).

[0306] If a subject experiences a Grade 3 or higher TEAE considered at least possibly related to study drug, the Investigator may reduce the subject's daily dose of Compound 1 down in increments of 200 mg. Study drug will be discontinued in subjects who experience clinically significant liver chemistry, QTcF, or other individual treatment stopping criteria.

Investigational Drug

[0307] Compound 1 is a small-molecule CRF_1 receptor antagonist and will be supplied as 200-mg, size 1, white, hard gelatin capsules.

Example 2.

[0308] Since elevated ACTH is the primary driver for TART growth, blocking ACTH synthesis using Compound 1, a small-molecule antagonist of the CRF_1 receptor, may be effective in reducing ACTH levels, thereby reducing the size of testicular lesions in TART and reducing the need for chronic supraphysiologic doses of GCs. In CAH, this mechanism of action has been

validated in a previous proof-of-concept study using a similar small-molecule CRF_1 receptor antagonist, which produced dose-dependent reductions in ACTH and 17-OHP in subjects with CAH. The safety profile of Compound 1 has been established in nonclinical toxicology studies and in 2 completed Phase 1 clinical studies in healthy volunteers. A Phase 2 study in subjects with CAH is currently ongoing.

Nonclinical Toxicology of Compound 1

[0309] The nonclinical safety profile of Compound 1 has been evaluated in repeat-dose range-finding and definitive toxicology studies of up to 91 days in duration in rats and dogs using the clinically relevant route (oral) and schedule of administration (once daily). In rats and beagle dogs administered oral Compound 1 once daily for 91 consecutive days at doses up to 2000 mg/kg/day, toxicologic findings were all mild and limited to the male reproductive tract, and all Compound 1-related findings were reversible following a 6-week treatment-free recovery period.

[0310] Additionally, a fertility toxicology study was conducted in rats. In this study, male rats were administered oral Compound 1 once daily at 0, 5, 20, or 1000 mg/kg for 85 to 87 days (70 days before mating, during mating, and ≤4 days between mating and necropsy). Male reproductive performance (mean day of mating, mating and fertility indices, and conception rates) was unaffected by Compound 1 treatment. Untreated females mated with the Compound 1-treated males had no ovarian and uterine effects.

[0311] In addition, a complete battery of standard genotoxicity tests consisting of in vitro bacterial reverse mutation and chromosome aberration assays and an in vivo (rat) bone marrow micronucleus assay has been conducted. Exploratory and definitive embryo-fetal development studies in rabbits and a bovine corneal opacity and permeability assay have also been conducted.

Clinical Experience with Compound 1

[0312] To date, a total of 53 subjects, all healthy adults aged 21 to 65 years, have been treated with at least 1 dose of Compound 1 in 2 Phase 1 studies. The first-in-human study of Compound 1 (Study I3C-FW-BLAA) evaluated single ascending doses of 2 mg to 800 mg. In this study, a small number of subjects experienced diarrhea and headache of mild or moderate severity. A subsequent study (Study I3C-FW-BLAB) evaluated repeat doses of 50 mg, 100 mg, or 200 mg Compound 1 once daily for 14 consecutive days. A small number of subjects in the repeat-dose study experienced dyspnea, rhinorrhea, palpitations, and headache. The Phase 1 studies showed no clinically significant alterations in safety laboratory values, vital signs, or electrocardiograms (ECGs). Compound 1 has been well tolerated in healthy human subjects, with no SAEs, AEs leading to discontinuation, or dose-limiting toxicities (DLTs). A maximum tolerated dose has not been reached in humans.

[0313] An ongoing Phase 2 open-label study (Study Compound 2) is currently evaluating the safety and efficacy of Compound 1 dosed once daily for 6 weeks in adults with classic CAH. In the initial Cohort A of this study, all subjects are dosed starting at 200 mg/day for 14 days, escalating for each subject to 600 mg/day for 14 days, then 1000 mg/day for 14 days if the subject experiences no DLTs at the previous dose level. (**FIGS. 2-3**).

Known Potential Risks

[0314] Because the reversible testicular effects (degeneration/atrophy) observed in rats and dogs treated with high-dose Compound 1 (2,000 mg/kg/day) are likely associated with on-target (mechanism-driven) effects, these effects are likely to be relevant to human risk. However, male reproductive studies in rodents showed no impairment in fertility at the highest dose tested (1,000 mg/kg/day). Additionally, there were no effects on clinical laboratory markers of testicular function (LH and FSH) when Compound 1 was administered to healthy male volunteers at doses up to 200 mg per day for 14 consecutive days. Clinical laboratory measures of testicular function (LH, FSH, inhibin B, and SHBG) will be assessed throughout this study to monitor the risk of testicular injury. Testicular ultrasounds and semen analysis will also be performed at screening/baseline and the end of the treatment period.

[0315] Laboratory abnormalities in liver function tests (LFTs) have been observed at significantly higher doses in nonclinical studies. However, these events were not observed in the Phase 1 clinical studies. Clinical laboratory measures of liver chemistry will be assessed throughout this study, and strict individual treatment stopping criteria for liver chemistry findings are in place.

[0316] Compound 1-induced effects on the thyroid gland were noted in dogs after 91 days of high-dose (2,000 mg/kg/day) treatment but not in any other nonclinical studies. Clinical pathology parameters of thyroid function (eg, T4, T3, thyroid-stimulating hormone [TSH]) were not evaluated in the Phase 1 studies but will be monitored throughout this study.

Known Potential Benefits

[0317] Early clinical studies suggests that Compound 1 is effective as a CRF_1 receptor antagonist, a mechanism of action that supports the clinical investigation of Compound 1 for treatment of TART. In CAH, this mechanism of action has been validated in a previous proof-of-concept study using a similar small-molecule CRF_1 receptor antagonist, which produced dose-dependent reductions in ACTH and 17-OHP in subjects with CAH.

Assessment of Potential Risks and Benefits

[0318] The mild and reversible toxicologic effects of Compound 1 on the male reproductive tract in nonclinical studies occurred in the context of animals with normal, rather than elevated, baseline hormone function. As a CRF_1 receptor antagonist, Compound 1 is intended to reduce

abnormally elevated ACTH levels, which in TART drives overstimulation of aberrant adrenal cells within the testes. In nonclinical models where baseline hormone levels are within normal ranges, clinical benefits are not expected, and indeed, toxicities may be expected as a result of androgen reduction outside of the normal range. In TART, where baseline ACTH and androgen levels are pathologically elevated, androgen reduction would be considered a positive, therapeutic effect.

[0319] Given the serious nature of TART and the limitations (and risks) of chronic steroid therapy, new treatment modalities are needed for patients with TART. Given the acceptable safety and tolerability profile of Compound 1 in healthy volunteers from the Phase 1 program, the low probability of demonstrating meaningful PD marker changes in healthy subjects, and the lack of any clear benefit to exposing more healthy subjects to study drug, the Sponsor believes the benefit-to-risk profile favors the entry of Compound 1 into Phase 2 in subjects with TART.

Example 3. Congenital Andrenal Hyperplasia (CAH)

[0320] CAH results from a genetic defect in the 21-hydroxylase enzyme. CAH patients are thus unable to produce cortisol. Without cortisol to provide negative feedback, patients over-produce ACTH. High ACTH promotes the overproduction of potent androgens (male sex hormones). Without wishing to be bound by theory, Compound 1 blocks CRF_1 receptor to prevent ACTH release by the pituitary and associated drive on the hypothalamic-pituitary-adrenal axis. Replacement of cortisol to physiological levels will correct cortisol deficiency, however, will not lower ACTH, resulting in hyperplasia of adrenal gland and associated overproduction of adrenal androgens.

[0321] As shown in **FIGS. 4** and **5A-5B**, the attenuation of ACTH demonstrates target engagement and functional CRF_1 receptor antagonism. 80% of subjects demonstrated reduction in ACTH. 70% of subjects demonstrated > 25% reduction in ACTH. 30% of subjects were in the normal range (*) after treatment with a CRF_1 receptor antagonist.

[0322] **FIGS. 6** and **7A-7B** demonstrate "control" of disease based on standard guidelines, which would then allow steroid taper. 80% of subjects demonstrated reduction in 17-OHP. 50% of subjects demonstrated > 25% reduction in ACTH. 50% of subjects were in the guideline range (*; 1200 ng/dL) after treatment with a CRF_1 receptor antagonist.

[0323] **FIGS. 8** and **9A-9B** demonstrate attenuation of morning rise in A4 upon treatment with a CRF1 antagonist, indicative of its ability to control excess andreogen production and associated symptoms. 100% of subjects demonstrated reduction in androstenedione (at various doses). 60% of subjects demonstrated > 25% reduction in androstenedione. 50% of subjects were within the normal reference range after treatment with a CRF_1 receptor antagonist.

[0324] FIGS. 10A-10B show approximately 25% shrinkage in TART in a 25 year old male with classic congenital adrenal hyperplasia following 6 weeks treatment with a CRF_1 receptor antagonist.

[0325] FIGS. 11A-11B demonstrate Compound 1 is a potent, selective, and functional CRF_1 receptor antagonist. In in vitro and ex vivo studies, Compound 1 was determined to be a potent and selective CRF_1 receptor antagonist with high affinity and selective for the $hCRF_1$ receptor. Compound 1 was found to have functional potency to inhibit adenylate cyclase activity (CRF_1 receptor-mediated downstream cascade), demonstrating in vitro efficacy as a CRF_1 receptor antagonist. Compound 1 was found to significantly inhibit CRF-induced locomotor behavior in response to i.c.v. administration of CRF (**FIG. 11B**).

[0326] FIGS. 12A-12B demonstrate single pill of CRF_1 receptor antagonist improves ACTH and 17-OHP in CAH patients.

[0327] FIG. 13 shows the subject level hormone changes (ACTH, 17-OHP, and androstenedione) by dose of a CRF_1 receptor antagonist.

Example 4. Assess reduction in excess ACTH and adrenal steroid production

[0328] The objectives of the study are: to assess the ability of Compound 1 to reduce excess ACTH and adrenal steroid production in participants with classic CAH on a stable gluococorticoid regimen; to evaluate the safety and tolerability of tildacerfont in participants with classic CAH; and to evaluate the pharmacokinetics (PK) of tildacerfont.

ELIGIBILITY CRITERIA

[0329] Inclusion Criteria:

- Adult males and females 18 years or older
- Diagnosis of classic CAH due to 21-hydroxylase deficiency
- Morning 17OHP ≥800 ng/dL, before taking any morning GC dose
- On a stable regimen of gluococorticoid replacement for ≥30 days before enrollment

[0330] Exclusion Criteria:

- Clinically significant unstable medical condition, illness, or chronic disease within 30 days of screening
- Clinically significant psychiatric disorder
- History of bilateral adrenalectomy or hypopituitarism

STUDY DESIGN

[0331] FIG. 14 depicts the proof of concept and escalating QD dosing at 10 PM for Cohort A and the single dose level and BID dosing at 10 AM and 10 pm for Cohort B.

BASELINE CHARACTERISTICS

[0332] Table 1 provides a description of the baseline characteristics displayed by Cohort A and Cohort B. In Cohort A, 10 partcipants were enrolled and received at least 1 dose of Compound 1. One participant withdrew early because of an occupation-related scheduling conflict. In Cohort B, 9 participants were enrolled and received at least one dose of Compound 1. One participant was withdrawn upon discovery of a major protocol violation at Baseline and did not have postbaseline efficacy/PK data. Thus, baseline characteristics are calculated for n=8 in Cohort B. One participant in Cohort B was taking both HC and prednisone.

Table 1 – Baseline characteristics of each cohort

Parameter	Cohort A (n=10)	Cohort B (n=8)
Demographics		
Age (years), mean (range)	39.3 (24-66)	36.8 (19-66)
Sex	5M, 5F	1M, 7F
Bmi (kg/m^2), mean ± SD	31.5 ± 12.0	31.1 ± 6.7
Daily GC dose (mg HC equivalents), mean range	28 (13-40)	35 (20-50)
Types of GCs	4 JC, 3 pred, 3 dex	2HC, 4 pred, 3 dex
17OHP at screening[a] (ng/dl), mean ± SD	5,985 ± 6,347	4,592 ±4,624

Abbreviations: 17OHP: 17-hydroxyprogesterone; BMI: body mass index; dex: dexamethasone; F: female; GC: glucocorticoid; HC: hydrocortisone; M: male; pred: prednisone; SD: standard deviation.
a 17OHP reference ranges: 27-199 ng/dL males, 15-290 ng/dL females.

SAFETY

[0333] Across both cohorts, Compound 1 was generally well tolerated. The most common type of adverse event (AE) was gastrointestinal disorders There were no serious AEs and no AEs leading to withdrawal.

PHARMACOKINETICS

[0334] Over a wide range of doses (200 to 1000 mg QD), Compound 1exhibited a predictable, dose-dependent PK profile.

EFFICACY

[0335] FIG. 15A-15D show the changes in levels of Androstenedione (A4) across both cohorts. Levels of A4 improved at ≥1 dose level for 10 out of 10 participants in Cohort A and 6 out of 8 participants in Cohort B. Excess A4 is associated with clinical sequelae in CAH. Attenuation of A4 demonstrates control of adrenal androgen.

[0336] FIG. 16A-16D show the changes in levels of 17-hydroxyprogesterone (17-OHP) across both cohorts. Levels of 17OHP improved at ≥1 dose level for 8 out of 10 participants in Cohort A and 6 out of 7 participants in Cohort B. 17OHP is a critical hormone in CAH diagnosis and management. Attenuation of 17OHP demonstrates control of adrenal steroid production.

[0337] FIG. 17A-17D show the changes in levels of adrenocorticotropic hormone (ACTH) across both cohorts. Levels of ACTH improved at ≥1 dose level for 8 out of 10 participants in

Cohort A and 5 out of 7 participants in Cohort B. Attenuation of ACTH demonstrates target engagement and fucntional CRF_1 receptor antagonism.

Table 2 – Percentage change from baseline in ACTH, 17OHP and A4.

	Cohort A			Cohort B
	200 mg QD (Week 2)	600 mg QD (Week 4)	1000 mg QD (Week 6)	200 mg BID (Weed 2)
ACTH	-25.7 (±51.6)	-60.1 (±37.5)	-32.7 (±47.0)	-36.5 (±58.0)
17OHP	+22.2 (±172.3)	-23.7 (±64.2)	-13.8 (±74.7)	-47.8 (±63.3)
A4	-12.1 (±47.4)	-33.2 (±36.5)	-28.6 (±44.6)	-16.0 (±81.4)

CONCLUSIONS

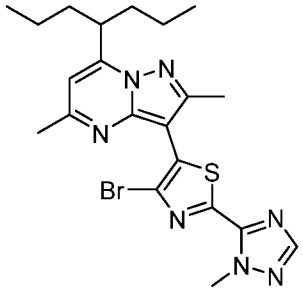
{0338} Compound 1, a potent and selective CRF_1 receptor antagonist, offers a novel nonsteroidal oral treatment approach for CAH. Compound 1 was well tolerated and efficacious in reducing excess ACTH, 17OHP, and androstenedione in patients with classic CAH. Compound 1 exhibited a predictable, dose-dependent PK profile suitable for the target population.

{0339} While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby

CLAIMS

WHAT IS CLAIMED IS:

1. A method of treating an endocrine disorder associated with corticotropin-releasing factor type-1 (CRF_1) activity, comprising administering to a subject in need thereof a small molecule CRF_1 antagonist, or a pharmaceutically acceptable salt thereof, wherein the small molecule CRF_1 antagonist is: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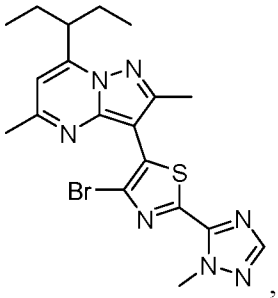
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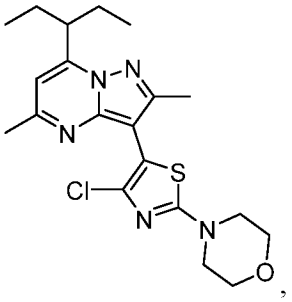
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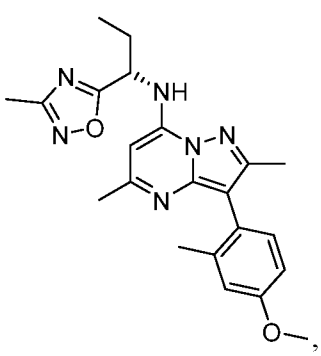
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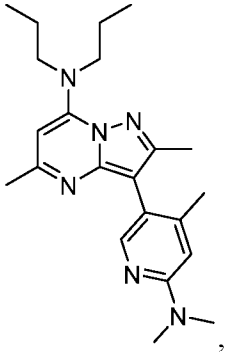
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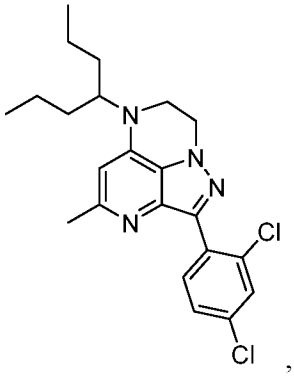
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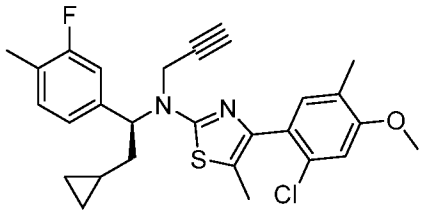
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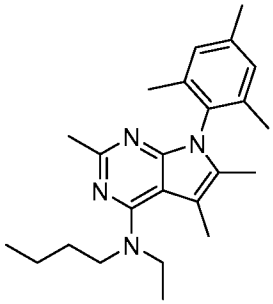
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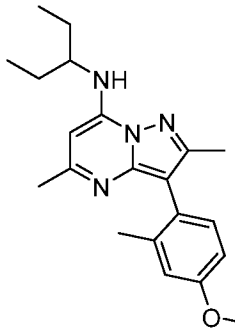
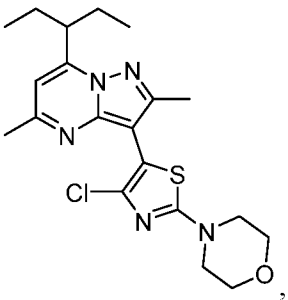
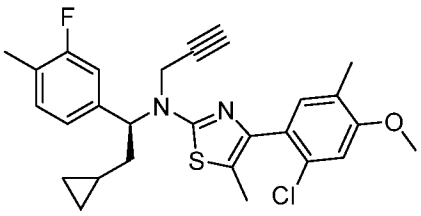

, or a pharmaceutically acceptable salt thereof.

2. The method of claim 1, wherein the small molecule CRF_1 antagonist is:



, or a pharmaceutically acceptable salt thereof.

3. The method of claim 1, wherein the small molecule CRF_1 antagonist is:



, or a pharmaceutically acceptable salt thereof.

4. The method of claim 1, wherein the endocrine disorder associated with CRF_1 activity is a testicular adrenal rest tumor (TART) or an ovarian adrenal rest tumor (OART).
5. The method of claim 1, wherein the subject has congenital adrenal hyperplasia (CAH).
6. The method of claim 1, wherein the subject is receiving a glucocorticoid in conjunction with the administering of the small molecule CRF_1 antagonist.
7. The method of claim 6, wherein the glucocorticoid comprises beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone, or a combination thereof.
8. The method of claim 7, wherein the glucocorticoid is hydrocortisone.
9. The method of claim 1, wherein the subject is an adolescent.
10. The method of claim 1, wherein the subject is at least 18 years old.
11. The method of claim 1, wherein the small molecule CRF_1 antagonist is administered at a dose of about 10 mg/day to about 400 mg/day to the subject.
12. The method of claim 11, wherein the small molecule CRF_1 antagonist is administered at a dose of about 50 mg.
13. The method of claim 11, wherein the small molecule CRF_1 antagonist is administered at a dose of about 100 mg.
14. The method of claim 11, wherein the small molecule CRF_1 antagonist is administered at a dose of about 200 mg.
15. The method of claim 11, wherein the small molecule CRF_1 antagonist is administered at a dose of about 400 mg.
16. The method of claim 1, wherein the small molecule CRF_1 antagonist is administered to the subject once per day.

17. The method of claim 1, wherein the small molecule CRF_1 antagonist is administered to the subject twice per day.
18. The method of claim 16, wherein the subject is in a fed state.
19. The method of claim 17, wherein the subject is in a fed state.
20. The method of claim 1, wherein the small molecule CRF_1 antagonist is administered orally.
21. The method of claim 1, wherein the small molecule CRF_1 antagonist is formulated as a capsule or tablet.

ABSTRACT OF THE DISCLOSURE

Provided herein are compounds and pharmaceutical compositions for the prevention and treatment of testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART).

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
19/043,275	**01/31/2025 07:27:41 PM Z ET**	**50535-710.304**

Title of Invention

METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	5486	FILED BY	Colleen Mensching
PATENT CENTER #	69010276	FILING DATE	-
CUSTOMER #	21971	FIRST NAMED INVENTOR	Alexis HOWERTON
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Victoria Cheng

Documents

TOTAL DOCUMENTS: 6

DOCUMENT		PAGES	DESCRIPTION	SIZE (KB)
Spruce Biosciences Inc. 50535-710.304 - ADS.pdf		9	Application Data Sheet	2180 KB
Spruce Biosciences Inc. 50535-710.304 - Figures.pdf		14	Drawings-other than black and white line drawings	1089 KB
Spruce Biosciences Inc. 50535-710.304 - Track 1 Request.pdf		2	Track One Request	174 KB
Spruce Biosciences Inc. 50535-710.304 - Specification.pdf		116	-	1638 KB
Spruce Biosciences Inc. 50535-710.304 - Specification-SPEC.pdf	(1-112)	112	Specification	1663 KB

Spruce Biosciences Inc. 50535-710.304 - Specification-CLM.pdf	(113-115)	3	Claims	124 KB
Spruce Biosciences Inc. 50535-710.304 - Specification-ABST.pdf	(116-116)	1	Abstract	87 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage

submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/14 (01-22)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50535-710.304
	Application Number	
Title of Invention	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Alexis		HOWERTON	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	San Francisco	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	611 Gateway Boulevard, Suite #740		
Address 2			
City	South San Francisco	State/Province	CA
Postal Code	94080	Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Hal		GERBER	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	San Francisco	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	611 Gateway Boulevard, Suite #740		
Address 2			
City	South San Francisco	State/Province	CA
Postal Code	94080	Country	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50535-710.304
	Application Number	
Title of Invention	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS	

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	21971		
Email Address	patentdocket@wsgr.com	Add Email	Remove Email

Application Information:

Title of the Invention	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS		
Attorney Docket Number	50535-710.304	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	14	**Suggested Figure for Publication (if any)**	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	21971		

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50535-710.304
	Application Number	
Title of Invention	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS	

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	18941832	2024-11-08
Prior Application Status	Abandoned		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
18941832	Continuation of	17667285	2022-02-08
Prior Application Status	Patented		Remove

Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
17/667285	Continuation of	17/081694	2020-10-27	11304950	2022-04-19

Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
17/081694	Continuation of	PCT/US2019/029486	2019-04-26
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2019/029486	Claims benefit of provisional	62/822815	2019-03-23
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2019/029486	Claims benefit of provisional	62/663951	2018-04-27

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50535-710.304
	Application Number	
Title of Invention	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS	

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50535-710.304
	Application Number	
Title of Invention	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50535-710.304
	Application Number	
Title of Invention	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	Spruce Biosciences, Inc.		
Mailing Address Information For Applicant:			
Address 1	611 Gateway Boulevard, Suite #740		
Address 2			
City	South San Francisco	**State/Province**	CA
Country	US	Postal Code	94080
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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Approved for use through 05/31/2024. OMB 0651-0032
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50535-710.304
	Application Number	
Title of Invention	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

Remove

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Victoria Cheng/			Date (YYYY-MM-DD)	2025-01-31
First Name	Victoria	Last Name	Cheng	Registration Number	79641

Additional Signature may be generated within this form by selecting the Add button. Add

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50535-710.304
	Application Number	
Title of Invention	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria,** V**A 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
19/043,275	**01/31/2025 07:27:41 PM Z ET**	**50535-710.304**

Title of Invention

METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	5486	FILED BY	Colleen Mensching
PATENT CENTER #	69010276	AUTHORIZED BY	Victoria Cheng
CUSTOMER #	21971	FILING DATE	-
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Alexis HOWERTON

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
DA / 232415	**E20251UJ29217440**	**Colleen Mensching**

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
1011	BASIC FILING FEE - UTILITY (PAPER FILING ALSO REQUIRES NON-ELECTRONIC FILING FEE UNDER 1.16(T))	350.00	1	350.00
1111	UTILITY PATENT APPL. SEARCH FEE	770.00	1	770.00
1311	PATENT APPL. EXAMINATION FEE	880.00	1	880.00
1817	REQUEST FOR PRIORITIZED EXAMINATION	4515.00	1	4515.00
1051	SURCHARGE- LATE FILING FEE, SEARCH FEE, EXAMINATION FEE, INVENTOR'S OATH OR DECLARATION, OR APPLICATION FILED WITHOUT AT LEAST ONE	170.00	1	170.00

	CLAIM OR BY REFERENCE			
1054	Non-DOC-X Filing Surcharge	430.00	1	430.00
1830	PROCESSING FEE, EXCEPT IN PROVISIONAL APPLICATIONS	150.00	1	150.00
1202	EACH CLAIM IN EXCESS OF 20	200.00	1	200.00
			TOTAL AMOUNT:	**$7,465.00**

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

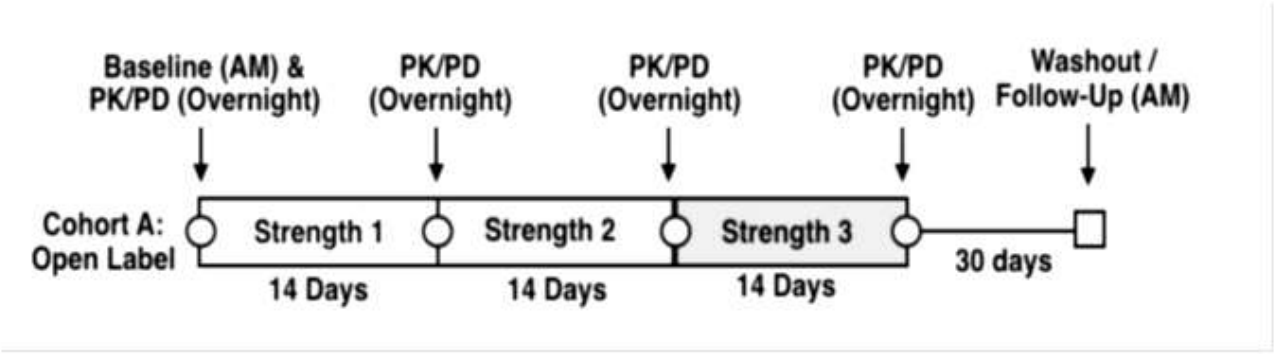
Baseline (AM) &
PK/PD (Overnight)
PK/PD
(Overnight)
PK/PD
(Overnight)
PK/PD
(Overnight)
Washout /
Follow-Up (AM)
Cohort A:
Open Label
Strength 1
Strength 2
Strength 3
30 days
14 Days
14 Days
14 Days

FIG. 1A

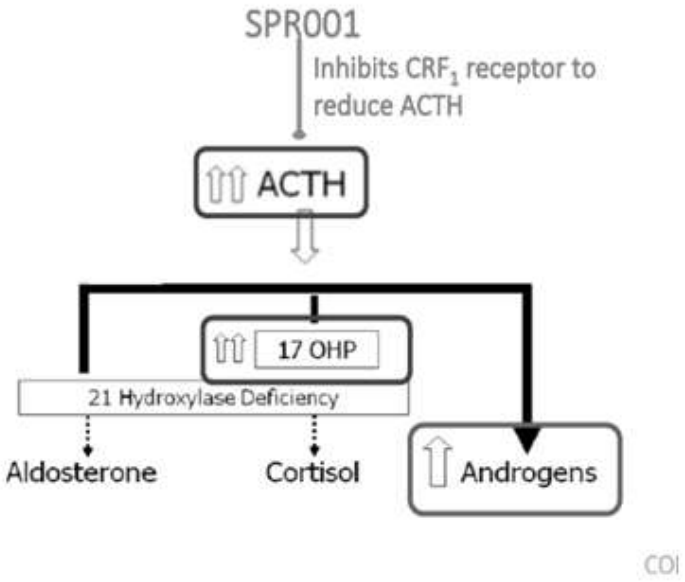
SPR001
Inhibits CRF1 receptor to
reduce ACTH
ACTH
17 OHP
21 Hydroxylase Deficiency
Aldosterone
Cortisol
Androgens

FIG. 1B

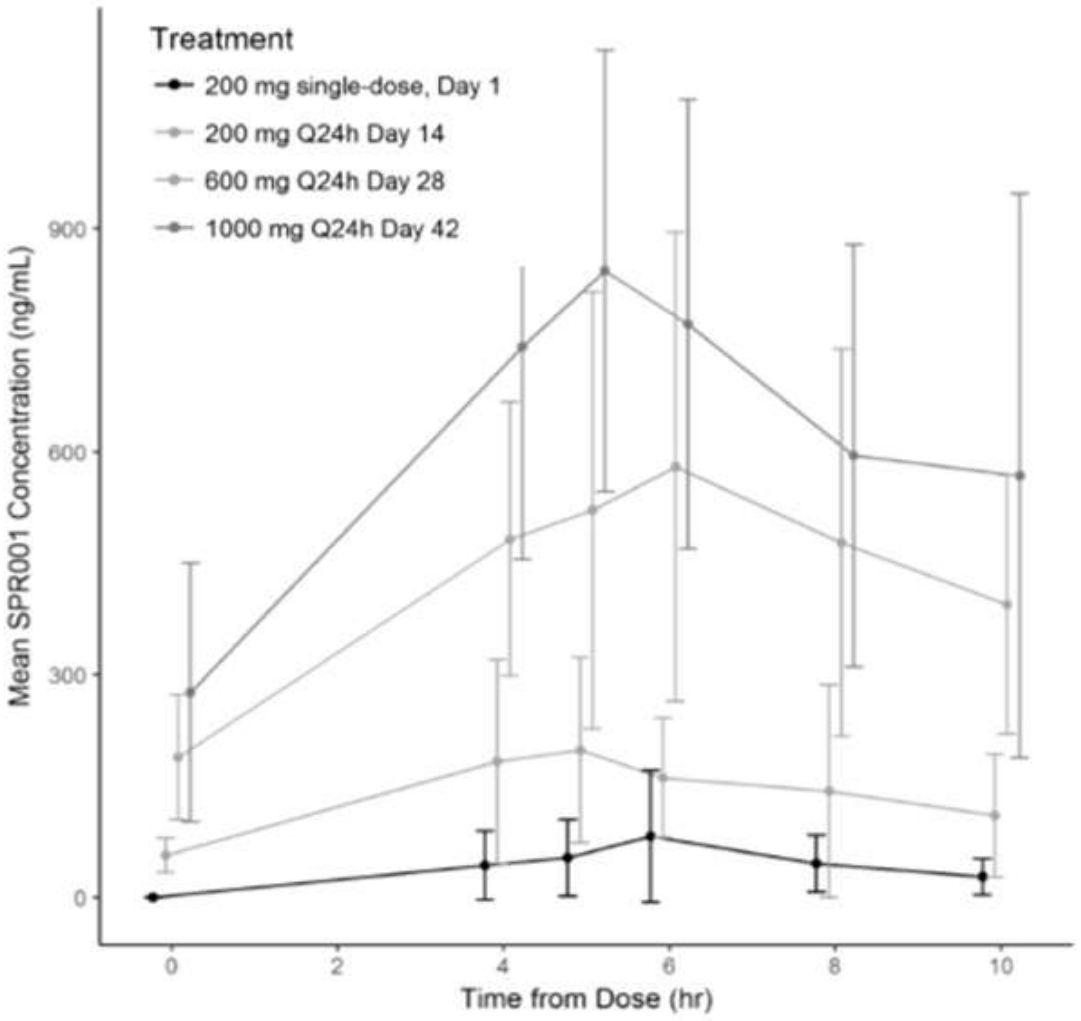
Treatment
200 mg single-dose, Day 1
200 mg Q24h Day 14
600 mg Q24h Day 28
1000 mg Q24h Day 42
Mean SPR001 Concentration (ng/mL)
900
600
300
0
0
2
4
6
8
10
Time from Dose (hr)

FIG. 2

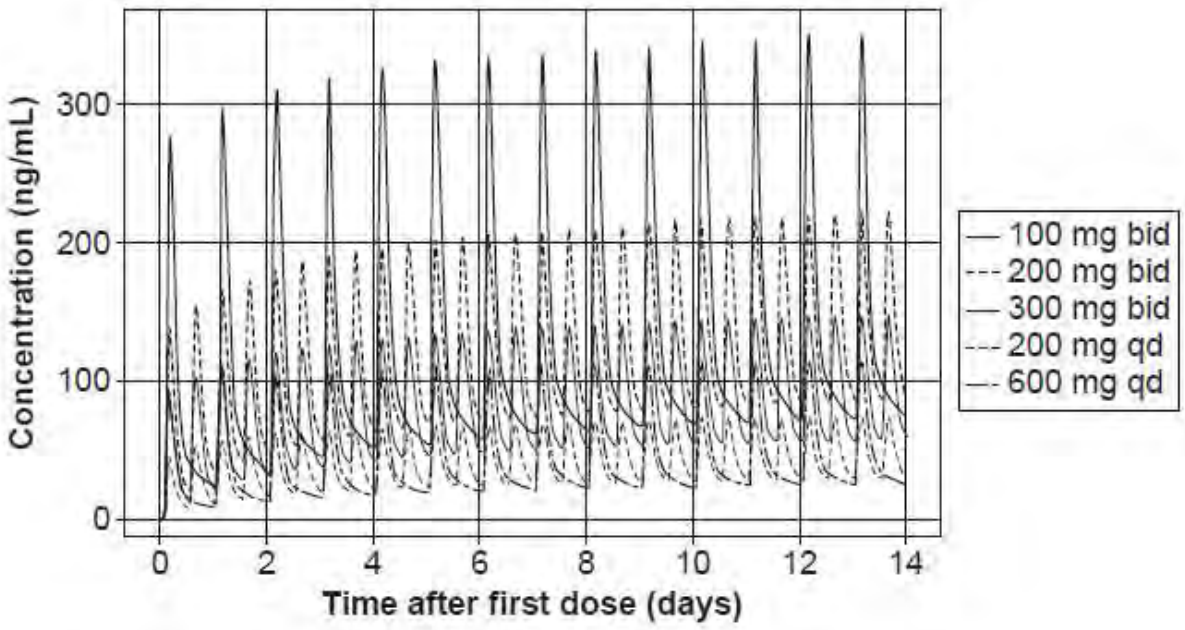
Concentration (ng/mL)
300
200
100
0
0
2
4
6
8
10
12
14
Time after first dose (days)
100 mg bid
200 mg bid
300 mg bid
200 mg qd
600 mg qd

FIG. 3

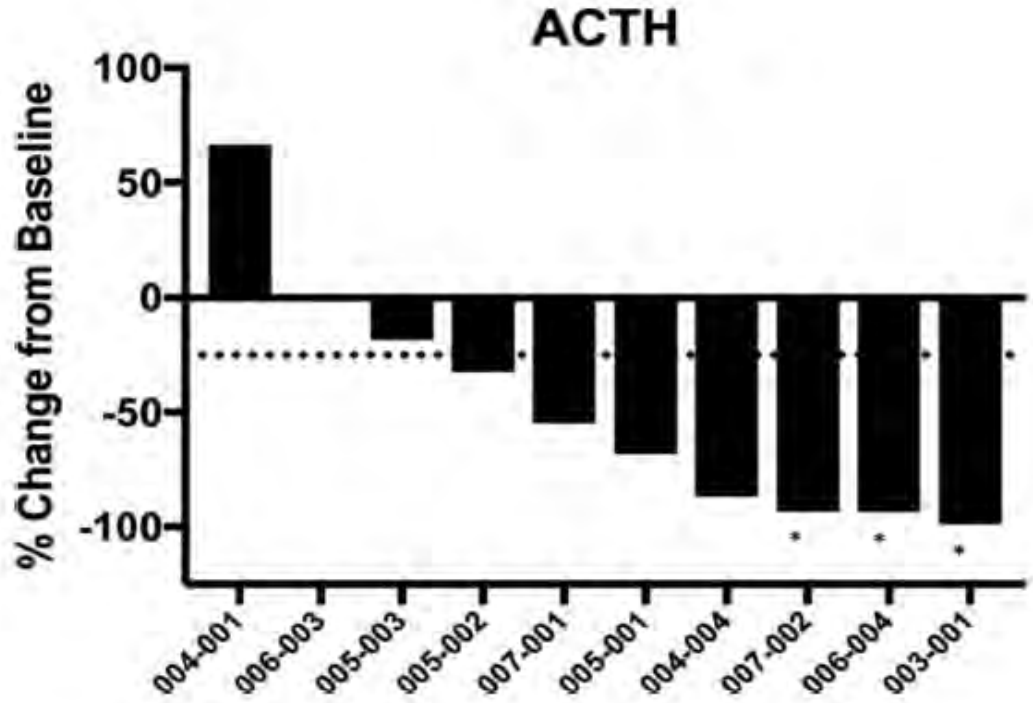
ACTH
% Change from Baseline
100
50
0
-50
-100
004-001
006-003
005-003
005-002
007-001
005-001
004-004
007-002
006-004
003-001

FIG. 4

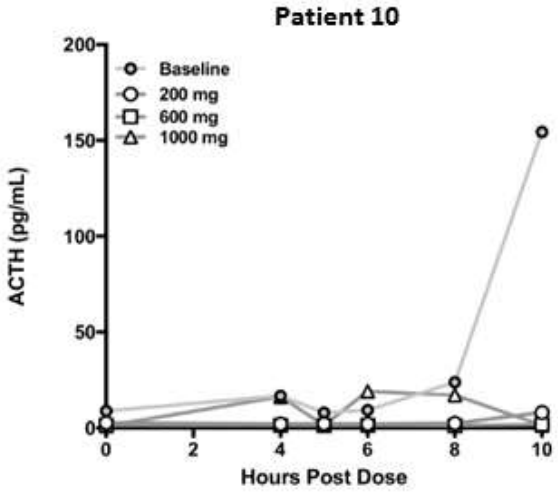

FIG. 5A

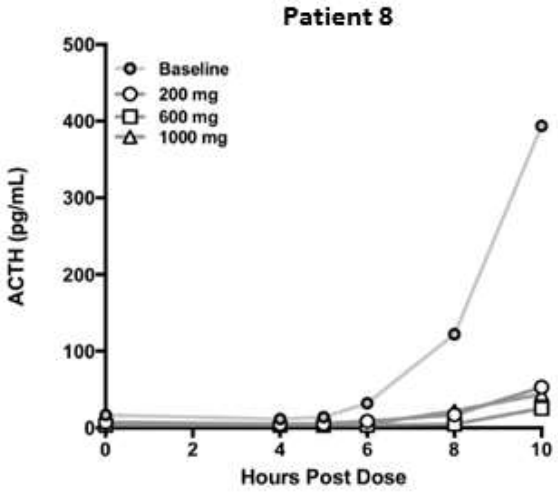

FIG. 5B

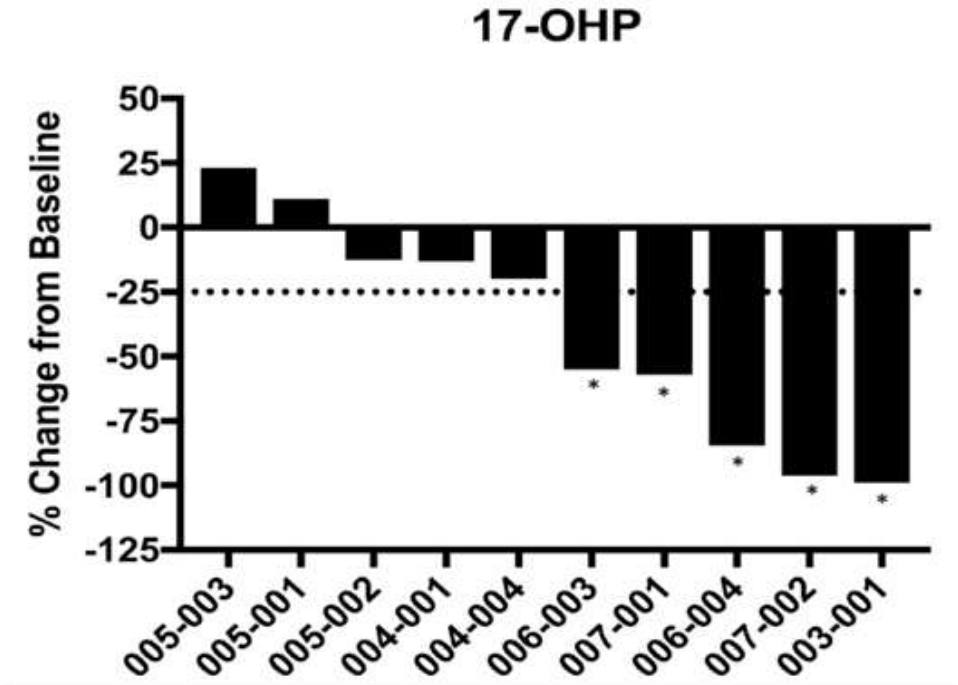

FIG. 6

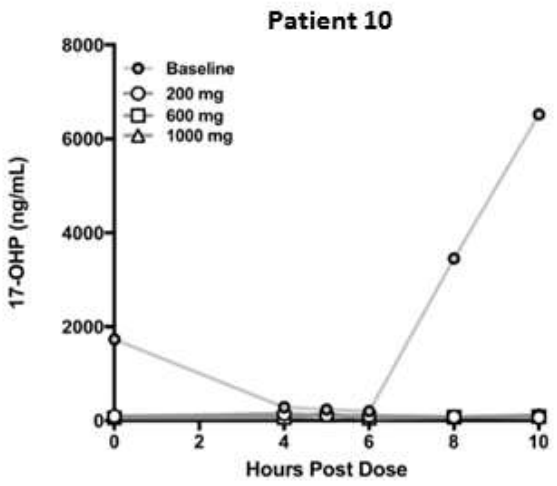

FIG. 7A

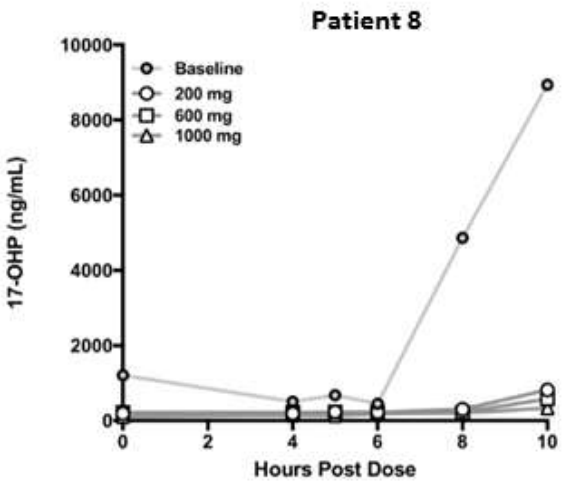

FIG. 7B

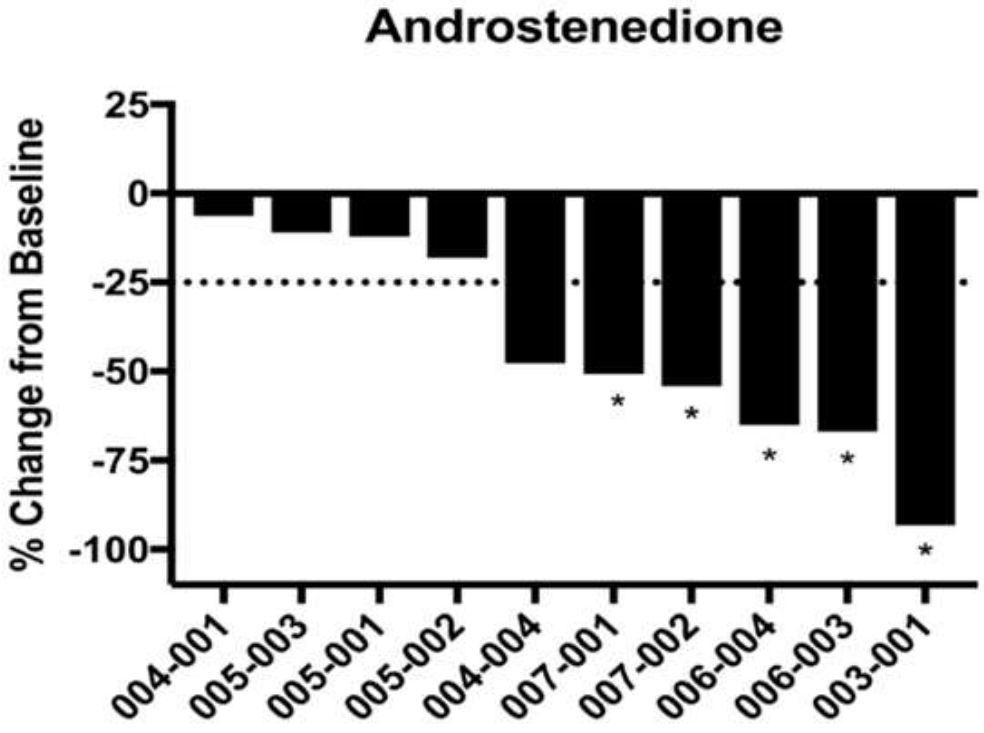

FIG. 8

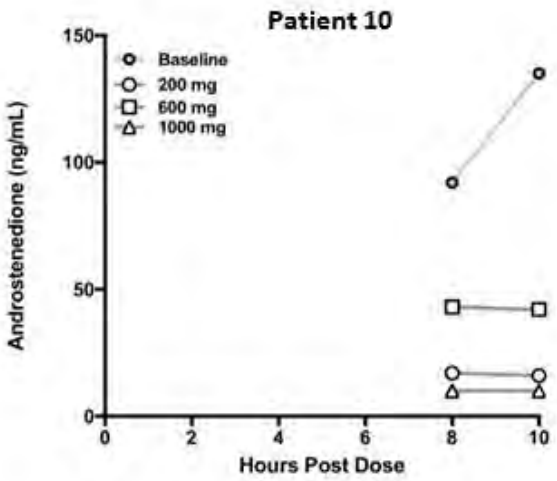

FIG. 9A

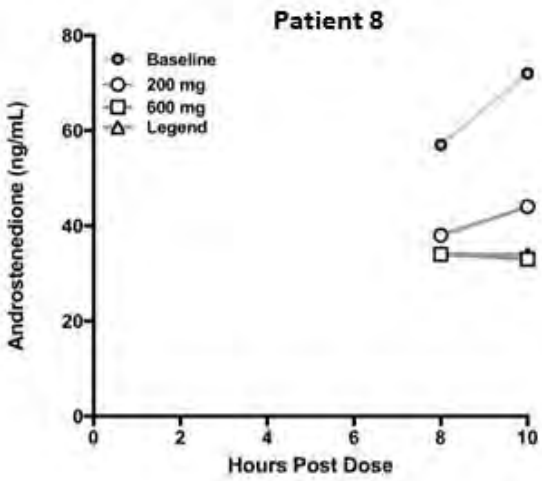

FIG. 9B

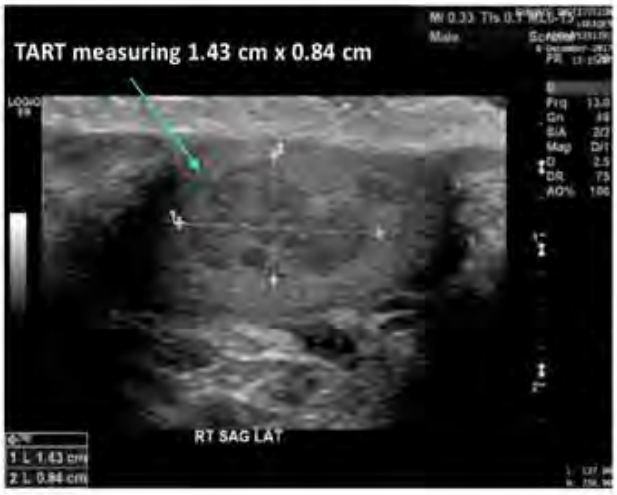

FIG. 10A

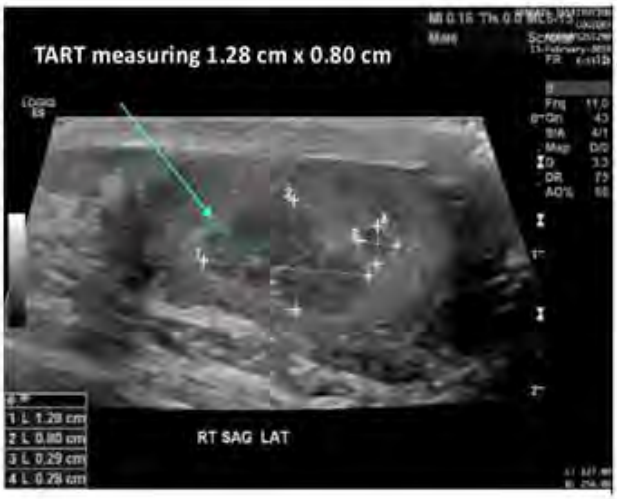

FIG. 10B

In Vitro Binding Assay

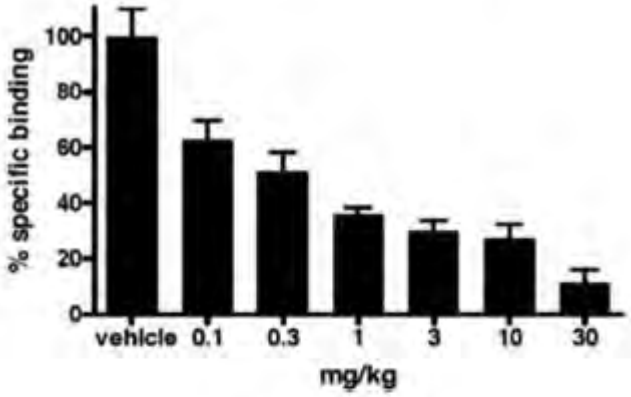

FIG. 11A

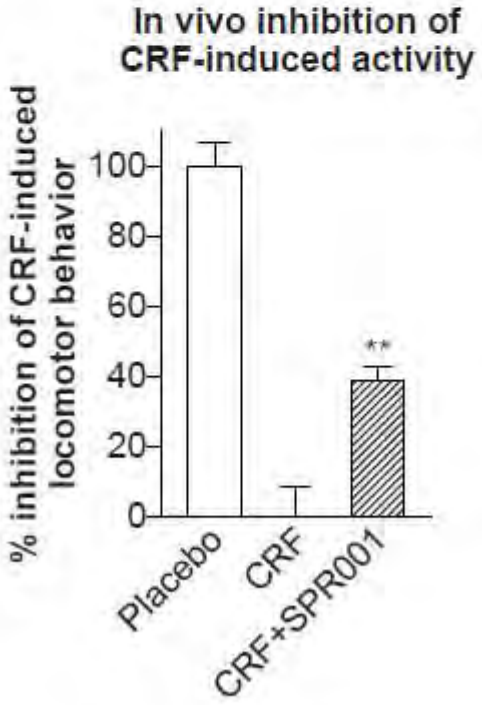

FIG. 11B

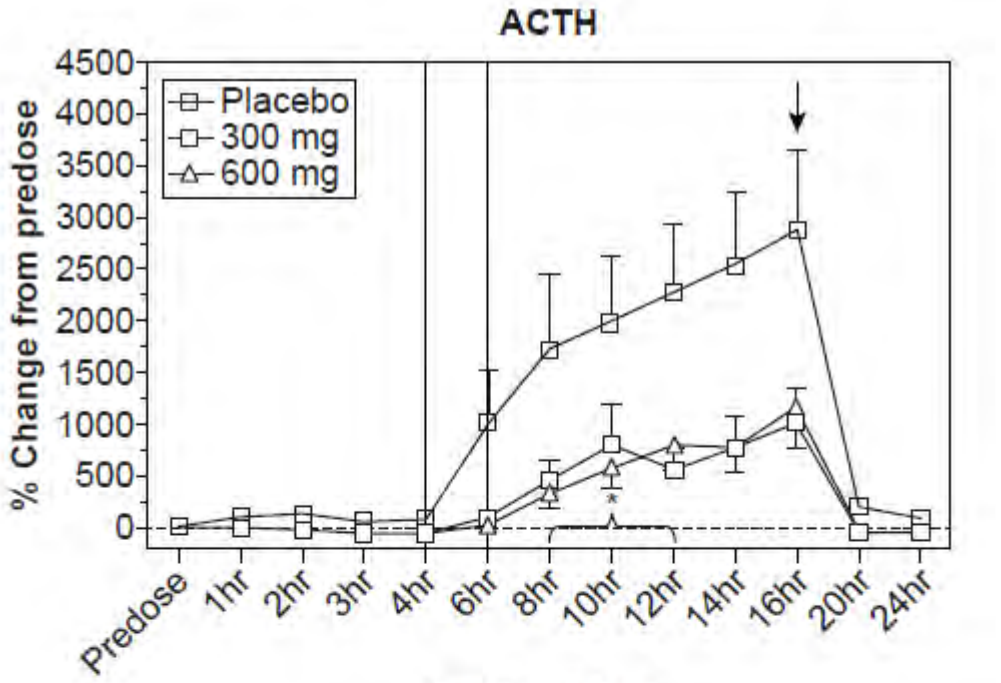

FIG. 12A

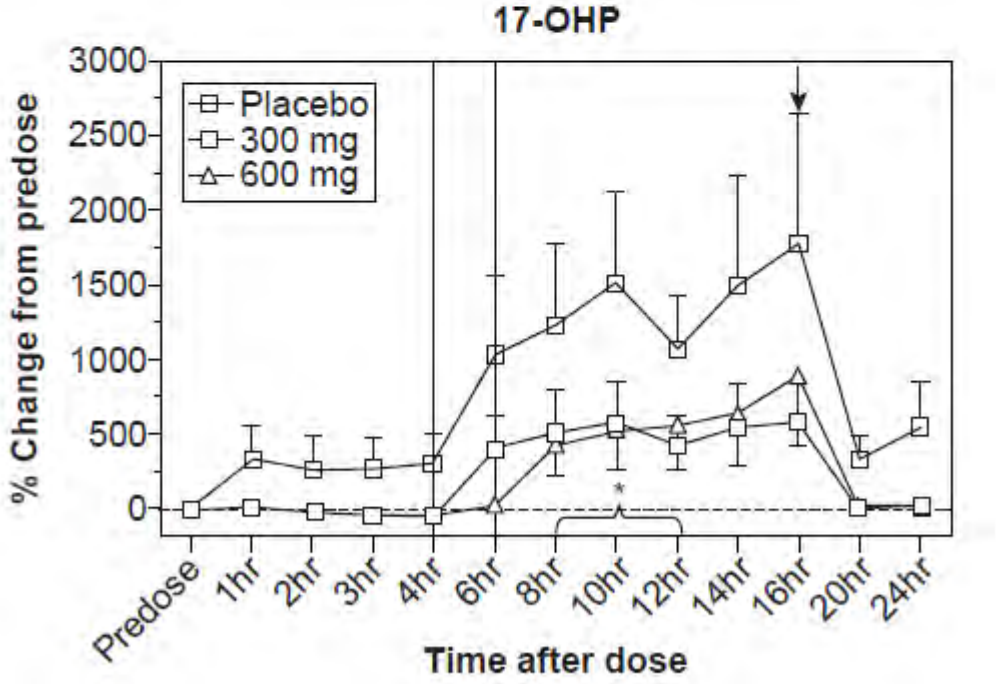

FIG. 12B

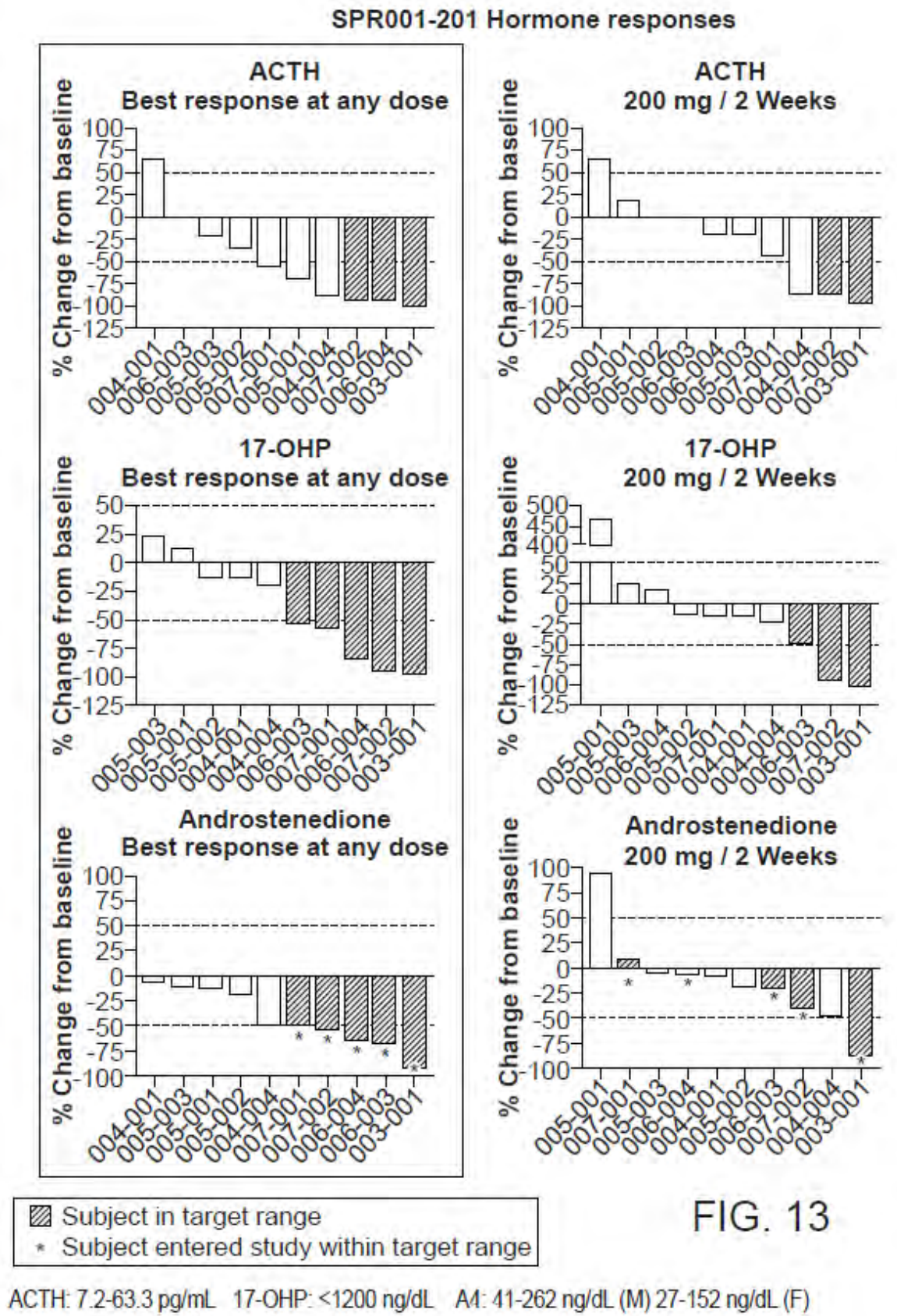

FIG. 13

ACTH: 7.2-63.3 pg/mL 17-OHP: <1200 ng/dL A4: 41-262 ng/dL (M) 27-152 ng/dL (F)

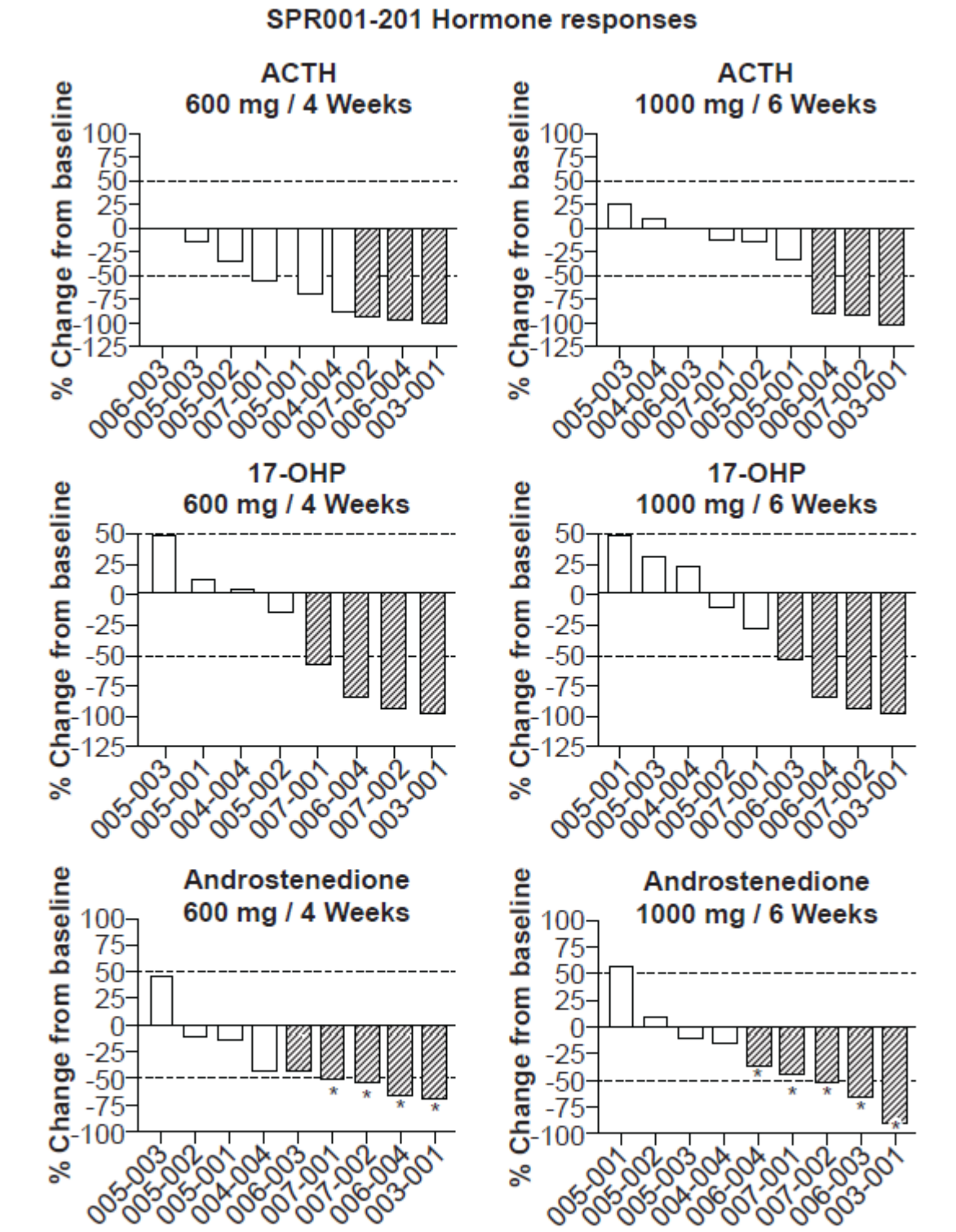

FIG. 13 (Cont.)

ACTH: 7.2-63.3 pg/mL 17-OHP: <1200 ng/dL A4: 41-262 ng/dL (M) 27-152 ng/dL (F)

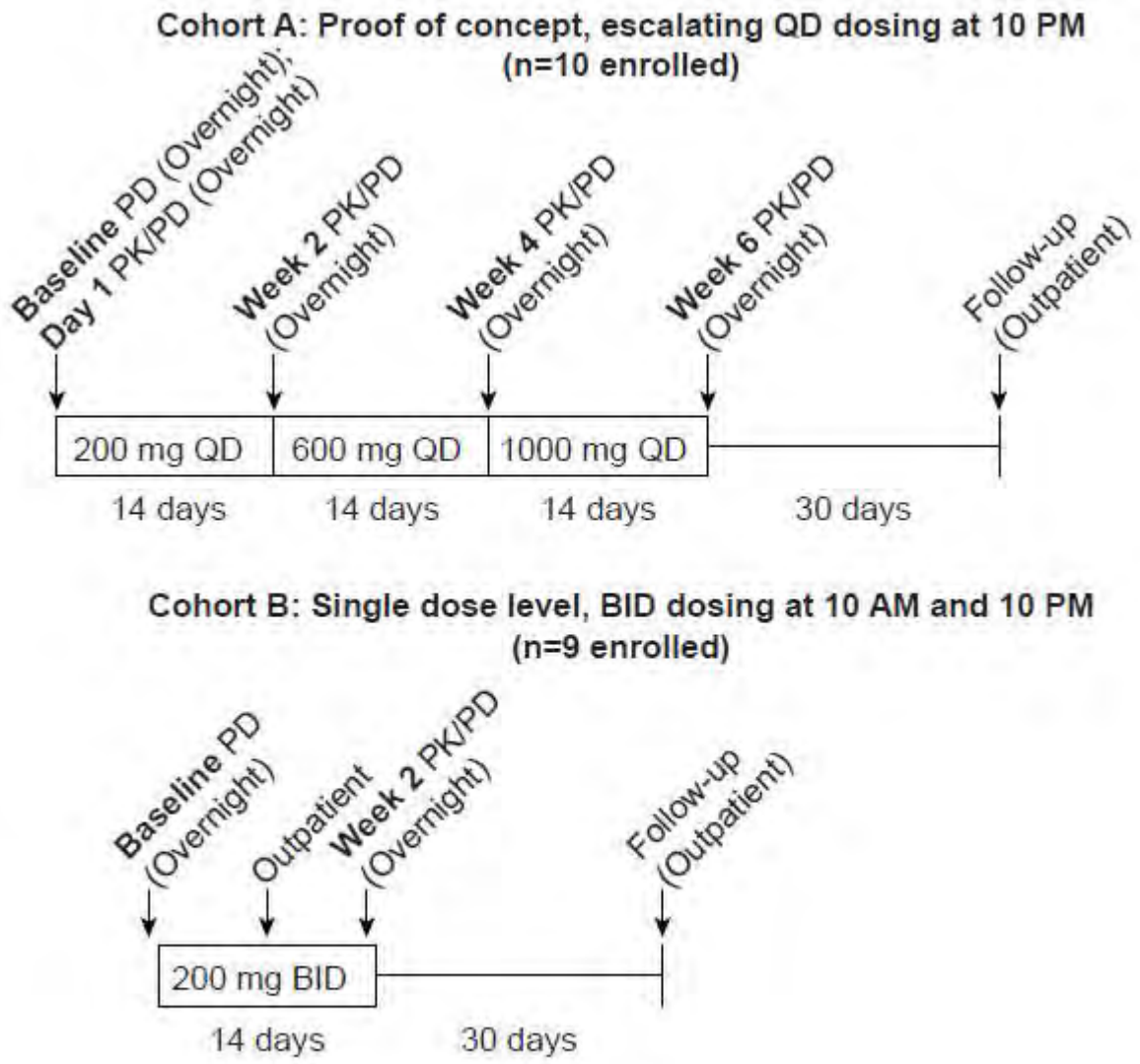
Cohort A: Proof of concept, escalating QD dosing at 10 PM
(n=10 enrolled)
Baseline PD (Overnight);
Day 1 PK/PD (Overnight)
Week 2 PK/PD
(Overnight)
Week 4 PK/PD
(Overnight)
Week 6 PK/PD
(Overnight)
Follow-up
(Outpatient)
200 mg QD
600 mg QD
1000 mg QD
14 days
14 days
14 days
30 days
Cohort B: Single dose level, BID dosing at 10 AM and 10 PM
(n=9 enrolled)
Baseline PD
(Overnight)
Outpatient
Week 2 PK/PD
(Overnight)
Follow-up
(Outpatient)
200 mg BID
14 days
30 days

FIG. 14

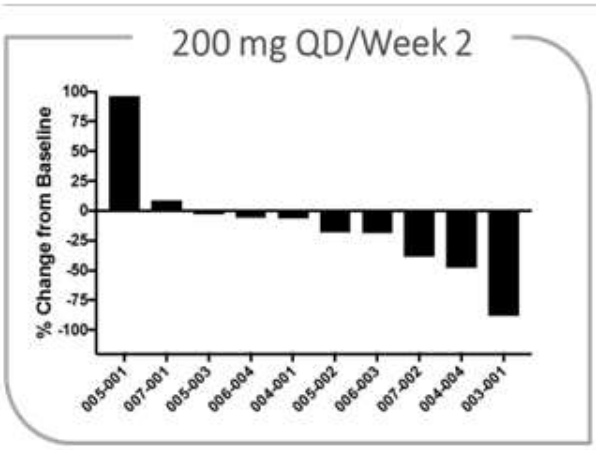

FIG. 15A

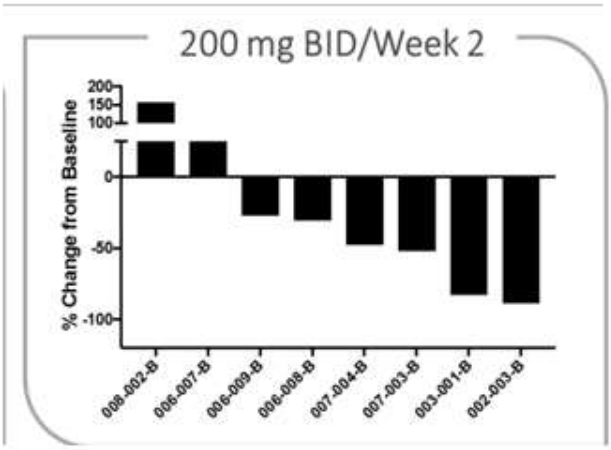

FIG. 15B

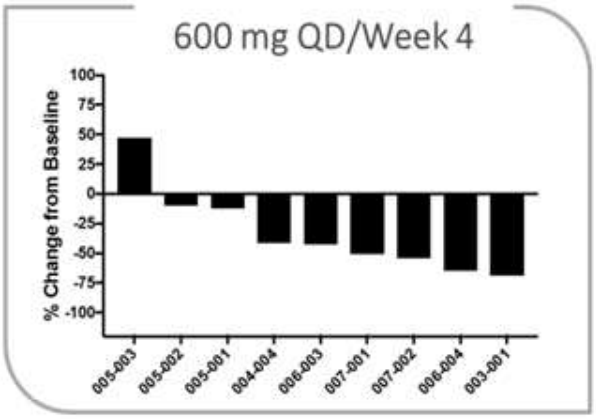

FIG. 15C

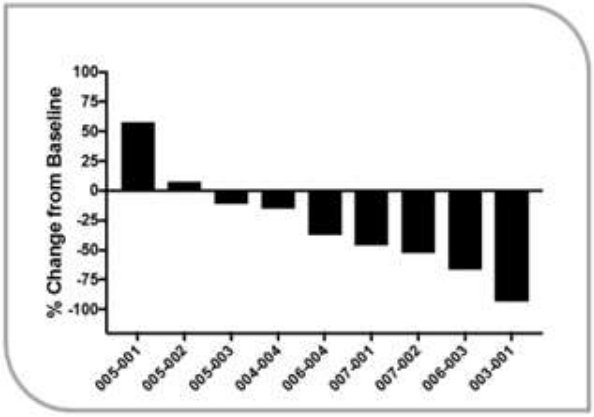

FIG. 15D

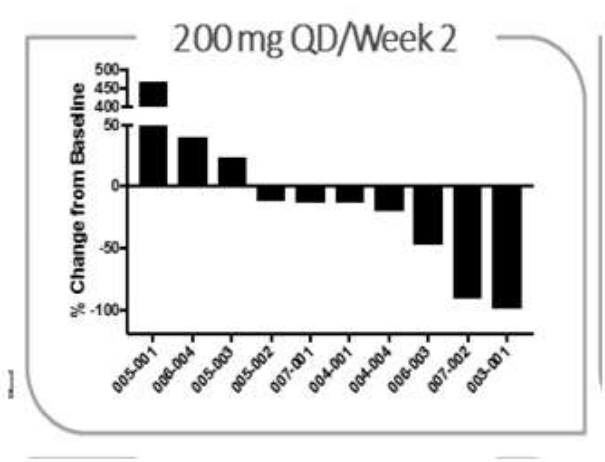

FIG. 16A

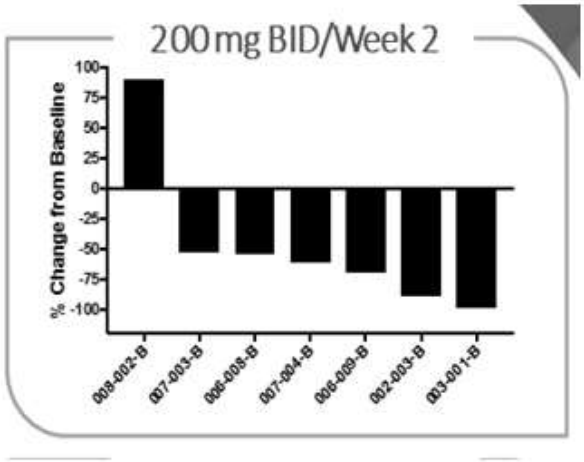

FIG. 16B

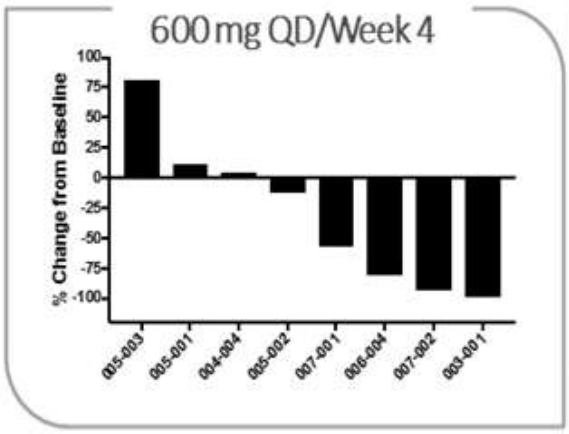

FIG. 16C

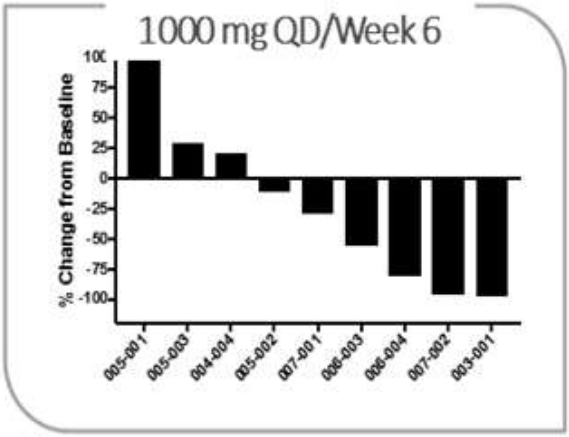

FIG. 16D

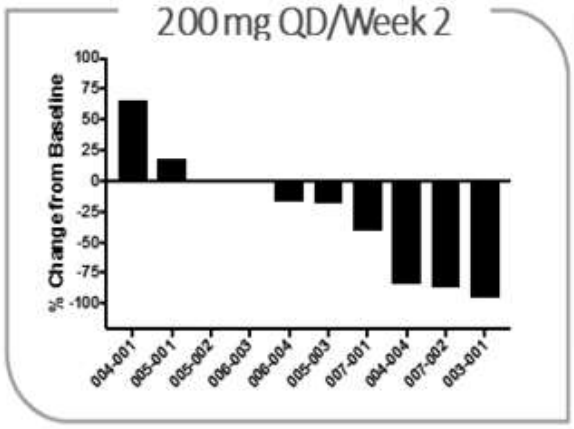

FIG. 17A

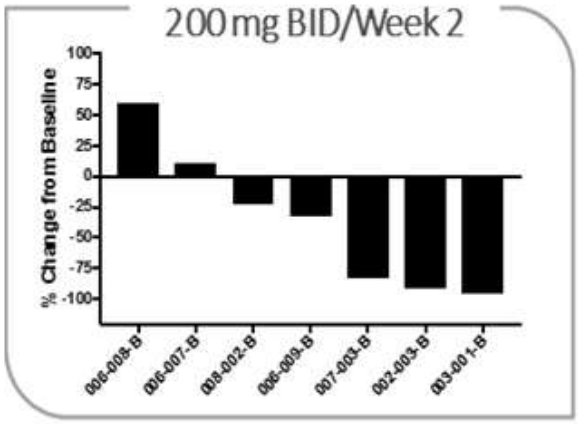

FIG. 17B

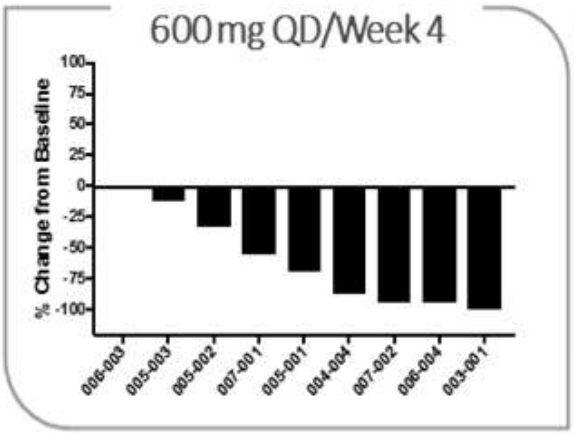

FIG. 17C

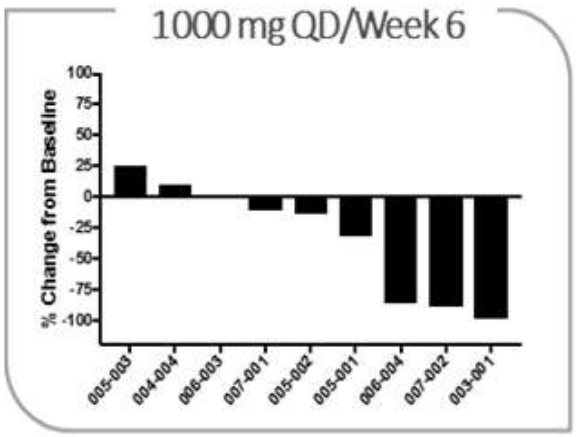

FIG. 17D

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor:	HOWERTON; Alexis et al.	Group Art Unit:	
Serial Number:	19/043,275	Examiner:	Not Yet Assigned
Filing or 371 (c) Date:	2025-01-31	**CONFIRMATION NO:**	**5486**
Title:	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS		

FILED ELECTRONICALLY ON: February 5, 2025

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT UNDER 37 CFR § 1.97

Commissioner for Patents:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP § 609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in § 1.56.

A. ☒ *37 CFR § 1.97 (b).* This Information Disclosure Statement should be considered by the Office because:

☐ (1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under § 1.53 (d);

-- OR --

☐ (2) It is being filed within 3 months of entry of the national stage as set forth in § 1.491 in an international application;

-- OR --

☒ (3) It is being filed before the mailing of a first Office action on the merits;

-- OR --

☐ (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under § 1.114.

B. ☐ *37 CFR § 1.97(c).* Although this Information Disclosure Statement is being filed after the period specified in *37 CFR § 1.97(b),* above, it is filed before the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, this Information Disclosure Statement should be considered because it is accompanied by one of:

☐ a statement as specified in §1.97 (e) provided concurrently herewith;

-- OR --

☐ a fee of [$280.00/112.00/56.00] as set forth in § 1.17 (p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.

C. ☐ *37 CFR § 1.97 (d).* Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, it is being filed before payment of the issue fee and should be considered because it is accompanied by:

i. a statement as specified in § 1.97 (e);

-- AND --

ii. a fee of [$280.00/112.00/56.00] as set forth in §1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.

D. ☐ *37 CFR §1.97 (e).* Statement.

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (c);

-- AND/OR --

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (d);

-- AND/OR --

☐ A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under 37 C.F.R. § 1.97(e) (1) as provided for under MPEP 609.04(b) V.

E. ☐ *Statement Under 37 C.F.R. §1.704(d).* Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office or is a communication that was issued by a

patent office in a counterpart foreign or international application or by the Office that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay. The Patent Term Adjustment Statement Under 37 CFR 1.704(d) on Form PTO/SB/133 is filed herewith.

F. ☒ *37 CFR §1.98 (a) (2).* The content of the Information Disclosure Statement is as follows:

☐ Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.

-- OR --

☒ Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 are not enclosed.

-- AND/OR --

☒ Copies of Foreign Patent Documents and/or Non Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).

-- AND/OR --

☒ Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98 (a) (2) (iii).

G. ☒ *37 CFR §1.98(a)(3).* The Information Disclosure Statement includes non-English patents and/or references.

☐ Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.

☐ Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.

-- OR --

☐ A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____

☒ Pursuant to 37 CFR §1.98(a) (3) (ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.

H. ☐ *37 CFR §1.98(d).* Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:

☐ Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.

Application in which the information was submitted: _____

Information Disclosure Statement(s) filed on: _____

AND

☐ The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

I. ☒ *Fee Authorization*. The Commissioner is hereby authorized to charge the above-referenced fees of $200.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 23-2415 (50535-710.304).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI

Dated: February 5, 2025

By: /Victoria Cheng/
Victoria Cheng, Reg. No. 79641

650 Page Mill Road
Palo Alto, CA 94304-1050
(650) 493-9300
Customer No. 21971

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,275
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	1	of	9	Attorney Docket Number	50535-710.304

U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	001	US-4328245	05-04-1982	YU; Cheng-Der et al.	
	002	US-4409239	10-11-1983	YU; Cheng-Der	
	003	US-4410545	10-18-1983	YU; Cheng-Der et al.	
	004	US-4861760	08-29-1989	MAZUEL; Claude et al.	
	005	US-4911920	03-27-1990	JANI; Rajni et al.	
	006	US-5212162	05-18-1993	MISSEL; Paul J. T. et al.	
	007	US-5403841	04-04-1995	LANG; John C. et al.	
	008	US-10849908	12-01-2020	HOWERTON; Alexis et al.	
	009	US-11007201	05-18-2021	HOWERTON; Alexis et al.	
	010	US-11304950	04-19-2022	HOWERTON et al.	
	011	US-11311549	04-26-2022	HOWERTON; Alexis et al.	
	012	US-11344557	05-31-2022	HOWERTON; Alexis et al.	
	013	US-11351177	06-07-2022	HOWERTON; Alexis et al.	
	014	US-12115166	10-15-2024	HOWERTON; Alexis et al.	
	015	US-20100196389	08-05-2010	EVANS-FREKE; Stephen	
	016	US-20100222339	09-02-2010	CHEN; Zhaogen et al.	
	017	US-20170020877	01-26-2017	GRIGORIADIS; Dimitri E.	
	018	US-20220143037	05-12-2022	HOWERTON; Alexis et al.	
	019	US-20220211711	07-07-2022	HOWERTON; Alexis et al.	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

PTO/SB/08a (07-09)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,275
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	2	of	9	Attorney Docket Number	50535-710.304

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	001	CN-101516887-A	08-26-2009	LILLY CO ELI [US]	Abstract:	☒
	002	CN-106102740-A	11-09-2016	NEUROCRINE BIOSCIENCES INC.	Abstract:	☒
	003	JP-2010504344-A	02-12-2010	ELI LILLY AND CO.	Machine Translation:	☒
	004	JP-2017503030-A	01-26-2017	NEUROCRINE BIOSCIENCES, INC.	Machine Translation:	☒
	005	JP-H0971528-A	03-18-1997	HAYASHIBARA BIOCHEM LAB [JP]	Abstract:	☒
	006	WO-0040529-A1	07-13-2000	ANGIOGENE PHARM LTD [GB], et al.		☐
	007	WO-0041669-A2	07-20-2000	ANGIOGENE PHARM LTD [GB], et al.		☐
	008	WO-0192224-A1	12-06-2001	ASTRAZENECA AB [SE], et al.		☐
	009	WO-0204434-A1	01-17-2002	ANGIOGENE PHARM LTD [GB], et al.		☐
	010	WO-0208213-A1	01-31-2002	ANGIOGENE PHARM LTD [GB], et al.		☐

Examiner Signature		Date Considered	

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Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,275
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	3	of	9	Attorney Docket Number	50535-710.304

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	011	WO-2007109853-A1	10-04-2007	VELACOR THERAPEUTICS PTY LTD [AU], et al.		☐
	012	WO-2008036579-A1	03-27-2008	LILLY CO ELI [US], et al.		☐
	013	WO-2015112642-A1	07-30-2015	NEUROCRINE BIOSCIENCES INC [US]		☐
	014	WO-2019036472-A1	02-21-2019	SPRUCE BIOSCIENCES INC [US]		☐
	015	WO-2019036503-A1	02-21-2019	SPRUCE BIOSCIENCES INC [US]		☐
	016	WO-2019210266-A1	10-31-2019	SPRUCE BIOSCIENCES INC [US]		☐
	017	WO-9722596-A1	06-26-1997	ZENECA LTD [GB], et al.		☐
	018	WO-9730035-A1	08-21-1997	ZENECA LTD [GB], et al.		☐
	019	WO-9732856-A1	09-12-1997	ZENECA LTD [GB], et al.		☐
	020	WO-9813354-A1	04-02-1998	ZENECA LTD [GB], et al.		☐
	021	WO-9902166-A1	01-21-1999	ANGIOGENE PHARM LTD [GB], et al.		☐

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				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	4	of	9	Attorney Docket Number	50535-710.304

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	001	AL-MUHAMMED, J. et al. In-vivo studies on dexamethasone sodium phosphate liposomes. Journal of microencapsulation 13(3):293-306 (1996).	☐
	002	BERGE, Stephen M. et al. Pharmaceutical Salts. Journal of Pharmaceutical Sciences 66(1):1-19 (1977).	☐
	003	CHEN, Hua-dong. et al. Ovarian adrenal rest tumors undetected by imaging studies and identified at surgery in three females with congenital adrenal hyperplasia unresponsive to increased hormone therapy dosage. Endocrine pathology 28(2):146-151 (2017).	☐
	004	CHONN, Archadio et al. Recent Advances in Liposomal Drug-delivery Systems. Current Opinion in Biotechnology 6(6):698-708 (1995).	☐
	005	Co-pending U.S. Application No. 18/941,832, inventors HOWERTON; Alexis et al., filed on November 08, 2024.	☐
	006	Co-pending U.S. Application No. 19/043,264, inventors HOWERTON; Alexis et al., filed on January 31, 2025.	☐
	007	Co-pending U.S. Application No. 19/043,303, inventors HOWERTON; Alexis et al., filed on January 31, 2025.	☐
	008	EYLES et al. Oral delivery and fate of poly(lactic acid) microsphere-encapsulated interferon in rats. J Pharm Pharmacol. 49(7):669-74 (1997).	☐
	009	GAO, Zhi-Hui. et al. Controlled Release of a Contraceptive Steroid From Biodegradable and Injectable Gel Formulations: in Vitro Evaluation. Pharmaceutical research 12(6):857-863 (1995).	☐
	010	JIN et al. Testicular adrenal rest tumors in a patient with untreated congenital adrenal hyperplasia. Korean J Pediatr 2011;54(3):137-140.	☐

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	011	KAPRARA, et al. The corticotropin releasing factor system in cancer: expression and pathophysiological implications. Cellular and molecular life sciences 67(8):1293-1306 (2010).	☐
	012	MENDES-DOS-SANTOS et al. Prevalence of Testicular Adrenal Rest Tumor and Factors Associated with Its Development in Congenital Adrenal Hyperplasia. Hormone Res. In Paed. 90:161-168 (2018).	☐
	013	MUNSON, Peter et al. Ligand: a Versatile Computerized Approach for Characterization of Ligand-binding Systems. Analytical Biochemistry 107(1):220-239 (1980).	☐
	014	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Decision Granting Institution of Post-Grant Review of U.S. Patent No. 11,007,201 dated December 1, 2023 (pp. 1-38) (PGR2022-00025).	☐
	015	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Final Written Decision of Post-Grant Review of U.S. Patent No. 10,849,908 dated November 26, 2024 (pp. 1-62) (PGR2021-00088).	☐
	016	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Final Written Decision of Post-Grant Review of U.S. Patent No. 11,007,201 dated November 27, 2024 (pp. 1-60) (PGR2022-00025).	☐
	017	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Petition for Post Grant Review of U.S. Patent No. 10,849,908 dated May 28, 2021 (pp. 1-90) (PGR2021-00088).	☐
	018	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Petition for Post Grant Review of U.S. Patent No. 11,007,201 dated February 18, 2022 (pp 1-92) (PGR2022-00025).	☐

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				First Named Inventor	HOWERTON; Alexis
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Sheet	6	of	9	Attorney Docket Number	50535-710.304

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	019	OSTRO, Mark J. et al. Use of Liposomes as Injectable-drug Delivery Systems. American Journal of Hospital Pharmacy 46(8):1576-1587 (1989).	☐
	020	PCT/US2019/029486 International Invitation to Pay Additional Fees dated June 4, 2019.	☐
	021	PCT/US2019/029486 International Search Report and Written Opinion dated August 12, 2019.	☐
	022	RAO. Recent developments of collagen-based materials for medical applications and drug delivery systems. J Biomater Sci Polym Ed. 7(7):623-45 (1995).	☐
	023	REICHMAN et al. Fertility in Patients with Congenital Adrenal Hyperplasia. Fertility and Sterility, Vol. 101, No. 2, 2014. 301-309.	☐
	024	REISCH et al. Testicular adrenal rest tumors develop independently of long-term disease control: a longitudinal analysis of 50 adult men with congenital adrenal hyperplasia due to classic 21-hydroxylase deficiency. J. Clin. Endocrinol. Metab. 98(11):E1820-E1826 (2013).	☐
	025	SMEETS et. al. Molecular characterization of testicular adrenal rest tumors in congenital adrenal hyperplasia: lesions with both adrenocortical and Leydig cell features. JCEM 100(3):E524-E530 (2015).	☐
	026	TIOSANO et al. Ovarian adrenal rest tumor in a congenital adrenal hyperplasia patient with adrenocorticotropin hypersecretion following adrenalectomy. Hormone Res. in Paediatrics 74:223-228 (2010).	☐
	027	U.S. Serial No. 17/081,694 Office Action dated February 26, 2021.	☐
	028	U.S. Serial No. 17/081,694 Office Action dated June 11, 2021.	☐
	029	U.S. Serial No. 17/667,285 Office Action dated December 29, 2023.	☐

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	030	U.S. Serial No. 17/667,285 Office Action dated June 10, 2024.	☐
	031	ZAAROUR et al. Bilateral Ovary Adrenal Rest Tumor in a Congenital Adrenal Hyperplasia Following Adrenalectomy. Endocrinol. Pract. 20(4):e69-e74 (2014).	☐

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Sheet	8	of	9	Attorney Docket Number	50535-710.304

TIMING STATEMENT

Please see 37 CFR 1.97(e) to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached timing statement under 37 CFR 1.97(e).

☒ A timing statement under 37 CFR 1.97(e) is not submitted herewith.

TIMING FEE

☐ The fee set forth in 37 CFR 1.17(p) has been submitted herewith.

COPIES

☐ An identification of an earlier application pursuant to 37 CFR 1.98(d)(1) is attached.

SIZE FEE ASSERTION

Please see 37CFR 1.17(v) and attached instructions for completing this form to make the appropriate selection of an assertion under 37 CFR 1.98. For the information disclosure statement (IDS) submitted herewith, the applicant or patent owner certifies the following with respect to the cumulative number of applicant-provided or patent-owner provided items of information submitted to date including those in the accompanying IDS (select only one):

☐ No IDS size fee is required under 37 CFR 1.17 (v) at this time.

☒ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(1).

☐ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(2).

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SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 11.18. Please see CFR 1.4(d) for the form of the signature.

Signature:	**/Victoria Cheng/**	Date (YYYY-MM-DD)	**2025-02-05**
Name/Print	Victoria Cheng	Registration Number	79641

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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(71) Applicant *(for all designated States except US)*: ANGIOGENE PHARMACEUTICALS LTD. [GB/GB]; 14 Plowden Park, Aston Rowant, Watlington, Oxfordshire OX9 5SX (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: DOUGHERTY, Graeme [GB/GB]; 4 Bonaly Grove, Edindurgh EH13 0QD (GB).

(74) Agent: BAILLIE, Iain, C.; Langner Parry, 52–54 High Holborn, London WC1V 6RR (GB).

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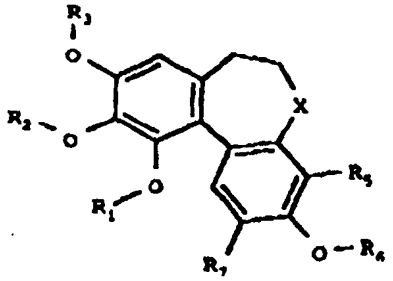
(54) Title: USE OF COLCHINOL DERIVATIVES AS VASCULAR DAMAGING AGENTS

(57) Abstract

Colchinol derivatives of formula (I) wherein R_1, R_2, R_3 and R_6 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, alkanoyl, PO_3H_2; X is carbonyl (CO), thiocarbonyl (CS), methylene (CH_2) or a group CHR_4; R_4 is OH, O–alkyl or NR_8R_9; R_5 and R_7 are each independently H, alkyl, halogen, hydroxy, alkoxy, nitro or amino; R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulfonyl, arylsulfonyl, aminosulfonyl, alkylaminosulfonyl, dialkylaminosulfonyl or arylaminosulfonyl; and R_9 is H, alkyl or cycloalkyl and the pharmaceutically acceptable salts, solvates, and hydrates thereof have been found to be useful for treatment of diseases involving angiogenesis. Some of these compounds are novel. Particularly preferred are those compounds in which R_6 is PO_3H_2.



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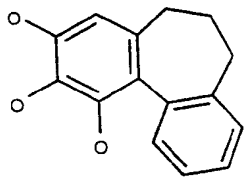
USE OF COLCHINOL DERIVATIVES AS VASCULAR DAMAGING AGENTS

This invention relates to vascular damaging agents and particularly to use in the preparation of agents for treatment of neovascularisation of a group of colchinol derivatives some of which are new compounds.

Formation of new vasculature by angiogenesis is a key pathological feature of several diseases (J Folkman, New England Journal of Medicine 333, 1757 (1995)). For example, for a solid tumour to grow it must develop its own blood supply upon which it depends critically for the provision of oxygen and nutrients; if this blood supply is mechanically shut off the tumour undergoes necrotic death. Neovascularisation is also a clinical feature of skin lesions in psoriasis, of the invasive pannus in the joints of rheumatoid arthritis patients and of atherosclerotic plaques. Retinal neovascularisation is pathological in macular degeneration and in diabetic retinopathy. In all these diseases reversal of neovascularisation by damaging the newly-formed vascular endothelium is expected to have a beneficial therapeutic effect.

Colchinol derivatives for example N-acetyl-colchinol are known. Anti-tumour effects have been noted on animal models (see for example - JNCI (Journal National Cancer Institute) Page 379-392 1952, Vol 13). However, the effect studied was that of gross damage (haemorrhage, softening and necrosis) and there is no suggestion of treatment of inappropriate angiogenesis by destruction of neovasculature.

A search of Chemical Abstracts (post 1955) based on the substructure

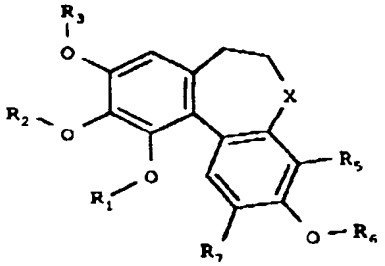


revealed a number of colchinol related structures.

To the extent that any of these compounds have been studied for anti-cancer activity it is because tubulin binding agents might be expected to be anti-mitotic and therefore to have a direct effect on tumour cells.

In the course of the work on the present invention, the issue of the relevance of tubulin-binding properties to possible effectiveness as anti-vascular agent was studied but no predictability was found. Thus docetaxel (Lancet, 344, 1267-1271, 1994), which is a tubulin-binding agent, had no vascular-damaging effects even when administered at its Maximum Tolerated Dose. Even when the present inventors tested some compounds structurally related to the present invention, the therapeutic window (ratio of MTD (Maximum tolerated dose) to MED (Minimum effective dose)) was found to be too small for potential clinical effectiveness.

According to the present invention there is provided the use of colchinol derivatives for the preparation of compositions for the treatment of diseases involving angiogenesis in which the colchinol derivative has the formula



I

wherein

R_1, R_2, R_3 and R_6 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, alkanoyl, PO_3H_2;

X is carbonyl (CO), thiocarbonyl (CS), methylene (CH_2) or a group CHR_4

R_4 is OH, O-alkyl or NR_8R_9;

R_5 and R_7 are each independently H, alkyl, halogen, hydroxy, alkoxy, nitro or amino;

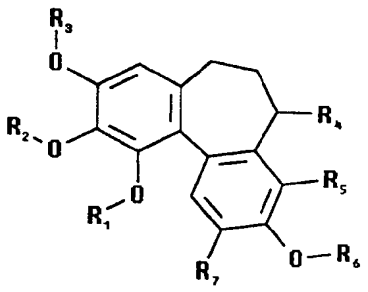
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;

and R_9 is H, alkyl or cycloalkyl

and the pharmaceutically acceptable salts, solvates, and hydrates thereof.

It is believed, though this is not limiting on the invention, that the use of compounds of the invention damages newly-formed vasculature, for example the vasculature of tumours, thus effectively reversing the process of angiogenesis as compared to known anti-angiogenic agents which tend to be less effective once the vasculature has formed.

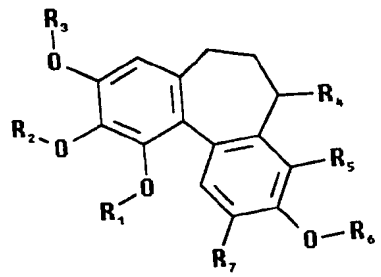
Certain of these compounds are novel. In one embodiment the novel compounds are those of formula I in which at least one of R_1, R_2, R_3, R_6 is PO_3H_2. In a particular preferred embodiment R_6 is PO_3H_2. Particularly preferred are compounds defined by the formula



II

wherein
R_1, R_2 and R_3 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, alkanoyl, or PO_3H_2;
R_6 is PO_3H_2;
R_4 is H or NR_8R_9;
R_5 and R_7 are each independently H, alkyl, halogen, alkoxy, nitro or amino;
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;
and R_9 is H, alkyl or cycloalkyl,
and the pharmaceutically acceptable salts, solvates and hydrates thereof.

In another aspect of the invention the novel compounds are of formula



IIA

wherein

R_1, R_2 and R_3 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, alkanoyl or PO_3H_2;

R_6 is H, optionally substituted alkyl, cycloalkyl, alkenyl, akynyl or PO_3H_2;

R_4 is H or NR_8R_9;

R_5 and R_7 are each independently H, alkyl, halogen, nitro or amino;

R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;

and R_9 is H, alkyl or cycloalkyl, with the proviso that, when R_1, R_2 and R_3 are all methyl groups and R_4 is hydrogen, acetylamino, acetylmethylamino, amino, methylamino or dimethylamino then R_6 is not hydrogen, methyl or hydroxyethyl, or acetoxyethyl,

and the pharmaceutically acceptable salts, solvates and hydrates thereof.

Preferred compounds used in the invention and of the invention are those in which R_1, R_2 and R_3 are alkyl and

those in which R_4 is acylamino.

As used herein the term "alkyl", (including any aliphatic structure chain related to alkyl) means a straight or branched-chain group containing from one to seven, preferably a maximum of four, carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, t-butyl and pentyl. Optional substituents which may be present on the alkyl groups include one or more substituents selected from halogen, amino, monoalkylamino, dialkylamino, hydroxy, alkoxy, alkylthio, alkylsulphonyl, acylamino, alkoxycarbonylamino, alkanoyl, acyloxy, carboxyl, sulphate or phosphate groups. Examples of alkoxy groups are methoxy, ethoxy, propoxy, isopropoxy, butoxy and t-butoxy. The term "halogen" means fluorine, chlorine, bromine or iodine.

An alkenyl group is an olefinic group containing from two to seven carbon atoms for example methylene, ethylene, n-propylene, i-propylene, n-butylene, i-butylene, s-butylene and t-butylene. An alkynyl group is of a group of 2-7 carbon atoms for example ethynyl, propynyl or butynyl group.

The term aryl alone or in combination means an unsubstituted phenyl group or a phenyl group carrying one or more, preferably one to three, substituents examples of which are halogen, alkyl, haloalkyl, hydroxy, nitro, cyano, amino and alkoxy. A haloalkyl group can carry one or more halogen atoms with the examples of such groups being trifluoromethyl and dichloromethyl.

The term heteroaryl is defined herein as a mono- or bi-cyclic aromatic group containing one to four heteroatoms selected in any combination from N, S or O atoms and a maximum of 9 carbon atoms. Examples of heteroaryl groups include pyridyl, pyrimidyl, furyl, thienyl, pyrrolyl,

pyrazolyl, indolyl, benzofuryl, benzothienyl, benzothiazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, imidazolyl, triazolyl, quinolyl and isoquinolyl groups.

The term aralkyl is defined herein as an alkyl group, as previously defined, in which one of the hydrogen atoms is replaced by an aryl or heteroaryl group as defined herein.

Where one or more functional groups in compounds of formulae I, II, IIA are sufficiently basic or acidic the formation of salts is possible. Suitable salts include pharmaceutically acceptable salts for example acid addition salts including hydrochlorides, hrdrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates and tartrates, salts derived from inorganic bases including alkali metal salts such as sodium or potassium salts, alkaline earth metal salts such as magnesium or calcium salts, and salts derived from organic amines such as morpholine, piperidine or dimethylamine salts.

Those skilled in the art will recognise that compounds of formulae I, II, IIA may exist as stereoisomers and/or geometrical isomers and accordingly the present invention includes all such isomers and mixtures thereof.

One useful group of compounds includes those in which R_1, R_2 and R_3 are each alkyl.

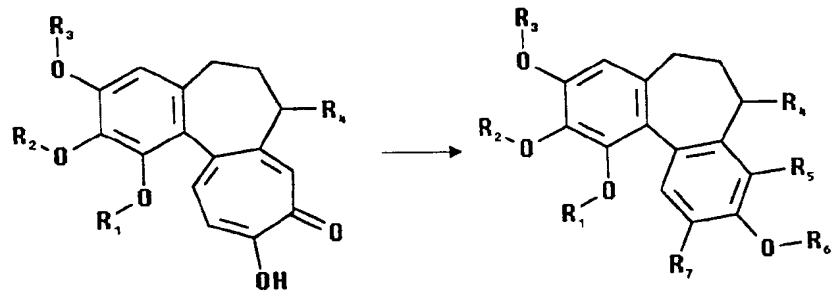
Another useful group of compounds includes those in which R_1, R_2 and R_3 are each alkyl and R_5 and R_7 are each hydrogen. A particularly useful subset of this group includes compounds in which R_1, R_2 and R_3 are each methyl and R_6 is hydrogen, alkyl or PO_3H_2.

Particularly useful compounds according to the invention include:

N-Acetylcolchinol-O-phosphate and its salts, solvates and hydrates.

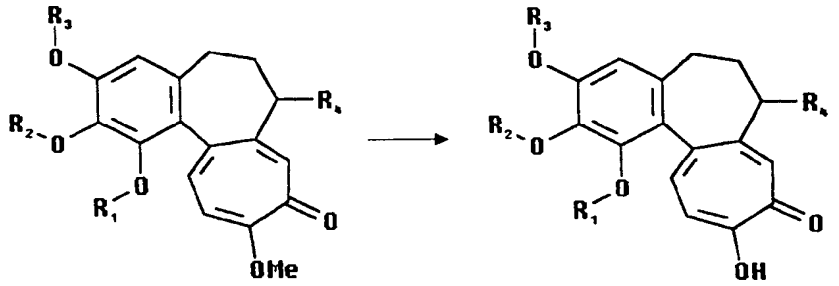
Compounds of formulae I, II or IIA may be prepared by a number of processes as generally described below and more specifically in the Examples hereinafter. In the following process description the symbols R_1, R_2, R_3, R_4, R_5, R_6 and R_7 when used in the formulae depicted are to be understood to represent those groups described above in relation to formulae I, II or IIA unless otherwise indicated. In the schemes described below it may be necessary to employ protecting groups which are then removed during the final stages of the synthesis. The appropriate use of such protecting groups and processes for their removal will be readily apparent to those skilled in the art.

Thus according to a further aspect of the invention compounds of formulae II or IIA in which R_5, R_6 and R_7 are each hydrogen may be prepared by treatment of a compound of formula (2) with alkaline hydrogen peroxide. The reaction may be conveniently performed in aqueous sodium hydroxide solution in the absence or presence of a cosolvent such as an alcohol, for example ethanol, at a temperature in the range for example 0- 100°C preferably at or near to 60°C.



(2) II or IIA

Intermediates of formulae (2) may be prepared by acid hydrolysis of compounds of formulae (3); The reaction is conveniently carried out in an aqueous acid such as hydrochloric acid at an elevated temperature, for example at or near 100°C.



(3) (2)

Compounds of formula (3) are either known or can be prepared from colchicine by conventional procedures.

Compounds of formulae I, II or IIA may also be prepared from other compounds of formulae I, II or IIA, by chemical modification. Examples of such chemical modifications that may be applied are standard alkylation, arylation, heteroarylation, acylation, thioacylation, sulphonylation, sulphation, phosphorylation, aromatic halogenation and coupling reactions. These reactions may be used to add new substituents or to modify existing substituents. Alternatively, existing substituents in compounds of formulae I, II or IIA may be modified by, for example oxidation, reduction, elimination, hydrolysis or other cleavage reaction to yield other compounds of formulae I, II or IIA.

Thus for example a compound of formulae II or IIA containing an amino group may be acylated on the amino group by treatment with, for example, an acyl halide or anhydride in the presence of a base, for example a

tertiary amine base such as triethylamine, in for example, a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In another general example of an interconversion process an amino group in a compound of formulae II or IIA may be sulphonylated by treatment with, for example, an alkyl or aryl sulphonyl chloride or an alkyl or aryl sulphonic anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for example a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In a further general example a compound of formulae II or IIA containing a hydroxy group can be converted into the corresponding dihydrogenphosphate ester by treatment with for example di-tert-butyl diethylphosphoramidite in the presence of a suitable catalyst for example tetrazole. In a solvent such as an ether solvent for example tetrahydrofuran at a temperature in the range -40 to 40°C, conveniently at or near room temperature, followed by treatment with an oxidising agent for example 3-chloroperoxy benzoic acid at a temperature in the range -78°C to 40°C preferably -40 to -10°C. The resulting intermediate phosphate triester is treated with an acid for example trifluoroacetic acid in a solvent such as a chlorinated solvent e.g. dichloromethane at a temperature in the range -30 to 40°C conveniently at or near 0°C to give the compound of formula (2) containing a dihydrogenphosphate ester.

In a further general example a compound of formula (2) containing an amide can be hydrolysed by treatment with

for example an acid such as hydrochloric acid in a solvent such as an alcohol, for example methanol at an elevated temperature conveniently at the reflux temperature.

In another general example an O-alkyl group may be cleaved to the corresponding alcohol (OH) by reaction with boron tribromide in a solvent such as a chlorinated solvent e.g. dichloromethane at a low temperature e.g. around -78°C.

In a further general example compounds of formulae II or IIA may be alkylated by reaction with a suitable alkylating agent such as an alkyl halide, an alkyl toluenesulphonate, an alkyl methanesulphonate or an alkyl triflate. The alkylation reaction can be carried out in the presence of a base for example an inorganic base such as a carbonate e.g. caesium or potassium carbonate, a hydride such as sodium hydride or an alkoxide such as potassium t-butoxide in a suitable solvent such as an aprotic solvent e.g. dimethylformamide or an ether solvent such as tetrahydrofuran at a temperature of around -10 to 80°C.

Preparation of a compound of formulae II or IIA as a single enantiomer or, where appropriate, diastereomer may be effected by synthesis from an enantiomerically pure starting material or intermediate or by resolution of the final product in a conventional manner.

Acid addition salts of the compounds of formulae II or IIA are prepared in a conventional manner by treating a solution or suspension of the free base II or IIA with about one equivalent of a pharmaceutically acceptable acid. Salts of compounds of formulae I, II or IIA derived from inorganic or organic bases are prepared in a conventional manner by treating a solution or suspension

of the free acid I, II or IIA with about one equivalent of a pharmaceutically acceptable organic or inorganic base. Alternatively both acid addition salts and salts derived from bases may be prepared by treatment of the parent compound with the appropriate ion-exchange resin in a standard fashion. Conventional concentration and recrystallisation techniques are employed in isolating the salts.

Compounds according to the invention are able to destroy tumour vasculature and vasculature that has been newly formed while leaving unaffected normal, mature vasculature. The ability of the compounds to act in this way may be determined by the tests described in the Examples hereinafter.

The compounds according to the invention are thus of particular use in the prophylaxis and treatment of cancers involving solid tumours and in the prophylaxis and treatment of diseases where inappropriate angiogenesis occurs such as diabetic retinopathy, psoriasis, rheumatoid arthritis, atherosclerosis and macular degeneration.

The compounds of the invention may be administered as a sole therapy or in combination with other treatments. For the treatment of solid tumours compounds of the invention may be administered in combination with radiotherapy or in combination with other anti-tumour substances for example those selected from mitotic inhibitors, for example vinblastine, paclitaxel and docetaxel; alkylating agents, for example cisplatin, carboplatin and cyclophosphamide, antimetabolites, for example 5-fluorouracil, cytosine arabinoside and hydroxyurea; intercalating agents for example adriamycin and bleomycin; enzymes, for example aspariginase; topoisomerase inhibitors for example etoposide, topotecan

and irinotecan; thymidylate synthase inhibitors for example raltitrexed; biological response modifiers for example interferon; antibodies for example edrecolomab; and anti-hormones for example tamoxifen. Such combination treatment may involve simultaneous or sequential application of the individual components of the treatment.

For the prophylaxis and treatment of disease the compounds according to the invention may be administered as pharmaceutical compositions selected with regard to the intended route of administration and standard pharmaceutical practice. Such pharmaceutical compositions may take a form suitable for oral, buccal, nasal, topical, rectal or parenteral administration and may be prepared in a conventional manner using conventional excipients. For example for oral administration the pharmaceutical compositions may take the form of tablets or capsules. For nasal administration or administration by inhalation the compounds may be conveniently delivered as a powder or in solution. Topical administration may be as an ointment or cream and rectal administration may be as a suppository. For parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) the composition may take the form of, for example, a sterile solution, suspension or emulsion.

The dose of a compound of the invention required for the prophylaxis or treatment of a particular condition will vary depending on the compound chosen, the route of administration, the form and severity of the condition and whether the compound is to be administered alone or in combination with another drug. Thus the precise dose will be determined by the administering physician but in general daily dosages may be in the range 0.001 to 100mg/kg preferably 0.1 to 50mg/kg.

BIOLOGICAL ACTIVITY

The following tests were used to demonstrate the activity and selectivity of compounds according to the invention.

Activity against tumour vasculature measured by radioactive tracer.

The following experiment demonstrates the ability of the compounds to damage selectively tumour vasculature.

Subcutaneous CaNT tumours were initiated by injecting 0.05ml of a crude tumour cell suspension, approximately 10^6 cells, under the skin overlying the rear dorsum of 12-16 week-old mice. The animals were selected for treatment after approximately 3-4 weeks, when their tumours reached a geometric mean diameter of 5.5-6.5mm. Compounds were dissolved in sterile saline and injected intraperitoneally in a volume of 0.1 ml per 10 g body weight. Tumour perfusion was measured 6 hours after intraperitoneal administration in tumour, kidney, liver, skin muscle, gut and brain by the ^{86}RbCI extraction technique (Sapirstein, Amer J Physiol, 193, 161-168, 1958). Tissue radioactivity measured 1 minute after an intravenous injection of ^{86}RbCI was used to calculate relative blood flow as a proportion of cardiac output (Hill and Denekamp, Brit J Radiol, 55, 905-913, 1982). Five animals were used in control and treated groups. Results were expressed as a percentage of the blood flow in the corresponding tissues in vehicle treated animals.

Activity against tumour vasculature measured by fluorescent dye.

The following experiment further demonstrates the ability of the compounds to damage tumour vasculature.

Tumour functional vascular volume in CaNT tumour-bearing mice was measured using the florescent dye Hoechst 33342

according to the method of Smith *et al* (Brit J Cancer 57, 247-253, 1988). Five animals were used in control and treated groups. The fluorescent dye was dissolved in saline at 6.25 mg/ml and injected intravenously at 10 mg/kg 6 hours after intraperitoneal drug treatment. One minute later, animals were killed and tumours excised and frozen; 10μm sections were cut at 3 different levels and observed under UV illumination using an Olympus microscope equipped with epifluorescence. Blood vessels were identified by their fluorescent outlines and vascular volume was quantified using a point scoring system based on that described by Chalkley, (J Natl Cancer Inst, 4, 47-53, 1943). All estimates were based on counting a minimum of 100 fields from sections cut at the 3 different levels. Compounds of the invention reduced tumour functional vascular volume by greater than 20% at doses of 50mg/kg or below.

The following non-limiting Examples illustrate the invention. In the Examples all ^{1}Hnmr were run at 300MHz unless otherwise specified. Column chromatography was performed on silica gel. All temperatures are in °C. The following abbreviations are used: THF - tetrahydrofuran; DMSO - dimethylsulphoxide; MCPBA - 3-chloroperoxybenzoic acid.

EXAMPLE 1

N-Acetylcolchinol-O-phosphate

A solution of N-acetylcolchinol (260mg, 0.76 mmol) in anhydrous THF (2ml) under an atmosphere of nitrogen was treated with di-t-butyl diethylphosphoramidite (189mg, 0.75mmol) and 1(H)-tetrazole (0.14g, 1.99mmol) and the solution stirred at 20° for 0.5h. The solution was cooled to -40° and a solution of 85% MCPBA (202mg, 0.99mmol) in anhydrous dichloromethane (2ml) at such a rate that the temperature remained below -10°. The solution was allowed to warm to room temperature, diethyl

ether (30ml) was added and the resulting solution washed successively with 10% aqueous sodium metabisulphite (two 25ml portions), 5% aqueous sodium bicarbonate (two 25ml portions), 5% aqueous citric acid (30ml), 5% aqueous sodium bicarbonate, and brine. The organic solution was concentrated under reduced pressure and the residue subjected to column chromatography to afford a white foam (170mg) containing N-acetylcolchinol-O-di-*t*-butylphosphate which was redissolved in dichloromethane (5ml), cooled to 0° and treated with trifluoroacetic acid (0.5ml).

The solution was allowed to warm to room temperature and stir 1 hr before being concentrated under reduced pressure and triturated with ether to give the title compound (110mg) as a white solid m.p. 233-235°. δH (d6-DMSO) 8.38(d,1H, J=8Hz), 7.27(d, 1H,J=7Hz), 7.12(d, 1H, J=8Hz), 7.10(s,1H), 6.77 (s, 1H), 4.48 (m, 1H), 3.81 (s, 3H), 3.76 (s, 3H), 3.49 (s, 3H), 2.5 (signal partially obscured by DMSO peak), 1.9-2.2 (m, 2H), 1.86 (s, 3H).

The activity of the phosphate compound was measured by the radioactive tracer assay described above: the compound of this Example gave a 65% decrease in tumour blood flow at a dose of 125mg/kg with no significant reduction in blood flow in skin, muscle, liver, kidney, gut or heart.

The phosphate compound was compared with the parent N-acetylcolchinol for the maximum tolerated dose (MTD) (no deaths in three animals), minimum effective dose (MED) measured by the fluorescent dye technique already described and the therapeutic window (MTD/MED).

	MTD mg/Kg body weight	MED mg/Kg body weight	Therapeutic Window (MTD/MED)
N-acetyl colchinol	125	30	4
N-acetyl colchinol-O-phosphate	750	50	15

Though the phosphate had a slightly higher MED the "window" was significantly greater. This was unexpected. The phosphate also gave greater solubility.

For comparison the "therapeutic windows" for colchicine (the closest structure to the present compounds and docetaxel (a tubulin-binding drug, marketed as "Taxotere", which has no vascular-damaging activity) and these data are presented in the following table:

Table 1 - Therapeutic windows of other tubulin-binding agents (by fluorescent dye technique)

Compound	MED (mg/kg body weight)	MTD (mg/kg body weight)	MTD/MED
Docetaxel	>30(No effect at 30)	30	<1
Colchicine	2.5	5	2

EXAMPLE 2

N-Ethylcolchinol

A solution of N-acetylcolchinol (500mg, 1.4mmol) in THF (15ml) was added dropwise over 15 minutes to a suspension of lithium aluminum hydride (106mg, 2.74mmol) in THF (10ml) with ice-bath cooling. The mixture was heated at reflux for 15h, allowed to cool and treated with further lithium aluminium hydride (53mg, 1.4mmol) before heating at reflux for a further 3h. The mixture was cooled (ice bath) and water (10ml) added dropwise before extraction with three portions of ethyl acetate. The combined,

dried (MgSO4) extracts were concentrated under reduced pressure to a green gum which, on trituration with ether, gave the title compound as a light-green solid. m.p. 185°C (dec.), m/e 343 (M+). Anal. Calculated for $C_{20}H_{25}NO_4$ H_2O: C, 66.46; H, 7.53; N, 3.88. Found: C,66.50; H, 7.17; N, 3.79.

EXAMPLE 3

N-Benzyloxycarbonylcolchinol

A solution of colchinol (625mg, 1.98mmol) in dry pyridine (10ml) was treated dropwise with benzylchloroformate (0.566ml, 3.97 mmol) and the mixture stirred 16h. Solvent was removed under reduced pressure, water added and the resulting mixture extracted with three portions of chloroform. The combined, dried ($MgSO_4$) extracts were concentrated under reduced pressure to a dark brown gum which was subjected to column chromatography on silica gel eluting with 50% ethyl acetate/petroleum ether. The resultant orange gum was crystallised from ether/petroleum ether to give the title compound (346mg) as a pale yellow solid. m.p. 79-81°C, m/e 449 (M+). Anal. Calculated for $C_{26}H_{27}NO_6$ $0.33H_20$; C, 68.57; H, 6.07; N, 3.08. Found: C,68.71; H, 6.18; N, 2.91.

EXAMPLE 4

N-(Phenylcarbamoyl) colchinol

A solution of colchinol (400mg, 1.27mmol) in dry pyridine (10ml) was treated dropwise with phenyl isocyanate (0.151ml, 1.39mmol) and the mixture stirred for 18h before heating at reflux for 2h. Solvent was removed under reduced pressure, water added and the resulting mixture extracted with three portions of chloroform. The combined, dried ($MgSO_4$) extracts were concentrated under reduced pressure to a dark brown gum which was subjected to column chromatography on silica gel eluting with 35% ethyl acetate/petroleum ether. The resultant gum was crystallised from ether/petroleum ether to give the title

compound (261mg) as a pale orange solid. m.p. 145-146°C, m/e 434 (M+).

EXAMPLE 5

N-Mesylcolchinol

A solution of N,O-dimesylcolchinol (234mg, 0.5mmol) in methanol (8ml) was treated with sodium hydroxide (40mg, 1mmol) and the mixture heated at reflux for 3h. Solvent was removed under reduced pressure and water (5ml) added. The solution was rendered neutral by the addition of 1M hydrochloric acid and extracted with three portions of dichloromethane. The combined, dried ($MgSO_4$) extracts were concentrated under reduced pressure to give the title compound (123mg) as a pink solid. m.p. 234-236°C, m/e 393 (M+).

The N,O-dimesylcolchinol used as starting material was prepared as follows: A solution of colchinol (500mg, 1.6mmol) in dry pyridine (15ml) was treated with mesyl chloride (0.135ml, 1.7mmol) and the mixture stirred at room temperature 36h. A further portion of mesyl chloride (0.135ml, 1.7mmol) was added and stirring continued 16h. Solvent was removed under reduced pressure and water (5ml) added. The solution was extracted with three portions of chloroform and the combined, dried ($MgSO_4$) extracts were concentrated under reduced pressure to give a brown gum which was subjected to column chromatography on silica gel eluting with ethyl acetate to give N,O-dimesylcolchinol (292mg) as a light orange solid.

EXAMPLE 6

N-Dimethylsulphamoylcolchinol

A solution of colchinol (50mg, 0.16mmol) in dry acetonitrile (3ml) and triethylamine (0.022ml, 0.16mmol) was treated with dimethylsulphamoyl chloride and the mixture stirred for 30 minutes before heating at reflux

for 15h. Solvent was removed under reduced pressure, water added and the resulting mixture extracted with three portions of chloroform. The combined, dried (Na_2SO_4) extracts were concentrated under reduced pressure to a dark brown gum which was subjected to column chromatography on silica gel eluting with ethyl acetate to give the title compound (46mg) as a pale orange gum which solidified. m.p. 82-85°C m/e 422 (M+).

EXAMPLE 7

N-Acetyl-O-methoxycarbonylmethylcolchinol

A solution of N-acetylcolchinol (500mg, 1.4mmol) in dry DMF (5ml) at 0°C was treated with methylbromoacetate (322mg, 2.1mmol) and sodium hydride (84mg of a 60% suspension in oil, 2.1mmol) and the mixture stirred for 30 minutes. Water (50ml) was added and the mixture extracted with four portions of ethyl acetate. The combined extracts were washed successively with four portions of water and two portions of saturated aqueous sodium chloride solution, dried ($MgSO_4$) and solvent removed under reduced pressure to give the title compound (280mg) as a white solid m.p. 82-83°C. m/e 429 2 (M+). Anal. Calculated for $C_{23}H_{27}NO_7$ $0.33H_20$; C, 63.45; H, 6.40; N, 3.22. Found: C, 63.53; H, 6.29; N, 3.17.

EXAMPLE 8

N-Acetyl-O-carboxymethylcolchinol

A solution of N-acetyl-O-methoxycarbonylmethylcolchinol (140mg, 0.33mmol) in acetonitrile (5ml) was treated with aqueous potassium hydroxide solution (1.0M, 5ml) and the mixture heated at 80°C for 30 minutes. The cooled mixture was adjusted to pH3 by addition of 2M hydrochloric acid and extracted with four portions of ethyl acetate. The combined extracts were washed with two portions of saturated aqueous sodium chloride solution, dried ($MgSO_4$) and concentrated under reduced

pressure. Addition of acetone (2ml) and hexane (1ml) produced the title compound (58mg) as a white solid m.p. 220-221°C. m/e 415.3 (M+). Anal. Calculated for $C_{22}H_{25}NO_7$ $0.33H_20$; C,62.71; H, 6.14; N, 3.32. Found: C, 62.63; H, 6.02; N, 3.26.

EXAMPLE 9

N-Acetyl-O-cyclopentylcolchinol

A solution of N-acetylcolchinol (200mg, 0.56mmol) in dry DMF (2ml) at 0°C was treated with sodium hydride (33mg of a 60% suspension in oil, 0.84mmol) followed by cyclopentyl bromide (125mg, 0.84mmol) and the mixture stirred 1h. A further portion of sodium hydride (17mg of a 60% suspension in oil, 0.42mmol) and of cyclopentyl bromide (63mg, 0.42mmol) and the mixture stirred overnight at room temperature. Water (10ml) was added and the mixture extracted with four portions of ethyl acetate. The combined extracts were washed with two portions of saturated aqueous sodium chloride solution, dried ($MgSO_4$) and concentrated under reduced pressure. The title compound (160mg) was obtained as a white solid m.p. 89-94°C. m/e 425.3 (M+). Anal. Calculated for $C_{25}H_{31}NO_5$: C,70.54; H, 7.35; N, 3.29. Found: C, 70.55; H, 7.35; N, 3.25.

EXAMPLE 10

N-Acetyl-10-nitrocolchinol

A solution of N-acetylcolchinol (100mg, 0.27mmol) in glacial acetic acid (20ml) was treated slowly with 20ml of a solution of concentrated nitric acid (0.34ml) in acetic acid (100ml) keeping the temperature at about 12°C. The mixture was stirred at room temperature for 18h, a further 1ml of the nitric acid/acetic acid solution added and stirring continued for 2h. The mixture was poured onto ice and extracted with three portions of ethyl acetate. The combined extracts were washed with two portions of saturated aqueous sodium

cloride solution, dried ($MgSO_4$) and concentrated under reduced pressure. Purification on silica gel eluting with ethyl acetate gave the title compound (50mg) as a pale yellow solid m.p. 117-8°C. m/e 401.9 (M+) Anal. Calculated for $C_{20}H_{22}N_2O_7$ $0.33H_2O$; C, 58.82; H, 5.56; N, 6.86. Found: C, 58.87; H, 5.66; N, 6.55.

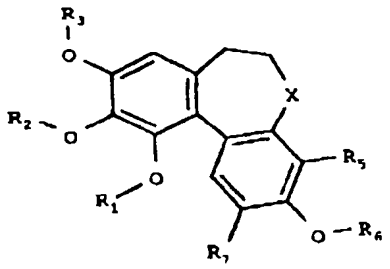
EXAMPLE 11

The activity against tumour vasculature was measured by the fluorescent dye technique described above for the compounds of Examples 1-10 administered at 50mg/kg and N-acetylcolchinol

Compound of Example	% decrease in vascular volume
1	89
2	38
3	43
4	37
5	38
6	30
7	12
8	49
9	59
10	28
N-acetylcolchinol	78

CLAIMS:

1. The use of colchinol derivatives for the preparation of compositions for the treatment of diseases involving angiogenesis in which the colchinol derivative has the formula



I

wherein

R_1, R_2, R_3 and R_6 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, alkanoyl, PO_3H_2;
X is carbonyl (CO), thiocarbonyl (CS), methylene (CH_2) or a group CHR_4
R_4 is OH, O-alkyl or NR_8R_9;
R_5 and R_7 are each independently H, alkyl, halogen, hydroxy, alkoxy, nitro or amino;
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;
and R_9 is H, alkyl or cycloalkyl
and the pharmaceutically acceptable salts, solvates, and hydrates thereof.

2. A use according to claim 1 wherein at least one of R_1, R_2, R_3 and R_6 is PO_3H_2.

3. A use according to claim 2 wherein R_6 is PO_3H_2.

4. A use according to any one of claims 1 to 3 in which R_1, R_2 and R_3 are alkyl.

5. A use according to any one of claims 1 to 4 in which R_4 is acylamino.

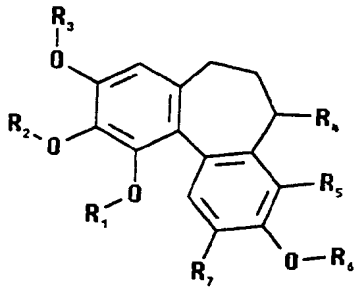
6. A use according to claim 1 in which R_6 is PO_3H, R_1, R_2 and R_3 are alkyl and R_4 is acylamino.

7. Compounds of formula I in which at least one of R_1, R_2, R_3 and R_6 is PO_3H_2.

8. A compound according to claim 7 in which R_6 is PO_3H_2.

9. A compound according to claim 8 in which R_1, R_2 and R_3 are alkyl and R_4 is acylamino.

10. A compound according to claim 7 of formula



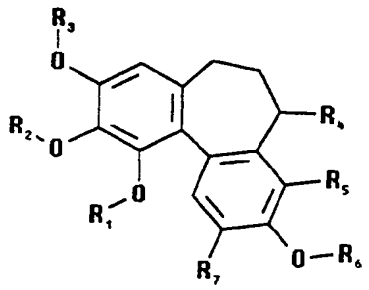
II

wherein

R_1, R_2 and R_3 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, alkanoyl, or PO_3H_2;

R_6 is PO_3H_2;
R_4 is H or NR_8R_9;
R_5 and R_7 are each independently H, alkyl, halogen, alkoxy, nitro or amino;
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;
and R_9 is H, alkyl or cycloalkyl,
and the pharmaceutically acceptable salts, solvates and hydrates thereof.

11. A compound of the formula



IIA

wherein
R_1, R_2 and R_3 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, alkanoyl or PO_3H_2;
R_6 is H, optionally substituted alkyl, cycloalkyl, alkenyl, akynyl or PO_3H_2;
R_4 is H or NR_8R_9;
R_5 and R_7 are each independently H, alkyl, halogen, nitro or amino;
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl,

aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylamnosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;

and R_9 is H, alkyl or cycloalkyl, with the proviso that, when R_1, R_2 and R_3 are all methyl groups and R_4 is hydrogen, acetylamino, acetylmethylamino, amino, methylamino or dimethylamino then R_6 is not hydrogen, methyl, hydroxyethyl, or acetoxyethyl,

and the pharmaceutically acceptable salts, solvates and hydrates thereof.

12. A compound according to claim 11 in which R_1, R_2 and R_3 are alkyl and R_4 is acylamino.

INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/GB 98/01977

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/66 A61K31/165 A61K31/27 C07C13/547

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	O. BOYE ET AL.: "Synthesis of carbon-14 labeled electrophilic ligands of the colchicine binding site of tubulin: chloroacetates of demethylthiocolchicines and of N-acetylcolchinol, isothiocyanates of 9-deoxy-N-acetylcolchinol." J. LABELLED COMPD. RADIOPHARM., vol. 33, no. 4, 1993, pages 293-299, XP002081866 ---	
A	R. BRECHT ET AL.: "Dihydrocolchicine 8,12-endoperoxide. a novel starting material for convenient syntheses of the allocolchicinoids N-acetylcolchinol O-methyl ether and androbiphenyline." LIEBIGS ANN., no. 11, 1997, pages 2275-2279, XP002081867 --- -/--	

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 October 1998	11/11/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Klaver, T

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/01977

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	GIL-JONG KANG ET AL: "n-acetylcolchinol O-methyl ether and thiocolchicine, potent nalogs of colchicine modified in the C-ring" J. BIOL. CHEM., vol. 265, no. 18, 1990, pages 10255-10259, XP002081868 ---	
A	DATABASE WPI Week 6800 Derwent Publications Ltd., London, GB; AN 66-13831f XP002081869 & JP 39 019635 B (SANKYO CO.) see abstract ---	
A	DATABASE WPI Week 6800 Derwent Publications Ltd., London, GB; AN 66-13830f XP002081870 & JP 39 019634 B (SANKYO CO.) see abstract -----	

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Bibliographic data

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Pub/Issue Date:	2016-11-09
Inventor(s):	GRIGORIADIS DIMITRI E|·E·
Applicant(s):	NEUROCRINE BIOSCIENCES INC
Classification:	A61K31/427AI; A61K31/4985AI; A61K31/519AI
Application number:	CN20158005270 2015-01-21
Priority number:	US201461929941P 20140121; US201461981033P 20140417; US201462069155P 20141027; WO2015US12315 20150121;

Abstract of CN106102740A

CRF1 receptor antagonists have the potential to directly inhibit ACTH release in patients with CAH and thereby allow normalization of androgen production while using lower, more physiologic doses of hydrocortisone, and thus reducing treatment-associated side effects.

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(71)申请人 纽罗克里生物科学有限公司
地址 美国加利福尼亚州

(72)发明人 季米特·E·格里戈里尔蒂斯

(74)专利代理机构 北京英赛嘉华知识产权代理有限责任公司 11204
代理人 王达佐 洪欣

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A61K 31/4985(2006.01)
A61K 31/519(2006.01)

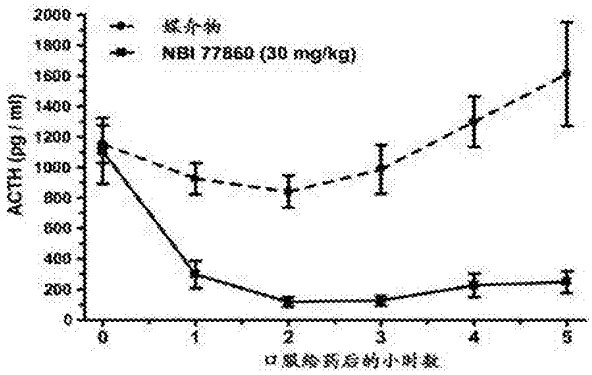
权利要求书2页 说明书22页 附图6页

(54)发明名称

治疗先天性肾上腺增生的CRF_1受体拮抗剂

(57)摘要

CRF_1受体拮抗剂可能直接抑制患有CAH的患者的ACTH释放，从而在使用多个较低生理剂量的氢化可的松时，使雄激素的产生正常化，并且因此减少治疗相关的副作用。



CN 106102740 A

1.治疗先天性肾上腺增生(CAH)的方法,其通过向有需要的对象施用具有超过30分钟的解离半衰期的CRF_1受体拮抗剂来进行。

2.如权利要求1所述的方法,其中所述CRF_1受体拮抗剂具有超过40分钟的解离半衰期。

3.如权利要求1所述的方法,其中所述CRF_1受体拮抗剂具有超过50分钟的解离半衰期。

4.如权利要求1所述的方法,其中所述CRF_1受体拮抗剂为化合物I(NBI-77860;2,5-二甲基-3-[2-甲基-4-(甲氧基)苯基]-N-[(1S)-1-(3-甲基-1,2,4-噁二唑-5-基)丙基]吡唑并[1,5-a]嘧啶-7-胺)。

5.如权利要求1所述的方法,其中所述CRF_1受体拮抗剂为NBI-30775、NBI-34041、SSR-126374、SSR-125543、安他拉明(N-丁基-N-乙基-2,5,6-三甲基-7-(2,4,6-三甲基苯基)吡咯并[3,2-e]嘧啶-4-胺)、或者DMP904。

6.如权利要求1-5中任一项所述的方法,其中所述CRF_1受体拮抗剂在临睡前施用。

7.如权利要求1-5中任一项所述的方法,其中所述CRF_1受体拮抗剂在预期的生理节律释放ACTH之时或者之前施用。

8.如权利要求7所述的方法,其中所述CRF_1受体拮抗剂在预期的生理节律释放ACTH之前3-4小时施用。

9.降低患有先天性肾上腺增生(CAH)的对象中的17-OHP水平和ACTH水平的方法,所述方法包括在临睡前向所述对象施用CRF_1受体拮抗剂。

10.如权利要求9所述的方法,其中所述CRF_1受体拮抗剂在预期的生理节律释放ACTH之时或者之前施用。

11.如权利要求9所述的方法,其中所述CRF_1受体拮抗剂在预期的生理节律释放ACTH之前3-4小时施用。

12.如权利要求9-11中任一项所述的方法,其中所述CRF_1受体拮抗剂为化合物I(NBI-77860;2,5-二甲基-3-[2-甲基-4-(甲氧基)苯基]-N-[(1S)-1-(3-甲基-1,2,4-噁二唑-5-基)丙基]吡唑并[1,5-a]嘧啶-7-胺)。

13.如权利要求9-11中任一项所述的方法,其中所述CRF_1受体拮抗剂为NBI-30775、NBI-34041、SSR-126374、SSR-125543、安他拉明(N-丁基-N-乙基-2,5,6-三甲基-7-(2,4,6-三甲基苯基)吡咯并[3,2-e]嘧啶-4-胺)、或者DMP904。

14.用于治疗先天性肾上腺增生(CAH)的CRF_1受体拮抗剂,其中所述CRF_1受体拮抗剂具有超过30分钟的解离半衰期。

15.如权利要求14所述的CRF_1受体拮抗剂,其中所述CRF_1受体拮抗剂具有超过40分钟的解离半衰期。

16.如权利要求14所述的CRF_1受体拮抗剂,其中所述CRF_1受体拮抗剂具有超过50分钟的解离半衰期。

17.如权利要求14所述的CRF_1受体拮抗剂,其中所述CRF_1受体拮抗剂为化合物I(NBI-77860;2,5-二甲基-3-[2-甲基-4-(甲氧基)苯基]-N-[(1S)-1-(3-甲基-1,2,4-噁二唑-5-基)丙基]吡唑并[1,5-a]嘧啶-7-胺)。

18.如权利要求14所述的CRF_1受体拮抗剂,其中所述CRF_1受体拮抗剂为NBI-30775、NBI-34041、SSR-126374、SSR-125543、安他拉明(N-丁基-N-乙基-2,5,6-三甲基-7-(2,4,6-三甲基苯基)吡咯并[3,2-e]嘧啶-4-胺)、或者DMP904。

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19.如权利要求14-18中任一项所述的CRF_1受体拮抗剂，其中所述CRF_1受体拮抗剂适于临睡前施用。

20.如权利要求14-18中任一项所述的CRF_1受体拮抗剂，其中所述CRF_1受体拮抗剂适于在预期的生理节律释放ACTH之时或者之前施用。

21.如权利要求14-18中任一项所述的CRF_1受体拮抗剂，其中所述CRF_1受体拮抗剂适于在预期的生理节律释放ACTH之前3-4小时施用。

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治疗先天性肾上腺增生的CRF_1受体拮抗剂

[0001] 发明背景

技术领域

[0002] 本文描述了治疗先天性肾上腺增生的组合物和方法。

[0003] 相关技术描述

[0004] 先天性肾上腺增生(CAH)是一组常染色体隐性基因病症,其导致很少或者没有皮质醇生物合成。最常见的疾病形式为由位于染色体6p21上的CYP21A2基因的突变引起的21-羟化酶缺乏症,这约占CAH病例的95%(对于综述,参见,例如,Speiser等人,Int.J.Pediatr.Endocrinol.2010:494173(2010))。这些突变的范围可以从合成肾上腺皮质中的皮质醇所需的酶活性的完全丧失至一系列部分丧失,这导致疾病严重度,所述疾病严重度是特异性突变的直接结果。这种连续的21-羟化酶缺乏症已经被广泛分为:被分组为典型CAH的失盐型和单纯雄性化型,以及被称为非典型CAH(NCCAH)或者"晚发型"CAH的轻微形式,所述轻微形式通常在儿童晚期或者成人早期被诊断出。非典型CAH患者是纯合或者混合的杂合子,通常具有典型CAH等位基因。这些患者具有足够的酶活性(>正常的20%-50%),使得他们在出生时不会患有失盐型缺乏症或者皮质醇缺乏症,具有正常的生殖器,并且许多人在整个一生都保持无症状(Trapp等人,Steroids 77(4):342-46(2012))。在较少见的疾病形式中,11β-羟化酶基因CYP11B1的突变导致CAH(11β-OH CAH),这占病例的5%。

[0005] 两种基因突变都导致先天性肾上腺增生、皮质醇缺乏症和过度的促肾上腺皮质激素(ACTH)产生以及雄激素的过度产生。这些患者需要终身管理糖皮质激素以及与该治疗相关的附带问题。因此,对于改善患有CAH的患者的健康、提升其幸福、改善其生命质量以及管理其相关病症的治疗方案存在重大需求。

[0006] 发明概述

[0007] 促肾上腺皮质激素释放因子(CRF)激活CRF_1受体(一类B G蛋白偶联受体(GPCR))。CRF_1拮抗剂可能直接抑制患有CAH的患者的ACTH释放,从而在使用多个较低生理剂量的氢化可的松时,使雄性激素的产生正常化并且减少治疗相关的副作用。

[0008] 在一个实施方案中,提供了通过向有需要的对象施用有效量的CRF_1拮抗剂治疗CAH的方法,其包括(但不限于)临睡前施用。

[0009] 在更具体的实施方案中,CRF_1拮抗剂具有超过30分钟的解离半衰期($t_{1/2}$),并且在另一实施方案中,CRF_1拮抗剂具有超过40分钟的解离半衰期($t_{1/2}$),以及在另一实施方案中,CRF_1拮抗剂具有超过50分钟的解离半衰期($t_{1/2}$)。

[0010] 实施方案1.治疗先天性肾上腺增生(CAH)的方法,其通过向有需要的对象施用具有超过30分钟的解离半衰期的CRF_1受体拮抗剂来进行。

[0011] 实施方案2.如实施方案1所述的方法,其中所述CRF_1受体拮抗剂具有超过40分钟的解离半衰期。

[0012] 实施方案3.如实施方案1所述的方法,其中所述CRF_1受体拮抗剂具有超过50分钟

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的解离半衰期。

[0013] 实施方案4.如实施方案1-3中任一项所述的方法，其中所述CRF_1受体拮抗剂为化合物I(NBI-77860;2,5-二甲基-3-[2-甲基-4-(甲氧基)苯基]-N-[(1S)-1-(3-甲基-1,2,4-噁二唑-5-基)丙基]吡唑并[1,5-a]嘧啶-7-胺)。

[0014] 实施方案5.如实施方案1-3中任一项所述的方法，其中所述CRF_1受体拮抗剂为NBI-30775、NBI-34041、SSR-126374、SSR-125543、安他拉明(antalarmin)(N-丁基-N-乙基-2,5,6-三甲基-7-(2,4,6-三甲基苯基)吡咯并[3,2-e]嘧啶-4-胺)或者DMP904。

[0015] 实施方案6.如实施方案1-5中任一项所述的方法，其中所述CRF_1受体拮抗剂在临睡前施用。

[0016] 实施方案7.如实施方案1-6中任一项所述的方法，其中所述CRF_1受体拮抗剂在预期的生理节律释放ACTH之时或者之前施用。

[0017] 实施方案8.如实施方案7所述的方法，其中所述CRF_1受体拮抗剂在预期的生理节律释放ACTH之前3-4小时施用。

[0018] 实施方案9.降低患有先天性肾上腺增生(CAH)的对象中的17-OHP水平和ACTH水平的方法，所述方法包括在临睡前向所述对象施用CRF_1受体拮抗剂。

[0019] 实施方案10.如实施方案9所述的方法，其中所述CRF_1受体拮抗剂在预期的生理节律释放ACTH之时或者之前施用。

[0020] 实施方案11.如实施方案9或者实施方案10所述的方法，其中所述CRF_1受体拮抗剂在预期的生理节律释放ACTH之前3-4小时施用。

[0021] 实施方案12.如实施方案9-11中任一项所述的方法，其中所述CRF_1受体拮抗剂为化合物I(NBI-77860;2,5-二甲基-3-[2-甲基-4-(甲氧基)苯基]-N-[(1S)-1-(3-甲基-1,2,4-噁二唑-5-基)丙基]吡唑并[1,5-a]嘧啶-7-胺)。

[0022] 实施方案13.如实施方案9-11中任一项所述的方法，其中所述CRF_1受体拮抗剂为NBI-30775、NBI-34041、SSR-126374、SSR-125543、安他拉明(N-丁基-N-乙基-2,5,6-三甲基-7-(2,4,6-三甲基苯基)吡咯并[3,2-e]嘧啶-4-胺)或者DMP904。

[0023] 实施方案14.用于治疗先天性肾上腺增生(CAH)的CRF_1受体拮抗剂，其中所述CRF_1受体拮抗剂具有超过30分钟的解离半衰期。

[0024] 实施方案15.如实施方案14所述的CRF_1受体拮抗剂，其中所述CRF_1受体拮抗剂具有超过40分钟的解离半衰期。

[0025] 实施方案16.如实施方案14所述的CRF_1受体拮抗剂，其中所述CRF1受体拮抗剂具有超过50分钟的解离半衰期。

[0026] 实施方案17.如实施方案14-16中任一项所述的CRF_1受体拮抗剂，其中所述CRF_1受体拮抗剂为化合物I(NBI-77860;2,5-二甲基-3-[2-甲基-4-(甲氧基)苯基]-N-[(1S)-1-(3-甲基-1,2,4-噁二唑-5-基)丙基]吡唑并[1,5-a]嘧啶-7-胺)。

[0027] 实施方案18.如实施方案14-16中任一项所述的CRF_1受体拮抗剂，其中所述CRF_1受体拮抗剂为NBI-30775、NBI-34041、SSR-126374、SSR-125543、安他拉明(N-丁基-N-乙基-2,5,6-三甲基-7-(2,4,6-三甲基苯基)吡咯并[3,2-e]嘧啶-4-胺)或者DMP904。

[0028] 实施方案19.如实施方案14-18中任一项所述的CRF_1受体拮抗剂，其中所述CRF_1受体拮抗剂适于临睡前施用。

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[0029] 实施方案20.如实施方案14-19中任一项所述的CRF_1受体拮抗剂，其中所述CRF_1受体拮抗剂适于在预期的生理节律释放ACTH之时或者之前施用。

[0030] 实施方案21.如实施方案14-20中任一项所述的CRF_1受体拮抗剂，其中所述CRF_1受体拮抗剂适于在预期的生理节律释放ACTH之前3-4小时施用。

[0031] 在其它实施方案中，以上以及本文所述的方法和用途还包括将糖皮质激素或者盐皮质激素的量从施用于患有CAH的发育完全的对象(例如，人对象)的GC(例如氢化可的松(HC)、强的松、泼尼松龙、地塞米松或者氟氢可的松)的推荐日剂量降低至少10％、15％、20％、30％、40％、50％、60％。在其它实施方案中，以上以及本文所述的方法和用途还包括将糖皮质激素或者盐皮质激素的量从施用于患有CAH的正在发育的对象(例如，人对象)的GC(例如氢化可的松)或者盐皮质激素(例如氟氢可的松)的推荐日剂量降低至少10％、15％、20％、30％、40％、50％、60％。

[0032] 参照以下详细说明，这些以及其它实施方案是显而易见的。为此，本文列出了各种参考文献，其更详细地描述某些背景信息、程序、化合物和组合物，并且将每一篇参考文献据此通过引用整体并入。

[0033] 本文未明确定义的术语应具有由本领域技术人员根据本公开内容和上下文所给出的含义。然而，除非作出相反说明，如说明书中所用，术语具有所指定的含义。在整个说明书中提及的“一个实施方案”或者“实施方案”，意指结合实施方案所述的具体的特征、结构或者特点被包括在至少一个实施方案中。因此，在整个说明书的不同地方出现的短语“在一个实施方案中”或者“在实施方案中”不必全部指同一实施方案。而且，具体的特征、结构或特点可以任何适合的方式组合在一个或多个实施方案中。

[0034] 此外，如在本说明书和所附权利要求中所用，单数形式“一个/一种(a)”、“一个/一种(a)”和“所述”包括复数指示物，除非上下文另外明确规定。因此，例如，关于“非人动物”可以指一种或者多种非人动物或者多个此类动物，并且关于“细胞”或者“所述细胞”包括提及的一个或者多个细胞以及本领域技术人员已知的其等同物(例如，多个细胞)等。当描述或者要求保护方法的步骤，并且所述步骤被描述为以特定顺序发生时，第一步骤“优先于”(即，先于)第二步骤发生(或者进行)的描述如果被改写为第二步骤“随后于”第一步骤发生(或者进行)的陈述，那么它们具有相同的含义。当提及数字或者数值范围时，术语“约”意指提及的数字或者数值范围为实验变异内的近似值(或者在统计实验误差内)，因此，数字或者数值范围可以在所述数字或者数值范围的1％至15％变化。还应当注意的是，术语“或者”通常采用包括“和/或”的含义，除非上下文另外明确规定。例如，当提及至少一种化合物或者至少一种组合物时，术语“至少一种”具有与术语“一种或者多种”相同的含义和理解。

[0035] 附图简述

[0036] 图1表示肾上腺的类固醇合成途径的示意图。最常见的CAH形式由导致皮质醇减少以及雄激素(例如睾酮)和雌激素增加的21-羟化酶(又称为21-α羟化酶)缺乏症所引起。更稀有的典型CAH类型为11β-羟化酶缺乏症。

[0037] 图2表示示例化合物I(NBI-77860)对切除肾上腺的大鼠中的ACTH浓度的影响的图。大鼠口服接受30mg/kg的化合物I(NBI-77860)。数据表示为ACTH的平均血浆浓度(±SEM)。

[0038] 图3A-C表示示例CRF_1受体拮抗剂(以它们的解离半衰期加以区分)对切除肾上腺

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的大鼠中的ACTH浓度的影响的图。

[0039] 图4表示实施例6中所述的临床试验的研究设计示意图，其被设计为评估NBI-77860的安全性、耐受性和血浆暴露，以及该化合物对HPA(下丘脑-垂体-肾上腺的)轴激素的内源水平的影响。

[0040] 图5表示实施例6中所述的临床试验的17-OHP水平(上图)和ACTH水平(下图)在给药后整个24小时期间的平均数据。

[0041] 图6表示在施用300mg和600mg NBI-77860和安慰剂后，具体单个对象的17-OHP水平(上图)和ACTH水平(下图)随时间的反应。

[0042] 发明详述

[0043] 如本文所述，已经发现CRF_1受体拮抗剂直接抑制患有CAH的患者中的ACTH释放，从而使雄激素的产生正常化。CRF_1受体拮抗剂的施用允许在患有CAH的患者中使用多个较低生理剂量的氢化可的松，因此减少治疗相关的副作用。

[0044] 通过免疫分析对CAH进行新生儿筛查，以对生命的最初72小时内获得的脚跟毛细血管血液样本中的17-OHP水平进行测量。通过可商购获得的解离增强镧系荧光免疫分析(DELFIA;PerkinElmer,Waltham Massachusetts)(White等人，J.Pediatr.163:10-12(2013))来分析血液样品的17-OHP。

[0045] 使用生物化学测试方法和分子基因测试方法的第二级筛选试验(在生命的第8天至第14天进行)被美国的九个州所采用，并且被额外的5个州强烈推荐。生物化学方法包括用有机溶剂萃取或者液相色谱随后串联质谱的免疫分析，以测量17-OHP、雄烯二酮和21-脱氧皮质醇与皮质醇的类固醇比值(参见，例如，Speiser等人，Int.J.Pediatr.Endocrinol.2010:494173,2010)。基因筛选寻找与CAH有关的CYP21A2突变。尽管未在美国得到广泛采用，但是增加第二筛选可能提高整个筛选过程的灵敏度，其中单独的第一筛选的灵敏度为约72％。

[0046] 在没有新生儿筛选的结果时，患有典型CAH的女婴通常由于存在模糊不清的生殖器而被鉴定。男性在出生时具有正常的生殖器，因此不进行诊断，除非进行新生儿筛选或者其它医学并发症引起注意。最初未被诊断患有CAH并且患有失盐型疾病的婴儿随后在生命的最初几周内在体重增加困难、呕吐、高钾血症以及低钠血症的情况下被诊断。

[0047] CAH的治疗基于在婴儿期至成人期使用来自诊断的各种药物使激素水平和类固醇水平正常化。糖皮质激素是CAH的目前标准治疗，并且被用于校正内源性皮质醇缺乏症以及降低来自脑垂体的升高的ACTH水平。与艾迪生氏病(肾上腺机能不全)的治疗不同，在所述治疗中，皮质醇替代是充分的，但CAH的治疗还必须降低ACTH的产生，以控制后续雄激素过量。因此，糖皮质激素治疗的目标包括皮质醇替代和ACTH的抑制，以防止女性的男性化和月经紊乱并且抑制男性的睾丸肾上腺残余瘤。需要盐皮质激素替代用于实现正常的血浆肾素活性以维持那些患有失盐型的CAH的患者中的正常血压、电解质平衡和容量状态。

[0048] 糖皮质激素治疗的方案必须支持正常的生理机能并且还应确保在可以引起强的应激反应的事件(例如，并发疾病、运动、低血压)期间可获得足够的皮质醇。仔细监测对于避免由于试图充分抑制雄激素产生而进行的糖皮质激素过度治疗引起的医源性库欣综合征(iatrogenic Cushing's syndrome)或者由于治疗不足引起的艾迪生综合征的形成也是必要的。盐皮质激素的过度治疗可以引起高血压，然而治疗不足可以导致低血压、盐缺失、

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疲劳和对糖皮质激素的需求增加。监测治疗功效的典型实验室试验包括测量17-OHP、雄烯二酮、睾酮、肾素活性和电解质的血浆浓度。

[0049] 患有CAH的成年患者具有增加的包括肥胖、高血压和胰岛素抗性在内的心血管疾病的风险因素的发生率(参见,例如,Kim等人,Semin.Reprod.Med.27(4):316-21(2009))。一大组CAH儿科患者和成年患者(n=244)的研究表明患者被开出各种糖皮质激素治疗方案,然而却频繁地遭受较差的激素控制和前述的不良后果(参见,例如,Finkielstain等人,J.Clin.Endocrinol Metab.97(12):4429-38(2012))。

[0050] CAH的治疗包括使用糖皮质激素(对于儿童通常为氢化可的松,但是对于成人通常为具有狭窄的治疗指数的更有效试剂,例如地塞米松)努力使皮质醇缺乏正常化,并且如果失盐需要,使用盐皮质激素(通常为氟氢可的松)。然而,实现充分抑制过量雄激素所需的糖皮质激素剂量通常远高于如在患有艾迪生氏病的患者中单独用于皮质醇替代的正常生理剂量。这种对糖皮质激素暴露的增加可以导致CAH患者的医源性库欣综合征、心血管风险因素增加、葡萄糖耐受不良和骨矿物质密度降低(参见,例如,Elnecave等人,J.Pediatr.Endocrinol.Metab.21:1155-62(2008);King等人,J.Clin.Endocrinol.Metab.91(3):8656-59(2006);Migeon等人,Endocrinol.Metab.Clin.North Am.30:193-206(2001))。

[0051] 促肾上腺皮质激素释放因子(CRF)从羊下丘脑分离并且被鉴定为41个氨基酸的肽。已经发现CRF引起内分泌、神经和免疫系统功能产生显著变化。据信CRF是来自垂体前叶的促肾上腺皮质激素("ACTH")、β-内啡肽和其它前阿黑皮素原(POMC)衍生肽的基础性和应激诱导的释放的主要生理调节剂(参见,例如,Vale等人,Science 213:1394-1397,1981)。CRF的分泌物经由与CRF_1受体(G蛋白偶联受体的B类家族的成员)结合引起ACTH从垂体前叶的促肾上腺皮质激素细胞释放。

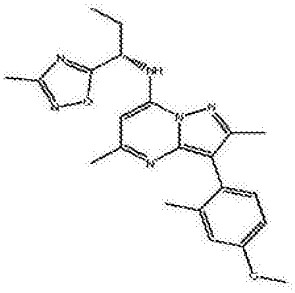
[0052] 由于CRF的生理学重要性,开发具有显著的CRF_1受体结合活性并且能够拮抗CRF_1受体的生物活性的小分子仍然是期望的目标,并且是用于治疗焦虑、抑郁、肠易激综合征、创伤后应激障碍和药物滥用的正在进行的研究和开发的主题。

[0053] 在下丘脑促肾上腺皮质激素释放因子(CRF)的控制下,脑垂体激素ACTH刺激胆固醇的吸收并且驱动孕烯醇酮的合成,从而启动肾上腺中的类固醇生成(参见图1)。肾上腺皮质由三个区组成,所述三个区产生不同类型的激素,许多激素通过这种途径被ACTH动员的胆固醇驱动。由于突变或者缺失导致的这些酶的缺乏引起底物浓度增加。在21-羟化酶基因(CYP21A2)中的突变或者缺失产生的最常见的CAH形式中,由于类固醇前体、孕酮和17-羟孕酮(17-OHP)的积累,有效的雄激素通过肾上腺来产生。在这些情况中,17-OHP的血浆水平可以达到正常浓度的10-1000倍。这些增长导致引起女性男性化的雄激素的过度产生,所述雄激素特别是雄烯二酮、睾酮和二羟基睾酮。此外,在CAH中的21-羟化酶缺乏引起糖皮质激素和盐皮质激素,特别是皮质醇和醛固酮的生物合成不足。皮质醇是下丘脑的CRF分泌和脑垂体的ACTH释放的关键性负反馈调节剂。糖皮质激素合成和释放的不足消除了对下丘脑和脑垂体的抑制,这引起ACTH水平增加。过度的ACTH刺激引起束状带和网状带的肥大,从而导致肾上腺增生。

[0054] 在实施方案中,可用于治疗CAH的CRF_1受体拮抗剂为NBI-77860(2,5-二甲基-3-[2-甲基-4-(甲氧基)苯基]-N-[(1S)-1-(3-甲基-1,2,4-噁二唑-5-基)丙基]吡唑并[1,5-

0330

a]嘧啶-7-胺(在本文又称为“化合物I”)),并且其具有以下结构。

[0055] 

化合物I

[0056] 化合物I是拥有结合pKi＝8.2的有效CRF_1拮抗剂(参见,例如,Tellew等人,Bioorg.Med.Chem.Lett.20:7259,2010和Int'l.Patent Appl.Publ.No.WO 2006/044958,以上两篇参考文献通过引用整体并入本文)。如本文所述,化合物I具有有效的降低ACTH的效果,如在切除肾上腺的大鼠中所示。

[0057] 在另一实施方案中,可用于治疗CAH的CRF_1受体拮抗剂为以下中所述的小分子拮抗剂:U.S.6,586,456、US 6,806,282、US 6,531,475、US 6,664,261、US 6,610,678、WO 98/08846、WO 98/11075、WO 99/10350、WO 2000/059888、WO 2006/044821、WO 2006/102194、WO 2006/107784、WO 2006/116412、WO 2006/126718、WO 2007/069565、WO 2007/069671、WO 2008/036541、WO 2008/036579、WO 2008/051533、WO 2008/082003、WO 2008/083070、WO2008/136377、WO 2009/008552、WO 2009/144632、WO 2010/014280、WO 2010/014687、WO 2010/015628、WO 2010/015655、WO 2010/062718、WO 2010/096426、WO 2011/043387、WO 2011/092293、WO 2011/095450、WO 2011/092290以及WO 2011/043381。

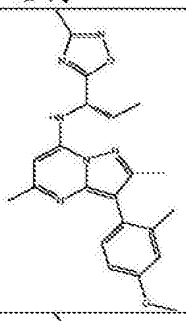
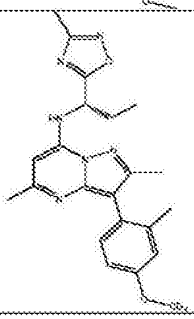
[0058] 在另一实施方案中,CRF_1受体拮抗剂为NBI-30775、CP-316,311、培沙舍封(pexacerfont)、emicerfont、SSR-125543[4-(2-氯-4-甲氧基-5-甲基苯基)-N-[(1S)-2-环丙基-1-(3-氟-4-甲基苯基)乙基]-5-甲基-N-(2-丙炔-1-基)-2-噻唑胺]、SSR-126374、ONO-2333、NBI-34041、JNJ-19567470、GSK586529、PF-00572778、CP-376395、安他拉明(N-丁基-N-乙基-2,5,6-三甲基-7-(2,4,6-三甲基苯基)吡咯并[3,2-e]嘧啶-4-胺)和DMP904。

[0059] 在另一实施方案中,CRF_1受体拮抗剂具有超过30分钟的解离半衰期($t_{1/2}$),在另一实施方案中,CRF_1受体拮抗剂具有超过40分钟的解离半衰期($t_{1/2}$),并且在另一实施方案中,CRF_1受体拮抗剂具有超过50分钟的解离半衰期($t_{1/2}$)。具体的CRF_1受体拮抗剂的解离半衰期由实施例3所公开的技术来测定。这些实施方案的代表性CRF_1受体拮抗剂包括化合物I、NBI-30775、NBI-34041、SSR-125543A、安他拉明(N-丁基-N-乙基-2,5,6-三甲基-7-(2,4,6-三甲基苯基)吡咯并[3,2-e]嘧啶-4-胺)和DMP904。

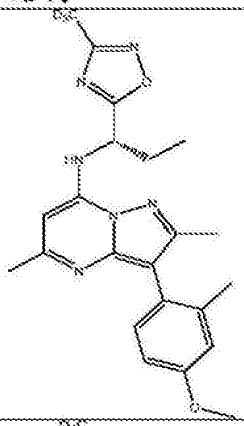
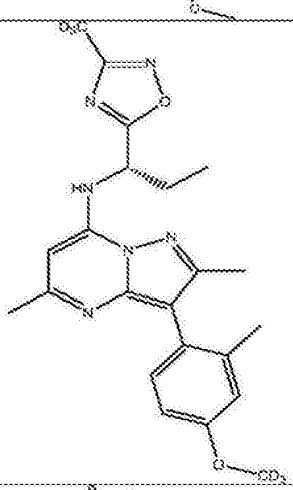
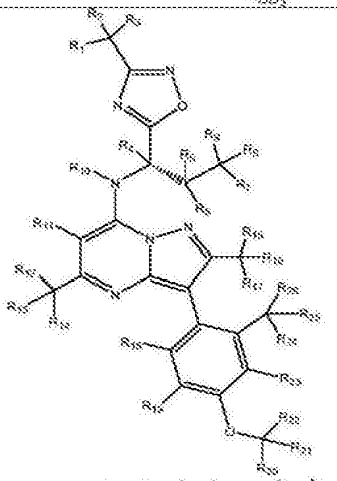
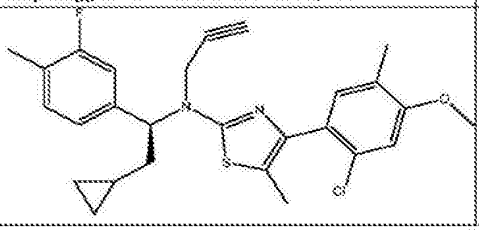
[0060] 关于本文所述的化合物,还应当理解,当特定位置被指定为氢时,该氢(H)可以用氘(D)替代。已知掺入代替氢的氘对所取代的化合物的生理和药理活性产生显著影响。为此,应当理解,氘替代氢意指氘在该位置的丰度基本上大于氘的天然丰度。因此,在一个实施方案中,代表性化合物包括以下化合物。

0331

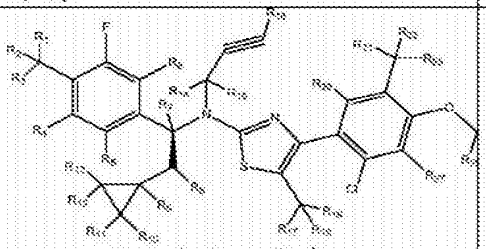
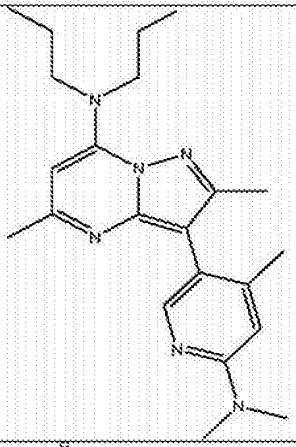
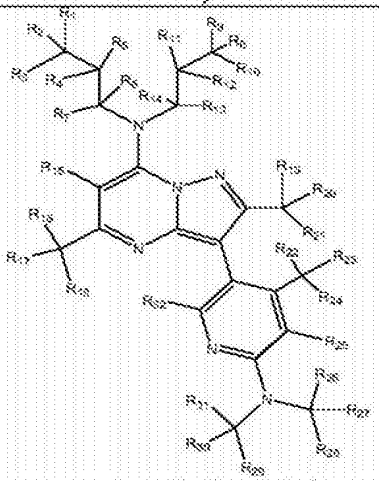
[0061]

化合物	結构	化学名称
NBI-77860		2,5-二甲基-3-[2-甲基-4-(甲氧基)苯基]-N-[(1S)-1-(3-甲基-1,2,4-噁二唑-5-基)丙基]吡唑并[1,5-a]嘧啶-7-胺
氘化NBI-77860		2,5-二甲基-3-[2-甲基-4-(d_3-甲氧基)苯基]-N-[(1S)-1-(3-甲基-1,2,4-噁二唑-5-基)丙基]吡唑并[1,5-a]嘧啶-7-胺

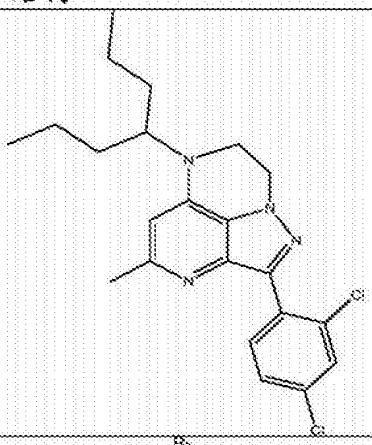
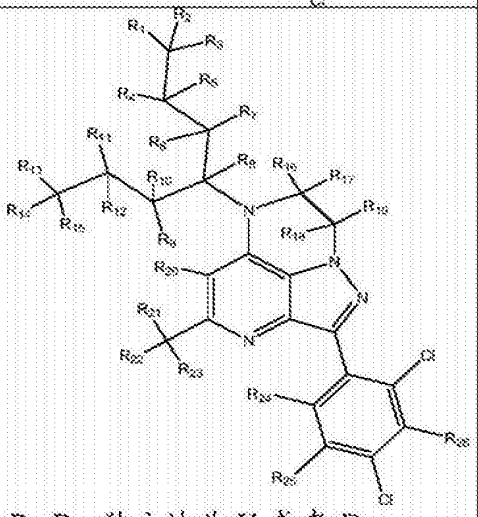
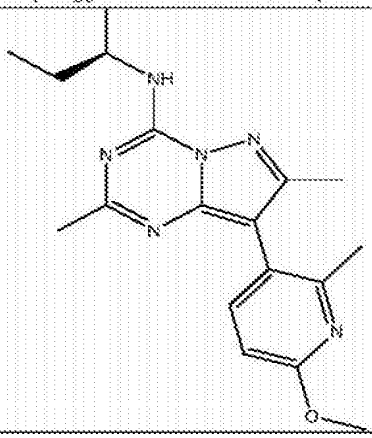
[0062]

化合物	结构	化学名称
氘化 NBI-77860		2,5-二甲基-3-[2-甲基-4-(甲氧基)苯基]-N-[(1S)-1-(3-d_3-甲基-1,2,4-噁二唑-5-基)丙基]吡唑并[1,5-a]嘧啶-7-胺
氘化 NBI-77860		2,5-二甲基-3-[2-甲基-4-(d_3-甲氧基)苯基]-N-[(1S)-1-(3-d_3-甲基-1,2,4-噁二唑-5-基)丙基]吡唑并[1,5-a]嘧啶-7-胺
氘化 NBI-77860	 R_1-R_{26} 独立地为 H 或者 D	
SSR-125543		4-(2-氯-4-甲氧基-5-甲基苯基)-*N*-[(1*S*)-2-环丙基-1-(3-氟-4-甲基苯基)乙基]-5-甲基-*N*-(2-丙炔-1-基)-2-噻唑胺

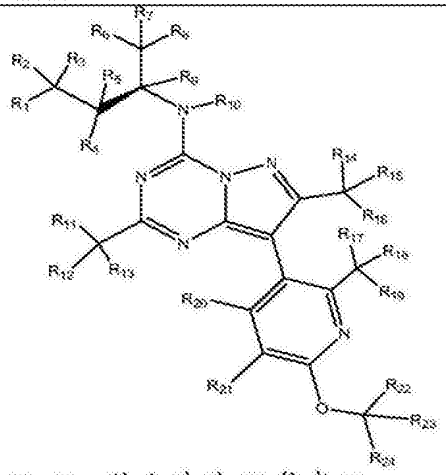
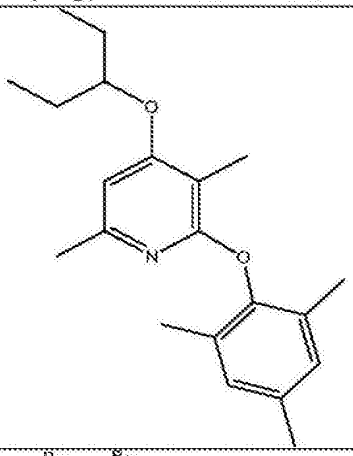
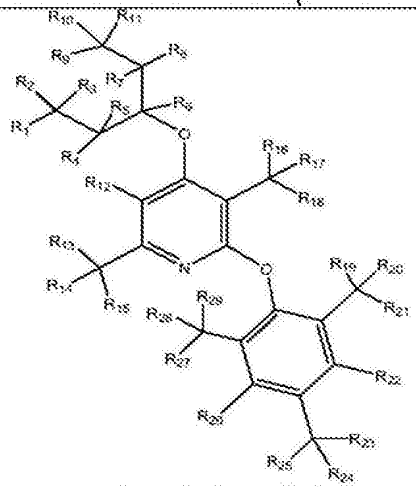
[0063]

化合物	結构	化学名称
氘化 SSR-125543	 R_1-R_{37} 独立地为 H 或者 D	
NBI-30775		5-[7-(二丙基氨基)-2,5-二甲基吡唑并[1,5-a]嘧啶-3-基]-N,N,4-三甲基吡啶-2-胺
氘化 NBI-30775	 R_1-R_{32} 独立地为 H 或者 D	

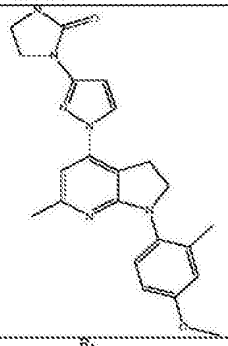
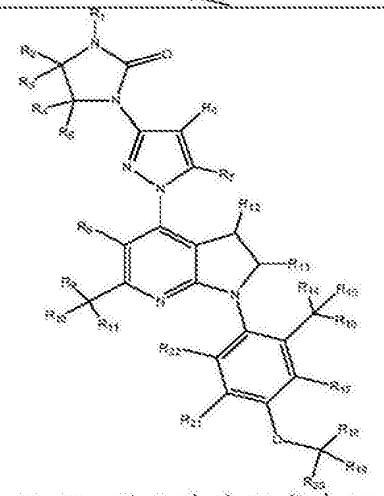
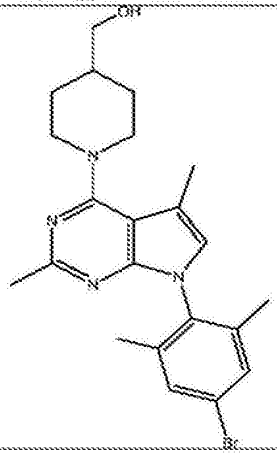
[0064]

化合物	結构	化学名称
NBI-34041		3-(2,4- 二 氯 苯 基)-9-(庚 -4- 基)-6- 甲 基 -1,2,5,9- 四 氮 杂 三 环 [6.3.1.0^{4,12}] 十 二 -2,4(12),5,7- 四 烯
氘 化 NBI-34041	 R_1-R_{26} 独立地为 H 或者 D	
培沙舍封		N-[(2R)- 丁 -2- 基]-8-(6- 甲 氧 基 -2- 甲 基 吡 啶 -3- 基)-2,7- 二 甲 基 吡 唑 并 [1,5-a][1,3,5] 三 嗪 -4- 胺

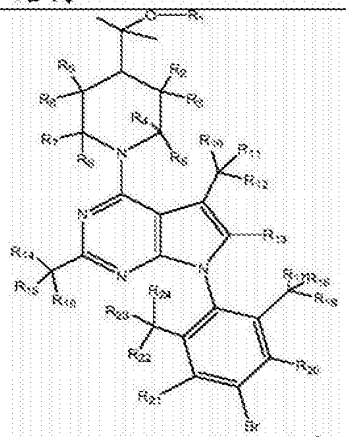
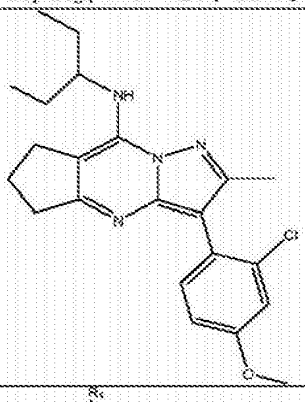
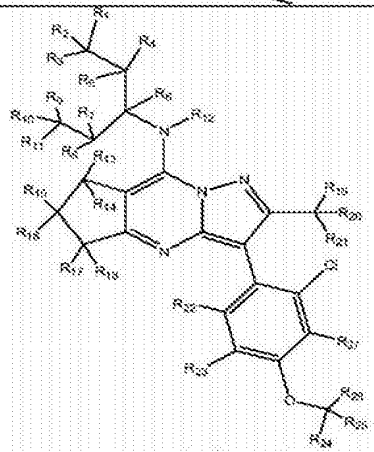
[0065]

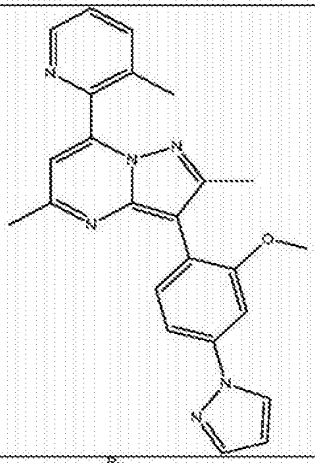
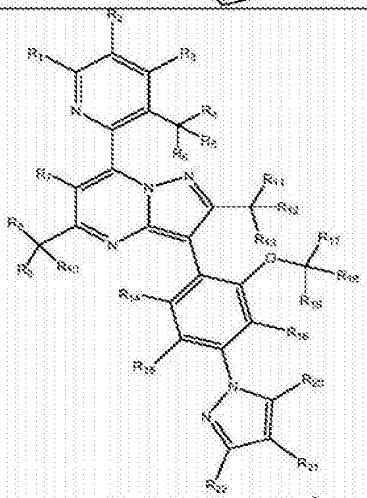
化合物	結构	化学名称
氘化培沙舍封	 R_1-R_{24}独立地为 H 或者 D	
CP-316,311		3,6-二甲基-4-(戊-3-基氧基)-2-(2,4,6-三甲基苯氧基)吡啶
氘化 CP-316,311	 R_1-R_{29}独立地为 H 或者 D	

[0066]

化合物	結构	化学名称
GSK876008		
氘化 GSK876008	 R_1-R_{22} 独立地为 H 或者 D	
CRA5626/R3 17573		

[0067]

化合物	結构	化学名称
氘化CRA5626/R317573	 R_1-R_{24}独立地为H或者D	
ONO2333Ms		10-(2-氯-4-甲氧基苯基)-11-甲基-N-(戊-3-基)-1,8,12-三氮杂三环[7.3.0.0^{3,7}]十二-2,7,9,11-四烯-2-胺
氘化ONO2333	 R_1-R_{27}独立地为H或者D	

化合物	结构	化学名称
NBI76169		
[0068] 氘化 NBI76169	 R_1-R_{33}独立地为H或者D	

[0069] 在另一实施方案中，任一前述化合物可以掺入稳定的或者放射性同位素。因此，还考虑使用与本文所述的那些相同的同位素标记的化合物，其中用具有与自然界中通常存在的原子质量或者质量数不同的原子质量或者质量数的原子替代一个或者多个原子。可以掺入这些化合物的同位素的实例包括氢、碳、氮、氧、磷、氟和氯的同位素，例如但不限于以上所述的氘(2H)，以及3H、13C、14C、15N、180、170、31P、32P、35S、18F和36Cl。某些同位素标记的化合物，例如掺入放射性同位素(例如3H和14C)的那些化合物也可用于药物或者底物组织分布测定。由于容易制备和可检测性，氚化氢(3H)和碳-14(14C)同位素为特别优选的。较重同位素(例如氘(2H))的取代可以由于较高的代谢稳定性而提供某些治疗优势，例如体内半衰期增加或者剂量需求降低，并且因此，在一些情况下，可以是优选的。同位素标记的化合物通常可以通过进行本领域中常规实践的程序来制备。

[0070] 对切除肾上腺的大鼠的血浆ACTH的作用

[0071] 肾上腺切除术消除大鼠中的循环皮质酮(主要的糖皮质激素)，并且去除下丘脑和脑垂体(促肾上腺皮质激素细胞)水平的HPA轴的负反馈控制，因此慢性升高血浆ACTH(参见，例如，Mims等人，J.Natl.Med.Assoc.69:145-47(1977))。已经证明静脉注射肽CRF_1受体拮抗剂将降低切除肾上腺(ADX)的大鼠中的高的血浆ACTH水平(参见，例如，Rivier等人，J.Med.Chem.12:42:3175-82(1999))。小分子NBI-77860(化合物I)重现了这些发现。在切除

肾上腺的大鼠中，化合物I能够有效降低ACTH。ACTH的最大降低与NBI-77860的最大血浆浓度有关；然而，降低ACTH的作用的持续时间超过药物血浆暴露。因此，在切除肾上腺的大鼠中，在口服施用后，NBI-77860的完整血浆暴露量与体内功效之间存在可预测的关系。

[0072] 化合物作为CRF受体拮抗剂的效果可以通过各种测定方法来确定。本文所述的CRF拮抗剂可以能够抑制CRF与其受体的特异性结合，因此拮抗活性与CRF有关。可以通过一种或者多种普遍接受的测定对化合物作为CRF拮抗剂的活性进行评估，所述测定包括实施例中所述的测定。可用于本文所述方法的CRF拮抗剂包括对CRF受体显示亲和性的化合物。

[0073] 不希望受理论束缚，在CAH的治疗中，CRF受体拮抗剂将可能阻止脑垂体促肾上腺皮质激素细胞释放ACTH，从而降低雄激素的产生，并且使皮质醇的替代为更精细的治疗范式。动物和人类研究已经显示化合物I(NBI-77860)对ACTH释放的药理学效应。监测治疗功效时内分泌学家所用的标准生物标志物评估可以用于监测该CRF_1受体拮抗剂的作用。17-OHP、雄烯二酮、睾酮、皮质醇和ACTH的血浆水平以及这些类固醇的尿液代谢物可以在儿童和成人中容易地测量，从而给出关于治疗影响的快速且有意义的数据。

[0074] 治疗的药物组合物和方法

[0075] 本公开内容还提供了用于治疗CAH的方法中的药物组合物，其包含本文所述的任一CRF拮抗剂化合物和药物可接受的赋形剂。药物可接受的赋形剂是生理学上和药学上适宜的无毒且非活性材料或者成分，所述材料或者成分不会干扰活性成分的活性；赋形剂还可以被称为载体。CRF拮抗剂化合物可以配制为药物组合物用于治疗或者防止性(或者预防性)治疗(例如，减少CAH疾病的恶化，或者疾病的一种或者多种症状的发生或者复发)。本文所述的方法和赋形剂是示例性的而非限制。药物可接受的赋形剂在药物领域是众所周知的并且在以下文献中进行了描述，例如，Rowe等人，Handbook of Pharmaceutical Excipients:A Comprehensive Guide to Uses,Properties,and Safety,第5版，2006，以及Remington:The Science and Practice of Pharmacy(Gennaro,第21版，Mack Pub.Co.,Easton,PA(2005))。药物可接受的赋形剂的实例包括无菌盐水以及处于生理学pH的磷酸盐缓冲盐水。可以将防腐剂、稳定剂、染料、缓冲液等提供在药物组合物中。此外，还可以使用抗氧化剂和悬浮剂。

[0076] 治疗和/或预防益处包括，例如改善的临床结果，治疗性治疗和预防性(prophylactic)或防止性(preventative)措施，其中目标是预防或减缓或延迟(减少)不期望的生理变化或病症，或者预防或减缓或延迟(减少)这类病症的扩展或严重性。如本文所讨论的，来自治疗对象的有益的或期望的临床结果包括但不限于减轻、减少或缓解由待治疗的疾病、病况或病症导致的症状或者与待治疗的疾病、病况或病症有关的症状；降低症状的发生率；改善生命质量；较长的无病状态(即，根据症状，进行疾病诊断，降低对象呈现所述症状的可能性或倾向性)；疾病范围的减小；稳定的(即，不恶化)疾病状态；疾病进展的延迟或减缓；疾病状态的改善或减轻；以及缓解(无论是部分还是全部)，无论是可检测的或不可检测的；和/或总存活期。“治疗”还可以意指相比于如果对象不接受治疗的预期生存，延长生存。需要治疗的对象包括已经患有病况或病症的那些，以及易患疾病、病况或病症或处于患有疾病、病况或病症的风险中的对象，以及将预防疾病、病况或病症(即，降低疾病、病症或病况发生的可能性)的那些。对象可以是人或者非人哺乳动物(例如，大鼠、小鼠、狗、猫、家畜、动物园动物)。

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[0077] 通常可以使用实验模型和/或临床试验确定最佳剂量。最佳剂量可以取决于对象的身体质量、体重或血容量。通常，本文所述的存在于一个剂量中的化合物的量为约0.1mg至约30mg/kg对象体重。在某些实施方案中，单剂量为约50-1000mg。通常优选使用足以提供有效治疗的最小剂量。通常可以由临床评估以及使用适于治疗或预防病况的测定来监测对象的治疗效果，所述测定是本领域普通技术人员所熟悉的，并且在本文中进行了描述。可以通过测定来自对象的生物流体，例如血液、血液部分(例如血清)和/或尿液和/或其它生物样品中的化合物水平来监测施用于对象的化合物水平。可以使用本领域实践的测定化合物的任何方法来测量治疗方案期间的化合物水平。

[0078] 包括至少一种用于治疗CAH或者相关疾病或者病症的本文所述的化合物的组合物的剂量可以取决于对象的状况，即，疾病的阶段、由疾病引起的症状的严重度、总体健康状态以及年龄、性别和体重以及对医学领域中的技术人员显而易见的其它因素。类似地，化合物的剂量可以根据医学领域的技术人员所理解的参数来确定。

[0079] 可以通过有效递送有效量的化合物的若干途径中的任一种，将包含至少一种本文所述的CRF_1拮抗剂化合物的本文所述的药物组合物施用于需要的对象。此类施用途径包括，例如，口服、肠胃外、肠内、直肠、鼻内、颊部、舌下、肌肉内和经皮。本文更详细地描述了通过这些施用途径和其它施用途径施用的组合物。

[0080] 所公开的化合物或者组合物的施用包括夜间施用或者睡前施用(即，临睡前施用)。如本文所用，临睡前施用是指旨在预期的生理节律释放ACTH之时或者之前(例如2-5小时前)递送临床相关浓度的CRF_1拮抗剂的给药。由于该ACTH释放通常在凌晨1-2点进行，并且因为大多数口服施用的药物具有数个小时的Tmax，所以期望例如在晚上10点给药，这比预期的生理节律释放ACTH提前3-4小时。这种相同的预脉冲临睡前给药可以适于轮班工人(例如，夜晚工作白天睡觉的那些工人)，在这种情况下，不一定在夜间进行施用。因此，施用取决于预期的生理节律释放ACTH并且可以根据个体(即，对象、患者)的具体工作和睡眠类型来变化。在某些实施方案中，将本文所述的CRF_1受体拮抗剂(例如，NBI-77860，或者NBI-30775、NBI-34041、SSR-126374、SSR-125543、安他拉明(N-丁基-N-乙基-2,5,6-三甲基-7-(2,4,6-三甲基苯基)吡咯并[3,2-e]嘧啶-4-胺)或者DMP904中的任一种)在预期的生理节律释放ACTH前(即，之前、提前)约2-5小时进行施用。在其它实施方案中，将CRF_1受体拮抗剂在预期的生理节律释放ACTH前约2-4小时或者3-5小时施用于对象。在更具体的实施方案中，将CRF_1受体拮抗剂在预期的生理节律释放ACTH前约3-4小时施用于对象。

[0081] 本文还提供通过施用CRF_1受体拮抗剂降低患有CAH的对象(即，患者、个体)的17-OHP和/或ACTH水平的方法。在某些实施方案中，在预期的生理节律释放ACTH之时或者之前施用拮抗剂。在其它某些实施方案中，在预期的生理节律释放ACTH之前约2-4小时或者约3-5小时施用CRF_1受体拮抗剂。在更具体的实施方案中，将CRF_1受体拮抗剂在预期的生理节律释放ACTH之前约3-4小时施用于对象。

[0082] 本文所述的方法可以导致雄激素(例如睾酮和雄烯二酮)的释放减少，所述方法包括将CRF_1受体拮抗剂以本文所述的方式施用于有需要的女性对象，所述方式引起对象的ACTH和17-OHP水平降低。糖皮质激素的剂量可以通过临床上显著的量伴随降低，这继而导致副作用减少。

[0083] 用于维持患有CAH且年青和正发育的患者的治疗以及用于维持发育完全的患者的

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治疗的糖皮质激素(GC)和盐皮质激素(MC)的量对于本领域技术人员是已知的。例如,指南描述于Speiser等人(J.Clin.Endocrinol.Metab.95:4133-60(2010)中,通过引用整体并入)中,并且显示在其中的表1和表2中。特别地,对于年青、正发育的CAH患者,本领域技术人员应理解,太高的GC给药可以阻止患者的身高生长。因此,本文所述的用CRF_1受体拮抗剂治疗患者的方法可以包括以临床上显著的方式降低GC的剂量。

[0084] 在某些实施方案中,通过施用CRF_1受体拮抗剂治疗CAH的方法还可以包括以低于治疗患有CAH的对象的目前推荐的GC剂量的剂量施用GC。当对象为发育完全的患者时,GC剂量(例如为了维持治疗发育完全的对象所推荐的氢化可的松(HC)、强的松、泼尼松龙、地塞米松或者氟氢可的松的剂量)可以从以下推荐剂量降低约10%、15%、20%、30%、40%、50%、60%或者更多:15-25mg/天HC;5-7.5mg/天强的松,4-6mg/天泼尼松龙;0.25-0.5mg/天地塞米松或者0.05-0.2mg/天氟氢可的松。在正在发育的CAH患者的对象中,GC(例如HC)的推荐剂量可以在接受如本文所述的CRF_1受体拮抗剂的对象中从10-15mg/m^2/天的总推荐剂量降低和/或0.05-0.2mg/天的氟氢可的松的总剂量可以在接受如本文所述的CRF_1受体拮抗剂的对象中各自降低约10%、15%、20%、30%、40%、50%、60%或者更多。在实施方案中,相对于安慰剂,本文所述的方法导致ACTH水平在临床上显著降低,所述方法包括向有需要的对象施用有效量的CRF_1拮抗剂。在具体的实施方案中,相对于安慰剂,向有需要的对象施用有效量的CRF_1拮抗剂导致ACTH水平在临床上显著降低,其中所述降低为至少25%。在另一实施方案中,相对于安慰剂,向有需要的对象施用有效量的CRF_1拮抗剂导致ACTH水平在临床上显著降低,其中所述降低为至少50%。关于施用糖皮质激素和盐皮质激素的指南,参见Speiser等人,同上。

[0085] 在另一实施方案中,相对于安慰剂,本文所述的方法导致17-OHP水平在临床上显著降低,所述方法包括向有需要的对象施用有效量的CRF_1拮抗剂。在另一具体实施方案中,相对于安慰剂,向有需要的对象施用有效量的CRF_1拮抗剂导致17-OHP水平在临床上显著降低,其中所述降低为至少25%。在另一实施方案中,相对于安慰剂,向有需要的对象施用有效量的CRF_1拮抗剂导致17-OHP水平在临床上显著降低,其中所述降低为至少50%。

[0086] 在实施方案中,相对于安慰剂,本文所述的方法导致ACTH和17-OHP水平在临床上都显著降低,所述方法包括向有需要的对象施用有效量的CRF_1拮抗剂。在某些实施方案中,相对于安慰剂,向有需要的对象施用有效量的CRF_1拮抗剂导致ACTH和17-OHP水平在临床上都显著降低,其中所述降低为至少25%。在另一具体实施方案中,相对于安慰剂,向有需要的对象施用有效量的CRF_1拮抗剂导致ACTH和17-OHP水平在临床上都显著降低,其中所述降低为至少50%。

[0087] 药物组合物可以是溶液的形式。可选地,它们可以是固体的形式,例如粉末、片剂等。包含本文所述的任一化合物的组合物可以配制用于持续释放或者缓慢释放。此类组合物通常可以使用众所周知的技术来制备以及通过例如口服、直肠或者皮下植入或者通过在所期望的靶位点植入来施用。持续释放制剂可以包含分散在载体基质中和/或包含在由速率控制膜包围的储器内的化合物。用于此类制剂中的赋形剂是生物相容的,并且还可以是生物可降解的;优选地,制剂提供相对恒定水平的活性组分释放。包含在持续释放制剂中的活性化合物的量取决于植入位点、释放速率和预期持续时间、以及待治疗或者预防的病况的性质。

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[0088] 对于口服制剂，至少一种本文所述的化合物可以单独使用或者与适当的添加剂组合使用以制备片剂、粉末、粒剂或者胶囊，所述适当的添加剂例如常规添加剂，例如乳糖、甘露醇、玉米淀粉或者马铃薯淀粉；粘合剂；崩解剂；润滑剂；并且如果需要，稀释剂、缓冲剂、湿润剂、防腐剂、着色剂和调味剂。化合物可以与缓冲剂配制来为化合物提供免受胃部环境和/或肠溶衣的低pH的保护。包含在组合物中的化合物可以与调味剂(例如在液体、固体或者半固体制剂中)和/或肠溶衣配制用于口服递送。口服制剂可以以明胶胶囊提供，所述明胶胶囊可以包含活性化合物以及粉末状载体，例如乳糖、淀粉、纤维素衍生物、硬脂酸镁、硬脂酸等。类似的载体和稀释剂可以用于制备压缩片剂。

实施例

[0089] 实施例1

[0090] CRF受体结合活性

[0091] 可以通过如Grigoriadis等人(参见，例如，Mol.Pharmacol第50卷，第679-686页，1996)和Hoare等人(参见，例如，Mol.Pharmacol 63:751-765,2003)中通常所述的标准放射性配体结合测定，评价本文所述的方法中使用的CRF拮抗剂对于CRF受体的结合活性。通过使用放射性标记的CRF配体，可以将测定用于评价本文所述的化合物与任何CRF受体亚型的结合活性。

[0092] 简而言之，结合测定涉及从CRF受体置换放射性标记的CRF配体。更具体地，使用来自人CRF受体稳定转染的细胞的1-10μg细胞膜在96孔测定板中进行结合测定。各孔接收包含目标化合物或者参考配体(例如，蛙皮降压肽、尿皮素I或者CRF)的约0.05mL测定缓冲液(例如，Dulbecco磷酸盐缓冲盐水、10mM氯化镁、2mM EGTA)、0.05mL[^{125}I]酪氨酸-蛙皮降压肽(最终浓度～150pM或者K_D大约如通过斯卡查德分析测定)以及包含CRF受体的0.1mL细胞膜悬浮液。将混合物在22℃孵育2小时，然后通过经玻璃纤维过滤器快速过滤以使结合的放射性配体与游离的放射性配体分离。在三次洗涤后，干燥过滤器并且使用闪烁计数器计算放射性(来自^{125}I的俄歇电子)。可以使用非线性最小二乘曲线拟合程序Prism(GraphPad Software Inc)或者XLfit(ID Business Solutions Ltd)分析所有的放射性配体结合数据。

[0093] 实施例2

[0094] CRF_1受体激动剂活性

[0095] 如Fleck等人(J.Pharmacology and Experimental Therapeutics,341(2):518-531,2012)(以下为“Fleck等人”并且通过引用整体并入)所报道，呈现了先前所鉴定的CRF_1受体拮抗剂的活性。此类活性被报道为由缔合速率常数(k_1)和解离速率常数(k_{-1})通过以下等式计算的动力学推导的亲和力(K_i)：

[0096] $K_i = k_{-1}/k_1$

[0097] 此外，如Fleck等人所报道，已经报道列于下表1中的CRF_1受体拮抗剂的动力学K_i。

[0098] 表1

[0099] 代表性CRF_1受体拮抗剂

0343

[0100]

配体	k_1 ($10^6M^{-1}min^{-1}$)	动力学 K_i (nM)
NBI-27914	9.4 ± 3	25
CP-316,311	13 ± 2	12
NBI-46200	6.2 ± 2	22

[0101]

配体	k_1 ($10^6M^{-1}min^{-1}$)	动力学 K_i (nM)
DMP696	7.7 ± 2	9.5
培沙舍封	2.6 ± 0.1	19
NBI-35965	20 ± 2	2.3
ONO-2333Ms	4.4 ± 2.2	15
安他拉明	3.4 ± 0.6	3.9
NBI-34041	8.3 ± 2.0	1.7
DMP904	18 ± 1	0.38
NBI-30775	14 ± 2.0	0.36
SSR125543A	33 ± 5	0.049

[0102] 通过这种相同的技术，发现化合物I(NBI-77860)的动力学Ki如下：

[0103] 表1(续)

[0104]

配体	k_1 ($10^6M^{-1}min^{-1}$)	动力学 Ki (nM)
NBI-77860	0.24 ± 0.05	48 ± 9

[0105] 实施例3

[0106] CRF_1受体拮抗剂的解离半衰期($T_{1/2}$)

[0107] 如用于本文所述的方法中的CRF_1受体拮抗剂的解离半衰期($t_{1/2}$)通过Fleck等人所述的技术来评价。如于其中所述的，将标记和未标记配体的解离速率常数表示为k_{-1}，而来自受体的药物解离半衰期($t_{1/2}$)(其与中值停留时间相等)由解离速率常数(k_{-1})通过以下等式来计算：

[0108] $t_{1/2}=0.693/k_{-1}$

[0109] 如Fleck等人所报道，已经报道列于下表2中的CRF_1受体拮抗剂的解离半衰期

0344

($t_{1/2}$)。

[0110] 表2

[0111] 代表性化合物的解离半衰期

配体	k_{-1} (min^{-1})	解离 $t_{1/2}$ (min)
NBI 27914	0.27 ± 0.07	2.6
CP-316,311	0.17 ± 0.04	4.1
NBI-46200	0.13 ± 0.002	5.3
DMP696	0.095 ± 0.02	7.3
培沙舍封	0.049 ± 0.001	14
NBI-35965	0.048 ± 0.005	16
ONO-2333Ms	0.063 ± 0.029	17
安他拉明	0.013 ± 0.002	53
NBI-34041	0.013 ± 0.002	53
DMP904	0.0072 ± 0.002	96
NBI-30775	0.0054± 0.0006	130
SSR125543A	0.0016± 0.0003	430

[0112]

[0113] 通过这种相同的技术,发现化合物I(NBI-77860)的解离半衰期如下。

[0114] 表2(续)

[0115]

配体	k_{-1}, min^{-1}	解离 $t_{1/2}$, min
NBI-77860	0.012 ± 0.0003	58

[0116] 因此,具有超过30分钟的解离半衰期($t_{1/2}$)的CRF_1受体拮抗剂包括(但不限于)安他拉明、NBI-34041、DMP904、NBI-30775、SSR125543A和NBI-77860(化合物I)。这些相同的化合物也代表具有超过40分钟的解离半衰期($t_{1/2}$)以及超过50分钟的解离半衰期($t_{1/2}$)的CRF_1受体拮抗剂。

[0117] 实施例4

[0118] 切除肾上腺的大鼠的ACTH的降低

[0119] 化合物I(NBI-77860)(参见,例如,Tellew等人,Bioorg.Med.Chem.Lett.2010,20:7259;WO2006044958)是有效的CRF_1拮抗剂,其拥有的结合pKi为8.2、动力学Ki为49nM(以上

0345

表1)以及解离$t_{1/2}$为58分钟(以上表2)。

[0120] 已经证明静脉注射肽CRF_1受体拮抗剂将降低切除肾上腺(ADX)的大鼠的高的血浆ACTH水平(参见,例如,Rivier等人,J.Med.Chem.12:42:3175-82(1999))。小分子NBI-77860重现了这些发现。当口服施用于ADX大鼠(n=6只/组)时,30mg/kg单剂量的NBI-77860显著降低血浆ACTH水平保持长达5小时(参见图2)。功效的持续时间与最大血浆浓度相关,时间进程超过药物血浆暴露。在切除肾上腺的大鼠中,在口服施用后,NBI-77860的完整血浆暴露量与体内功效之间存在可预测的关系。

[0121] Fleck等人还报道了解离半衰期不同的CRF_1受体拮抗剂对切除肾上腺的大鼠的血浆ACTH水平的影响;即,NBI 30775、NBI 34041和NBI 35965。在最大剂量(10mg/kg)时,全部三种配体剧烈降低ACTH(注射1小时后,图3A-图3C)。在较长的持续时间后,在NBI 35965与其它两种配体之间出现了明显的差别。对于NBI 35965而言,ACTH水平经2小时恢复至媒介物水平(图3C),然而对于NBI 30775和NBI 34041而言,反应持续4-6小时。

[0122] 实施例5

[0123] 化合物I在人中的药效动力学作用

[0124] 在本研究中,通过观察相对于安慰剂以及单剂量的阿普唑仑(0.75mg),单次口服剂量的NBI-77860(化合物I)对由甲吡酮(0.04g/kg)产生的HPA轴反应的作用来评价NBI-77860(化合物I)对人类对象的药效动力学作用。甲吡酮阻断肾上腺中皮质醇的合成,由此模拟CAH的皮质醇缺乏,并且与急剧折转升高的ACTH水平有关。

[0125] 通过在给药后30min至4h测定ACTH值(AUC(30min至4h))进行主要分析。在该分析中,当与安慰剂比较时,进行了以下观察。使用400mg阿普唑仑和400mg NBI-77860治疗,观察到ACTH AUC(30min至4h)显著降低。使用50mg NBI-77860治疗,观察到ACTH AUC(30min至4h)的非显著降低。使用10mg NBI-77860治疗,观察到AUC(30min至4h)增加。

[0126] 在给药后2h至4h(暴露达到C_{max}值时的时间)对ACTH值(AUC(2h至4h))进行的分析中,相对于安慰剂治疗期间,ACTH水平在400mg和50mg NBI-77860治疗期间存在显著降低;然而,在使用10mg剂量NBI-77860的情况下,未观察到降低。

[0127] 实施例6

[0128] 临床研究

[0129] 在本研究中,在标题为"评价NBI-77860在患有先天性肾上腺增生的成年女性中的安全性和耐受性的1期、单盲、安慰剂对照的、固定顺序、单剂量研究(A Phase 1,Single-Blind,Placebo-Controlled,Fixed-Sequence,Single-Dose Study to Evaluate the Safety and To Tolerability of NBI-77860 in Adult Females with Congenital Adrenal Hyperplasia)"(IND 117,388)的临床研究中评价NBI-77860(化合物I)。该研究在典型的成年女性CAH患者中为单盲、安慰剂对照的、单中心、固定顺序的单剂量临床试验。其被设计为评估NBI-77860的安全性、耐受性和血浆暴露,以及该化合物对HPA轴激素的内源性水平的影响。

[0130] 总共8名女性对象(年龄为19至58岁,医学诊断为患有典型的21-羟化酶缺乏CAH)在三个单独治疗期间被施用单次临睡前剂量(hs)为300mg、600mg的NBI-77860以及安慰剂(参见图4中的研究设计示意图)。对象的同时甾体治疗的常规早晨给药被延迟,直至给药后16小时之后,收集血液样品(即,直到约1400h)。

0346

[0131] 本研究的PD端点包括该患者人群中的目标HPA轴生物标志物;即,17-羟孕酮(17-OHP;作为主要的PD端点)、促肾上腺皮质激素(ACTH)、雄烯二酮、睾酮和血清皮质醇水平。PD变量的初始分析为检查各生物标志物的分组对象数据,所述数据被表示为相对于安慰剂条件,来自两种活性给药条件的给药前水平的平均变化百分比。图5提供了在给药后整个24小时期间的17-OHP和ACTH的平均水平的数据。

[0132] 相对于安慰剂,在这些CAH患者中施用NBI-77860后,在给药后的整个时期观察来自17-OHP和ACTH的给药前水平的一致性和临床上有意义的降低。除了群组平均数据之外,评价了个体反应并且治疗"应答者"被保守地定义为在早晨峰值期间活性NBI-77860相对于安慰剂的情况下,17-OHP和ACTH至少降低50%的那些对象。在研究中,该应答者分析产生相当大的50%的应答者比率(在初始的安慰剂治疗期间,这些对象中没有应答者)。此外,300mg剂量对17-OHP和ACTH产生与600mg剂量几乎相同的作用。图6提供了单个对象对这些生物标志物反应的实例。

[0133] 可以组合以上所述的各种实施方案以提供其它实施方案。本说明书中提及的和/或应用数据表中列出的所有美国专利、美国专利申请公开、美国专利申请、外国专利、外国专利申请和非专利公开,包括2014年1月21日提交的美国临时申请序列号61,929,941、2014年4月17日提交的美国临时申请序列号61,981,033、2014年10月27日提交的美国临时申请序列号62/069,155,通过引用整体并入本文。如果需要采用各种专利、申请和公开的理念以提供其它实施方案,可以修改实施方案的方面。

[0134] 可以根据以上详细描述对实施方案进行这些和其它变化。通常,在以下权利要求中,使用的术语不应解释为将权利要求限制于本说明书和权利要求公开的具体实施方案中,而是应解释为包括所有可能的实施方案以及该权利要求书所享有的等同物的全部范围。因此,权利要求不受公开内容的限制。

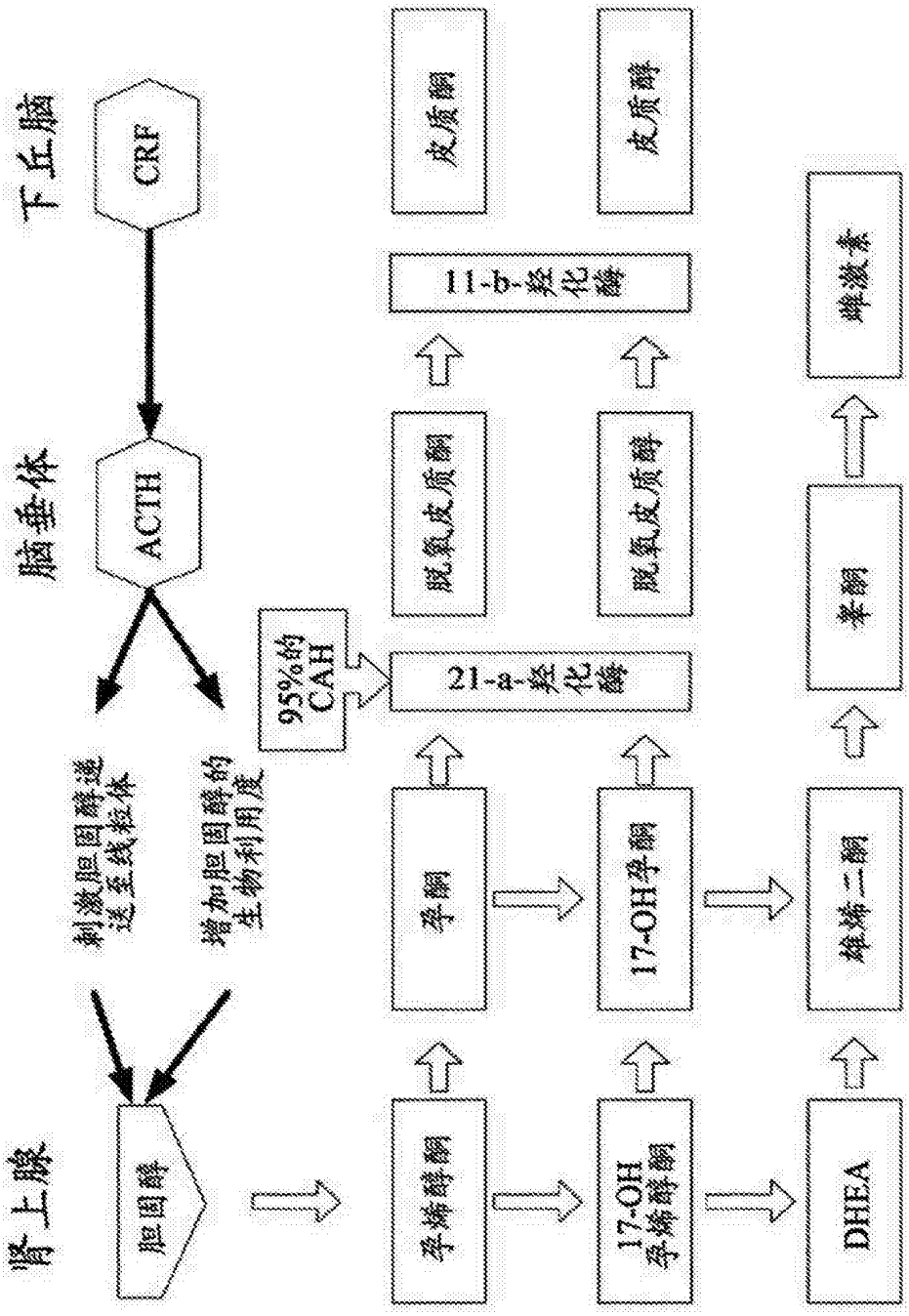

下丘脑
CRF
脑垂体
ACTH
肾上腺
胆固醇
刺激胆固醇进入线粒体
增加胆固醇的生物利用度
95%的CAH
孕烯醇酮
孕酮
17-OH孕烯醇酮
17-OH孕酮
DHEA
雄烯二酮
睾酮
雌激素
21-a-羟化酶
11-b-羟化酶
脱氧皮质酮
脱氧皮质醇
皮质酮
皮质醇

图1

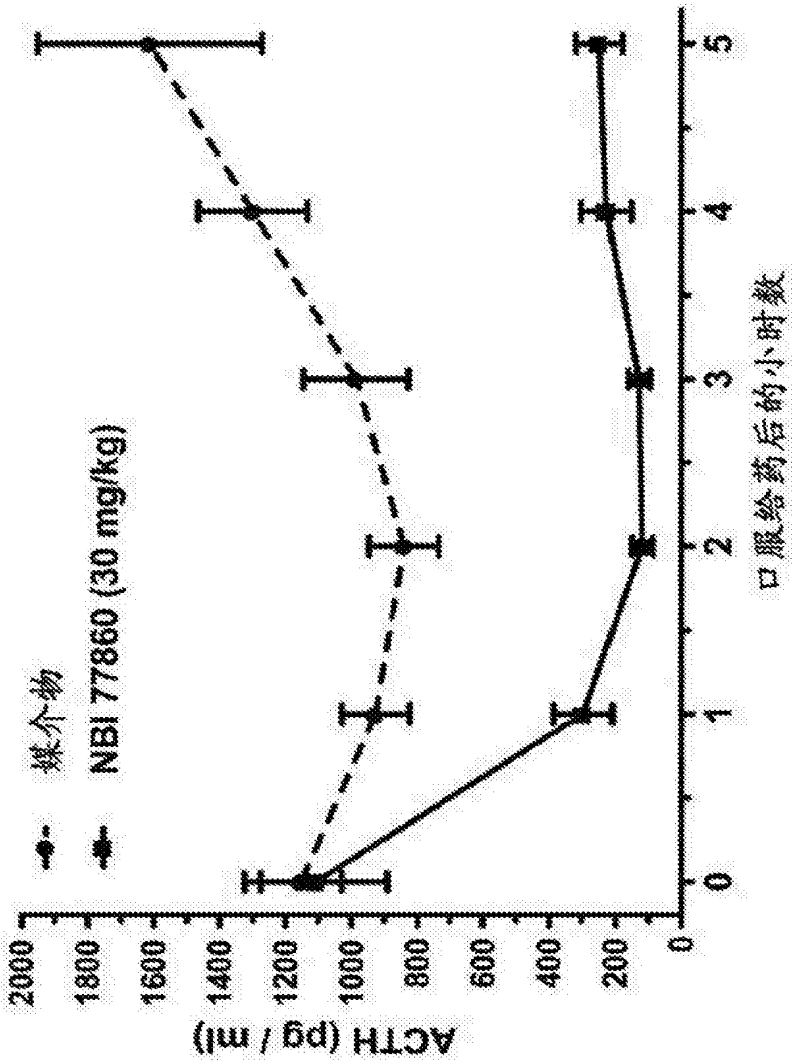

媒介物
NBI 77860 (30 mg/kg)
ACTH (pg / ml)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
口服给药后的小时数
0
1
2
3
4
5

图2

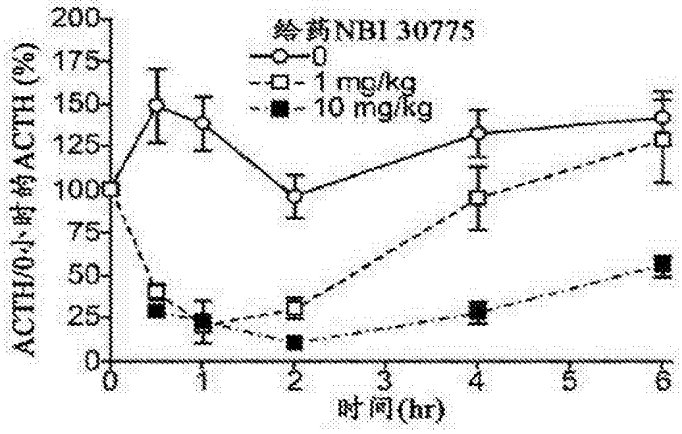

给药NBI 30775
0
1 mg/kg
10 mg/kg
ACTH/0小时的ACTH (%)
200
175
150
125
100
75
50
25
0
0
1
2
3
4
5
6
时间(hr)

图3A

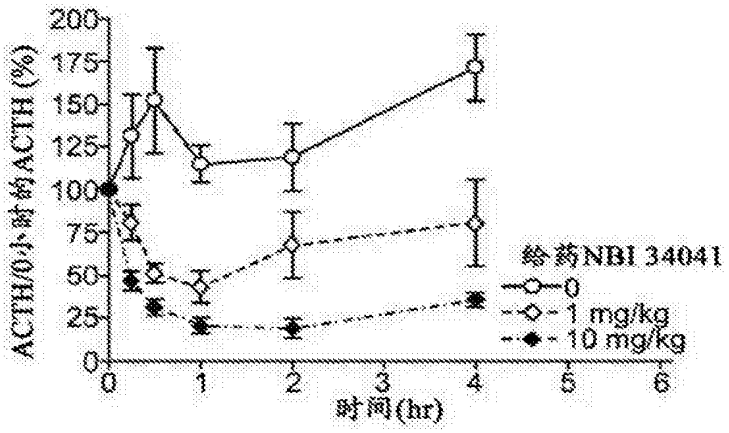

ACTH/0小时的ACTH (%)
200
175
150
125
100
75
50
25
0
0
1
2
3
4
5
6
时间(hr)
给药NBI 34041
0
1 mg/kg
10 mg/kg

图3B

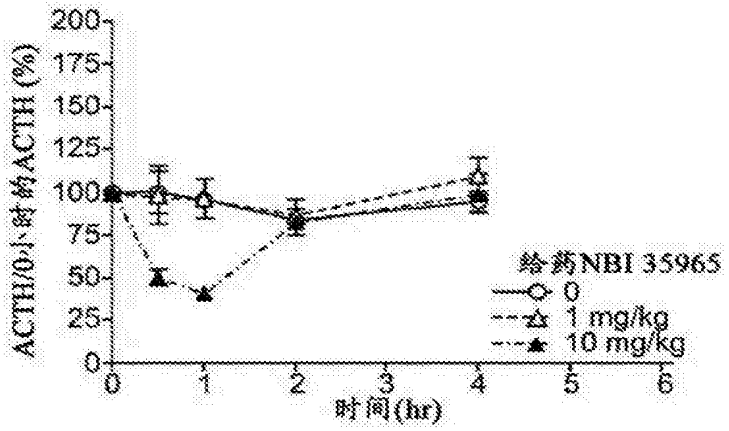

ACTH/0小时的ACTH (%)
200
175
150
125
100
75
50
25
0
0
1
2
3
4
5
6
时间(hr)
给药NBI 35965
0
1 mg/kg
10 mg/kg

图3C

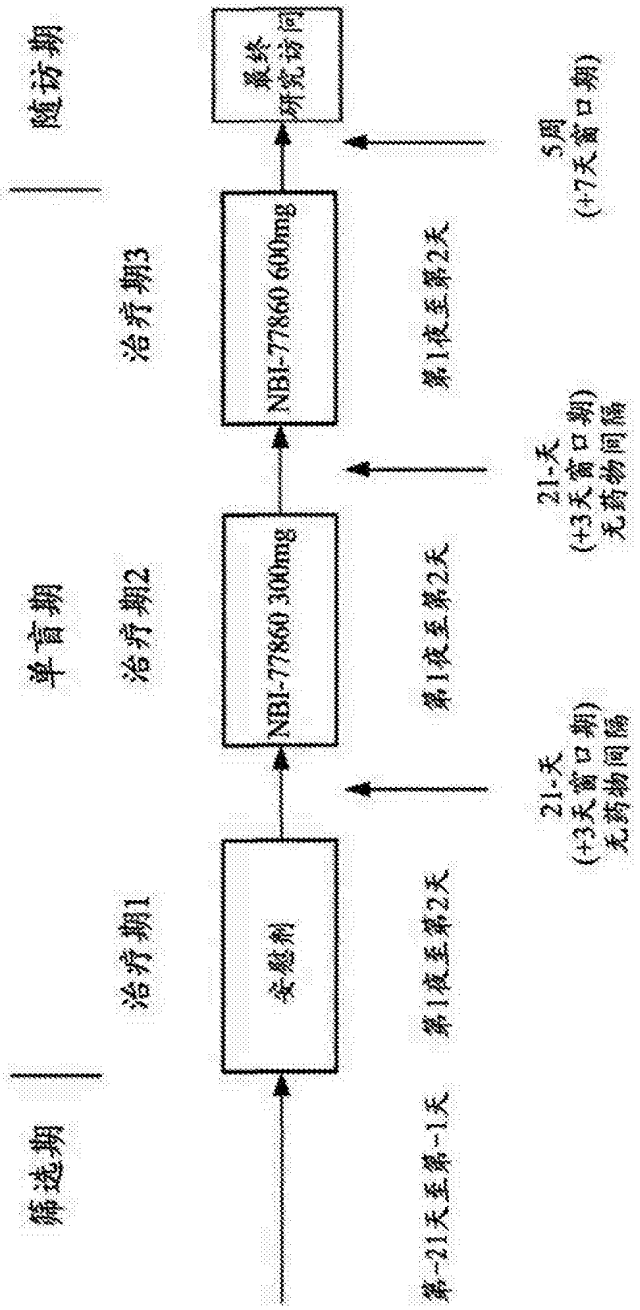
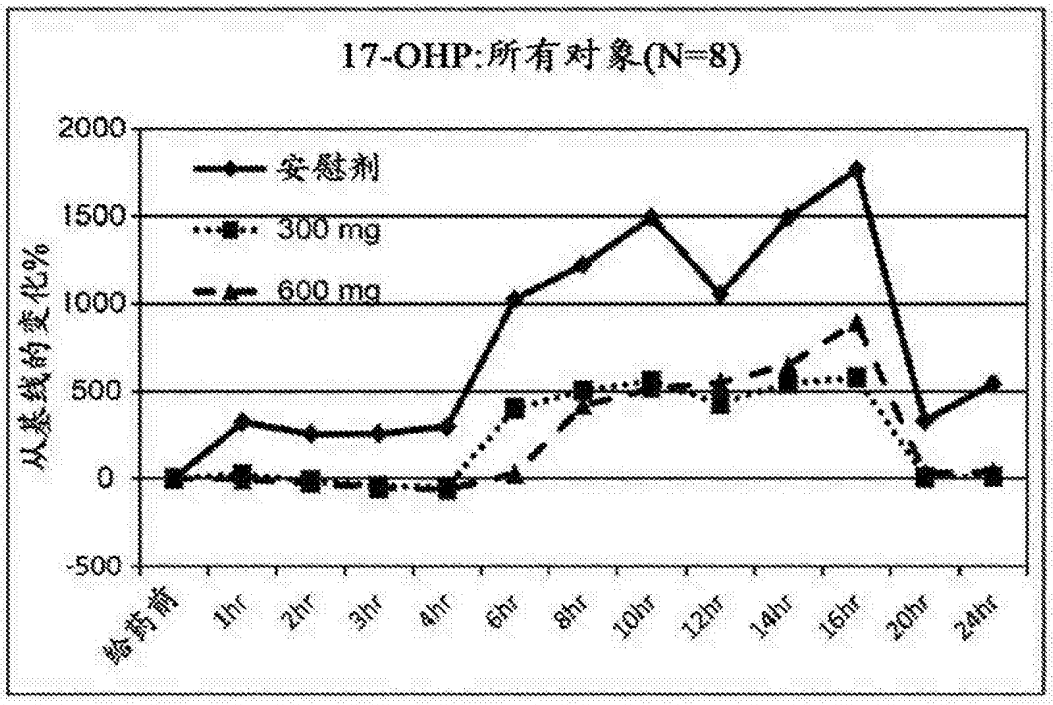

筛选期
随访期
单盲期
治疗期1
治疗期2
治疗期3
安慰剂
NBI-77860 300mg
NBI-77860 600mg
最终研究访问
第-21天至第-1天
第1夜至第2天
第1夜至第2天
第1夜至第2天
21-天
(+3天窗口期)
无药物间隔
21-天
(+3天窗口期)
无药物间隔
5周
(+7天窗口期)

图4


17-OHP:所有对象(N=8)
2000
1500
1000
500
0
-500
从基线的变化%
安慰剂
300 mg
600 mg
给药前
1hr
2hr
3hr
4hr
6hr
8hr
10hr
12hr
14hr
16hr
20hr
24hr

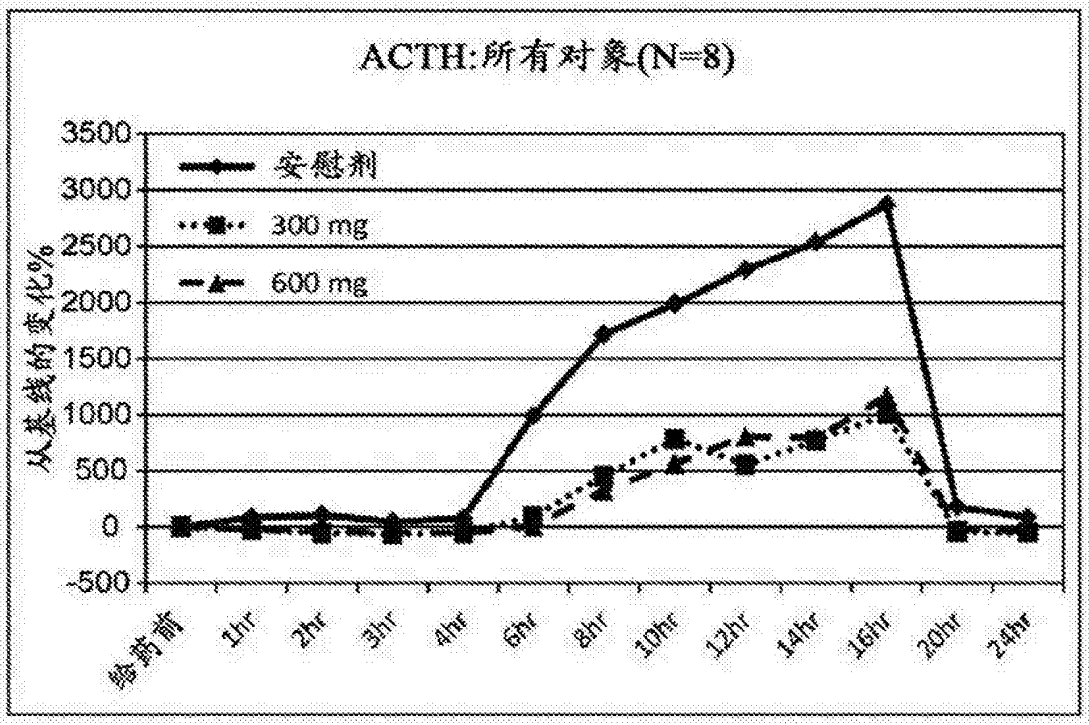

ACTH:所有对象(N=8)
3500
3000
2500
2000
1500
1000
500
0
-500
从基线的变化%
安慰剂
300 mg
600 mg
给药前
1hr
2hr
3hr
4hr
6hr
8hr
10hr
12hr
14hr
16hr
20hr
24hr

图5

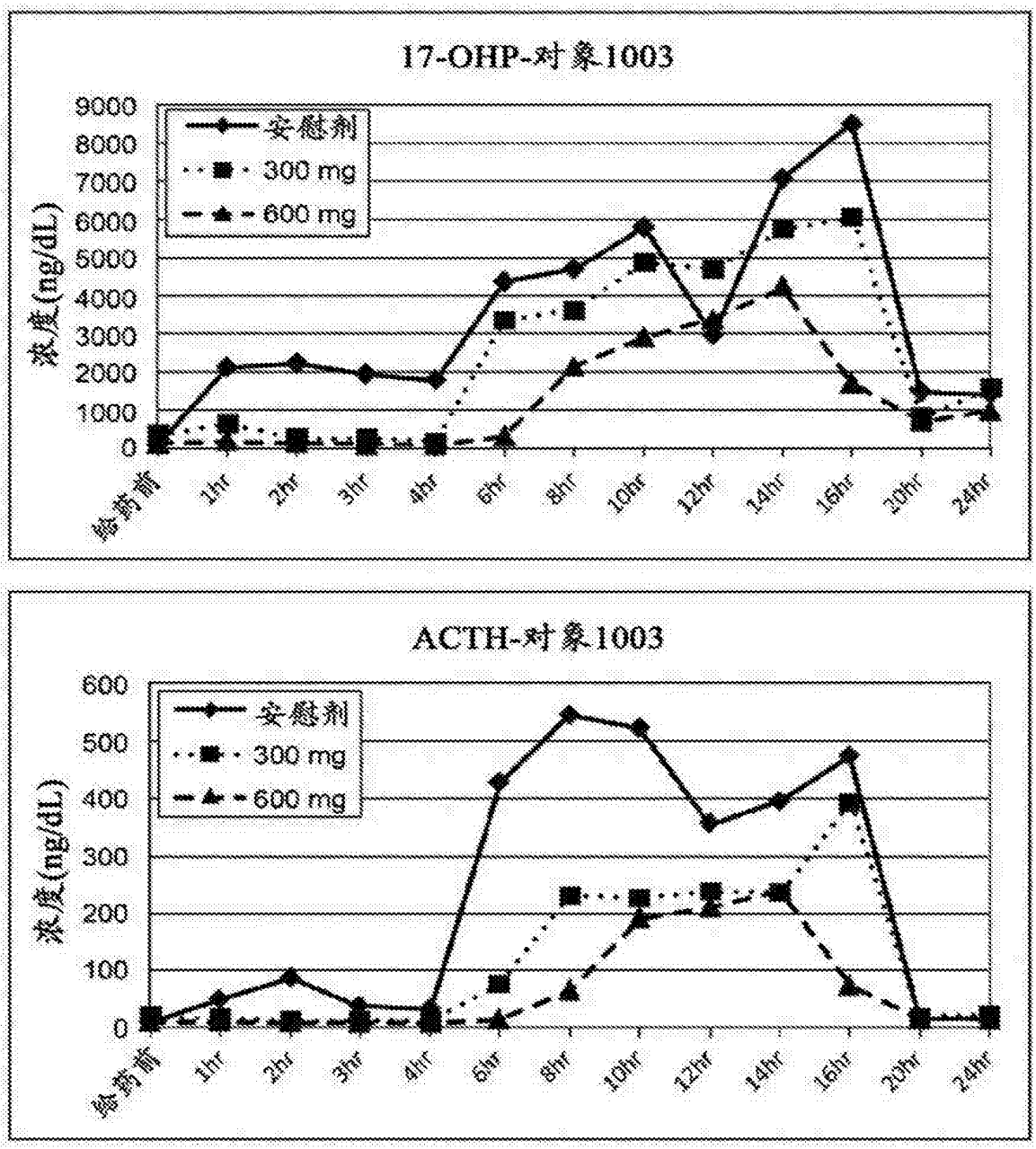

17-OHP-对象1003
浓度(ng/dL)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
安慰剂
300 mg
600 mg
给药前
1hr
2hr
3hr
4hr
6hr
8hr
10hr
12hr
14hr
16hr
20hr
24hr
ACTH-对象1003
浓度(ng/dL)
600
500
400
300
200
100
0
安慰剂
300 mg
600 mg
给药前
1hr
2hr
3hr
4hr
6hr
8hr
10hr
12hr
14hr
16hr
20hr
24hr

图6

Patents JP2010504344A

Thiazolepyrazolopyrimidines as CRF1 receptor antagonists

Abstract

translated from Japanese

The present invention relates to compounds of formula I, pharmaceutical compositions thereof and their use as corticotropin releasing factor 1 (CRF1) receptor antagonists in the treatment of psychiatric and neuroendocrine disorders, nervous system diseases and metabolic syndrome. .

Classifications

C07D487/04 Ortho-condensed systems

View 22 more classifications

JP2010504344A

Japan

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Other languages: Japanese

Inventor: チャオゲン・チェン, チャフィク・アムドウチ・アムドウチ, エリック・ジェイムズ・ヘンプル, フィリップ・アーサー・ヒップスカインド, ジェイソン・ケネス・マイヤーズ, 愛宇 高▲桑▼, ジェイムズ・リー・トス

Worldwide applications

2007 JP BR CA AU MX US RS DE CN PL NZ SI WO EP ES EA DK UA MY PT KR AT AR CL PE TW 2008 IL TN GT NO MA 2010 HK HR CY

Application JP2009529316A events

2006-09-20 Priority to US82626406P

2007-09-17 Application filed by イーライ リリー アンド カンパニー

2008-06-17 First worldwide family litigation filed

2010-02-12 Publication of JP2010504344A

2010-10-28 Publication of JP2010504344A5

2013-03-13 Application granted

2013-03-13 Publication of JP5161228B2

Status Active

2027-09-17 Anticipated expiration

Show all events

Info: Patent citations (10), Non-patent citations (1), Cited by (14), Legal events, Similar documents, Priority and Related Applications

External links: Espacenet, Global Dossier, Discuss

Claims (17)

Hide Dependent

translated from Japanese

Compound of formula I

(Where

R^1 and R^2 are independently ethyl or n-propyl;

R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl or methoxy;

R^4 is hydrogen, Br, $R^a R^b$ N-, methoxymethyl, n-butyl, acetamide, pyridin-4-yl, morpholin-4-yl,

And

R^a and R^b are independently hydrogen, C_1 -C_3 alkyl, $H_2NCH_2CH_2$ --, $(CH_3)_3COC(O)NHCH_2CH_2$ --, or $CH_3CH_2CH_2NHCH_2CH_2$-)

Or a pharmaceutically acceptable salt thereof. R^3 is Cl, Br, or trifluoromethyl, a compound or a pharmaceutically acceptable salt thereof according to claim 1. The compound or a pharmaceutically acceptable salt thereof according to claim 1 or 2, wherein R^3 is Cl or Br. R^4 is $R^a R^b$ N-, pyridin-4-yl, morpholin-4-yl, or

The compound according to any one of claims 1 to 3 or a pharmaceutically acceptable salt thereof. R^4 is morpholin-4-yl or

The compound according to any one of claims 1 to 4 or a pharmacologically acceptable salt thereof. The compound according to any one of claims 1 to 4, or a pharmacological thereof, wherein R^4 is $R^a R^b$ N-, and R^a and R^b are independently C_1 -C_3 alkyl. Acceptable salt. 3- [4-Bromo-2- (2-methyl-2H- [1,2,4] triazol-3-yl) -thiazol-5-yl] -2,5-dimethyl-7- (1-propyl- The compound or a pharmaceutically acceptable salt thereof according to claim 1, which is (butyl) -pyrazolo [1,5-a] pyrimidine. 3- (4-Bromo-2- (2-methyl-2H- [1,2,4] triazol-3-yl) -thiazol-5-yl) -7- (1-ethyl-propyl) -2,5 The compound according to claim 1, which is -dimethyl-pyrazolo [1,5-a] pyrimidine, or a pharmaceutically acceptable salt thereof. 3- (4-Chloro-2-morpholin-4-yl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine, The compound according to claim 1 or a pharmaceutically acceptable salt thereof. A pharmaceutical composition comprising the compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, and a pharmacologically acceptable excipient. Patient depression, anxiety, alcohol abuse or drug abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, seizures, sleep disorders,

allergies, migraine, premenstrual syndrome, infertility, sexual dysfunction, Congenital adrenal hyperplasia, Cushing's disease, premature birth, stress-induced gastric ulcer, inflammatory disorder, pituitary or ectopic tumors, chronic fatigue syndrome, fibromyalgia, visceral pain or multiple sclerosis A method of treatment comprising administering to a patient in need thereof a therapeutically effective amount of a compound according to any one of claims 1 to 9 or a pharmaceutically acceptable salt thereof. .Method. A method for treating anxiety or depression in a patient, wherein the patient in need thereof has a therapeutically effective amount of the compound of any one of claims 1 to 9 or a pharmacologically acceptable salt thereof. Administering a possible salt. A method of treating a patient's alcohol or drug abuse, wherein the patient in need thereof has a therapeutically effective amount of the compound of any one of claims 1 to 9 or a pharmacologically acceptable salt thereof. Administering a possible salt. 10. A compound according to any one of claims 1 to 9 or a pharmaceutically acceptable salt thereof for use in therapy. Depression, anxiety, alcohol abuse or drug abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, seizures, sleep disorders, allergies, migraine, premenstrual syndrome, infertility, sexual dysfunction, congenital in the treatment of adrenal hyperplasia, Cushing's disease, premature birth, stress-induced gastric ulcer, inflammatory disorder, pituitary or ectopic pituitary tumor, chronic fatigue syndrome, fibromyalgia, visceral pain or multiple sclerosis The compound or pharmacologically acceptable salt thereof according to any one of claims 1 to 9, for use. 10. A compound according to any one of claims 1 to 9 or a pharmaceutically acceptable salt thereof for use in the treatment of anxiety or depression. 10. A compound according to any one of claims 1 to 9 or a pharmaceutically acceptable salt thereof for use in the treatment of alcohol abuse or drug abuse.

Description

translated from Japanese

The present invention relates to novel thiazolepyrazolopyrimidine compounds, pharmaceutical compositions thereof and their use as CRF1 receptor antagonists in the treatment of psychiatric and neuroendocrine disorders, nervous system diseases and metabolic syndrome.

Corticotropin releasing factor (CRF) is a 41 amino acid peptide that is a major physiological regulator of proopiomelanocortin (POMC) -derived peptide secretion from the anterior pituitary gland. In addition to its endocrine role in the pituitary gland, due to the immunohistochemical localization of CRF, this hormone has a broad extrahypothalamic distribution of the central nervous system and as a neurotransmitter or neuromodulator in the brain Proven to produce a wide range of autonomic effects, electronic physiological effects and effects on action consistent with the role. There is also evidence that CRF plays an important role in integrating responses to physiological, psychological and immunological stressors in the immune system.

CRF is a psychiatric and nervous system disease, including depression and neuroanxiety, and Alzheimer's disease, Huntington's disease, progressive supranuclear palsy, amyotrophic lateral sclerosis, Parkinson's disease, epilepsy, migraine, alcohol abuse And drug abuse and related withdrawal symptoms, obesity, metabolic syndrome, congenital adrenal hyperplasia, Cushing disease, hypertension, stroke, irritable bowel syndrome, stress-induced gastric ulcer, premenstrual syndrome, sexual dysfunction, premature birth, It has been associated with inflammatory disorders, allergies, multiple sclerosis, visceral pain, sleep disorders, pituitary or ectopic pituitary tumors, chronic fatigue syndrome and fibromyalgia.

CRF receptor subtypes (CRF1 and CRF2) have been identified and these are heterogeneously distributed in the brain, suggesting a possible functional diversity For example, widely distributed brain CRF1 receptors are strongly associated with the emotion associated with exposure to environmental stressors. Importantly, the CRF1 receptor, but not the CRF2 receptor, appears to mediate the selection of anxiety-like behavior. The more discrete septal / hypothalamic distribution and availability of another endogenous ligand suggests a different functional role for the CRF2 receptor. For example, a novel CRF family of neuropeptides with a preferential affinity for the CRF2 receptor over the CRF1 receptor may cause an appetite without producing the behavior-activated appearance observed with selective CRF1 agonism. It has been reported to suppress. In other cases, CRF2 agonism produces effects similar to those reported for CRF1 antagonists or CRF1 gene deletions. For example, although CRF2 agonists have been proposed as anti-obesity agents, CRF1 antagonists may also be important treatments for obesity.

Certain pyrrolo [2,3-d] pyrimidines, pyrrolo [3,2-d] pyrimidines, pyrazolo [1,5-a] pyrimidines, 1,2,3-triazolo [4,5-b useful as CRF antagonists] Pyridine and pyrazolo [1,5-a] -1,3,5-triazine are described in Patent Document 1, Patent Document 2, Patent Document 3, and Patent Document 4.

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The present invention provides novel thiazolepyrazolopyrimidines useful as CRF1 receptor antagonists. In view of the above, it would be desirable to discover new effective and selective antagonists of CRF1 as potentially beneficial therapeutics for the treatment of psychiatric and neuroendocrine disorders, nervous system diseases and metabolic syndrome. In addition, novel compounds with preferred bioavailability and pharmacokinetic profiles over known CRF antagonists (eg CP154526 and NBI30775) because the majority of commercially available CNS drugs and cardiovascular drugs exhibit unfavorable bioavailability and pharmacokinetic profiles. It is also desirable to discover.

In one embodiment, the invention provides a compound of formula I

Wherein R^1 and R^2 are independently ethyl or n-propyl, R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl or methoxy, R^4 is hydrogen, Br, $R^a R^b N$-, methoxymethyl, n-butyl, acetamide, pyridin-4-yl, morpholin-4-yl,

R^a and R^b are independently hydrogen, C_1-C_3 alkyl, $H_2NCH_2CH_2$ ---, $(CH_3)_3COC(O)NHCH_2CH_2$ ---, or $CH_3CH_2CH_2NHCH_2CH_2$ - in which) or to provide a

pharmaceutically acceptable salt thereof.

In another embodiment, the present invention provides a pharmaceutical composition comprising a compound of formula I or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient.

In another embodiment, the invention relates to patient depression, anxiety, alcohol abuse or drug abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, seizures, sleep disorders, allergies, migraine, premenstrual Syndrome, infertility, sexual dysfunction, congenital adrenal hyperplasia, Cushing's disease, premature birth, stress-induced gastric ulcer, inflammatory disorder, pituitary or ectopic pituitary tumor, chronic fatigue syndrome, fibromuscular A method of treating pain, visceral pain or multiple sclerosis comprising administering to a patient in need thereof a therapeutically effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof. About.

In another embodiment, the present invention relates to depression, anxiety, alcohol abuse or drug abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, seizures, sleep disorders, allergies, migraine, premenstrual syndrome, infertility, sexual dysfunction, congenital adrenal hyperplasia, Cushing's disease, premature birth, stress-induced gastric ulcer, inflammatory disorder, pituitary or ectopic pituitary tumor, chronic fatigue syndrome, fibromyalgia, It relates to the use of a compound of formula I or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of visceral pain or multiple sclerosis.

In another embodiment, the present invention relates to depression, anxiety, alcohol abuse or drug abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, seizures, sleep disorders, allergies, migraine, premenstrual syndrome, infertility, sexual dysfunction, congenital adrenal hyperplasia, Cushing's disease, premature birth, stress-induced gastric ulcer, inflammatory disorder, pituitary or ectopic pituitary tumor, chronic fatigue syndrome, fibromyalgia, It relates to a compound of formula I or a pharmaceutically acceptable salt thereof for use in the treatment of visceral pain or multiple sclerosis.

In another embodiment, the present invention relates to a therapeutic compound of formula I or a pharmaceutically acceptable salt thereof.

As used above and throughout the description of the present invention, the following terms shall be understood to have the following meanings unless otherwise indicated.

"Alkyl" means a saturated aliphatic hydrocarbon group which may be straight or branched having 1 to 5 carbon atoms in the chain.

"Pharmaceutically acceptable excipient" refers to a pharmacologically acceptable pharmaceutical carrier, solution, or additive to enhance pharmaceutical properties. Such excipients must be compatible with the other ingredients of the formulation and not deleterious to the user. Such excipients are well known to those skilled in the art (see, eg, Remingtons Pharmaceutical Sciences, 19th Edition, Mack Publishing Company, 1995).

"Pharmaceutically acceptable salt" refers to the relatively non-toxic, inorganic and organic acid addition salts and base addition salts of the compounds of the present invention. These salts can be prepared in situ in the final isolation and purification steps of the compound. In particular, acid addition salts can be prepared by reacting the purified compound in the free base form separately with a suitable organic or inorganic acid and isolating the resulting salt (see, eg, Remingtons Pharmaceutical Sciences, No. 1). 19th edition, see Mack Publishing Company, 1995).

A "therapeutically effective amount" or "effective amount" is the biological or medical response of a tissue, system, animal or human being sought by a researcher, veterinarian, physician or other clinician, or the desired response thereto It means the amount of a compound of formula I of the invention or a pharmaceutical composition containing a compound of formula I of the invention that causes a therapeutic effect.

Terms such as "treatment", "treat", "treating" and the like are intended to include both slowing the progression of the disorder and directing recovery. These terms also refer to one or more symptoms of the disorder or condition, even if the disorder or condition is not actually resolved, and the progression of the disorder or condition is not slowed by itself and is not likely to recover. Also includes mitigating, improving, mitigating, eliminating or reducing. The term "treatment" and similar terms also include preventive (eg, prophylactic) treatment and treatment for pain relief. Prevention of a disease results in prolonging or delaying the onset of symptoms of the disease.

Symbols in molecular structure
Indicates the bonding position for the specific substituent.

When any variable occurs more than one time in any constituent or in formula I, its definition when each appears is independent of its definition when it appears elsewhere. Also, combinations of substituents and / or variables are acceptable only if such combinations result in stable compounds. In selecting the compounds of the present invention, those skilled in the art will recognize that various substituents, ie R^1, R^2, etc., should be selected in accordance with the well-known principles of chemical structure connectivity. I will.

Under standard nomenclature used throughout this disclosure, the terminal portion of the designated side chain is described first, followed by the adjacent functionality toward the point of attachment. For example, an arylcarbonylaminoalkyl substituent is equivalent to aryl-C (O) -NH-alkyl-.

The present invention contemplates the following embodiments, which can be further combined differently:

(A) a compound of formula I wherein R^1 and R^2 are ethyl;

(B) a compound of formula I wherein R^1 and R^2 are n-propyl;

(C) R^3 is Cl, Br, compound of formula I is methyl or trifluoromethyl;

(D) the compound of formula I, wherein R^3 is Cl;

(E) the compound of formula I, wherein R^3 is Br;

(F) R^4 is $R^a R^b N$-, pyridin-4-yl, morpholin-4-yl, or
A compound of formula I which is

(G) the compound of formula I, wherein R^4 is morpholin-4-yl;

(H) R^4 is
A compound of formula I which is

(I) the compound of formula I, wherein R^a and R^b are independently C_1-C_3 alkyl;

(J) the compound of formula I, wherein R^1 and R^2 are ethyl, R^3 is Cl, and R^4 is morpholin-4-yl;

(K) the compound of formula I, wherein R^1 and R^2 are n-propyl, R^3 is Cl, and R^4 is morpholin-4-yl;

(L) a compound of formula I wherein R^1 and R^2 are ethyl, R^3 is Br and R^4 is morpholin-4-yl;

(M) R^1 and R^2 are n- propyl, R^3 is Br, compound of formula I R^4 is morpholin-4-yl;

(N) R^1 and R^2 are ethyl, R^3 is Cl, R^4 is
A compound of formula I which is

(O) R^1 and R^2 are n-propyl, R^3 is Cl, and R^4 is
A compound of formula I which is

(P) R^1 and R^2 are ethyl, R^3 is Br, and R^4 is
A compound of formula I which is

(Q) R^1 and R^2 are n-propyl, R^3 is Br, and R^4 is
A compound of formula I which is

(R) the use of a compound of formula I or a pharmaceutically acceptable salt thereof for the treatment of depression or neuroanxiety;

(S) the use of a compound of formula I or a pharmaceutically acceptable salt thereof for the treatment of alcohol abuse or drug abuse;

(T) the use of a compound of formula I or a pharmaceutically acceptable salt thereof for the treatment of alcohol or drug abuse and related withdrawal symptoms;

(U) a compound of formula I or a pharmaceutically acceptable salt thereof showing a Ki value of 500 nM or less for CRF1 binding;

(V) a compound of formula I or a pharmaceutically acceptable salt thereof showing a Ki value of 50 nM or less for CRF1 binding;

(W) a compound of formula I or a pharmaceutically acceptable salt thereof showing a Ki value of 5 nM or less for CRF1 binding;

(X) a compound of formula I or a pharmaceutically acceptable salt thereof that exhibits a Ki value of 500 nM or less for CRF1 binding and selectively binds to CRF1 over CRF2 (ie, a smaller Ki);

(Y) a compound of formula I or a pharmaceutically acceptable salt thereof that exhibits a Ki value of 50 nM or less for CRF1 binding and selectively binds to CRF1 over CRF2 (ie, a smaller Ki);

(Z) a compound of formula I or a pharmaceutically acceptable salt thereof that exhibits a Ki value of 5 nM or less for CRF1 binding and selectively binds to CRF1 over

CRF2 (ie, a smaller Ki); and / or Alternatively (aa) certain exemplified compounds (such as Example 15) that have better bioavailability and pharmacokinetic profiles than some known CRF antagonists (eg, CP154526 and NBI30775).

The compounds of the present invention are preferably formulated as pharmaceutical compositions that are administered by a variety of routes. Such compositions are preferably for oral administration. Such pharmaceutical compositions and processes for preparing such pharmaceutical compositions are well known in the art (see, eg, Remington: The Science and Practice of Pharmacy, A. Gennaro et al , 19th Edition, Mack Publishing Co. , 1995).

The compounds of formula I are generally effective over a wide dosage range. For example, daily dosages typically range from about 0.0001 to about 30 mg / kg body weight. In some cases, dosage levels below the lower limit of the aforementioned range may be sufficient, while in some cases even higher doses may be used without causing any harmful side effects, and therefore The above dosage ranges are not intended to limit the scope of the invention in any way. The amount of the compound actually administered will depend on the condition being treated, the route of administration selected, the actual compound (s) being administered, the age, weight and response of the individual patient, it should be understood that it will be determined by the physician in view of the relevant circumstances, including the severity of the patient's symptoms.

The compounds of formula I are CRF-1 antagonists and are therefore useful for the treatment of medical conditions that can be treated by reducing CRF1 receptor tone or stimulation. Corticotropin releasing factor (CRF), a 41 amino acid peptide that is a major physiological regulator of peptide secretion from proopiomelanocortin (POMC) from the anterior pituitary gland [J. Rivier et al., Proc. Natl. Acad. Sci (USA) 80: 4851 (1983); Vale et al., Science 213: 1394 (1981)] has been associated with a number of medical conditions. For example, in addition to its endocrine role in the pituitary gland, because of the immunohistochemical localization of CRF, this hormone has a broad extra-thalamic distribution of the central nervous system and is a neurotransmitter or neuromodulator in the brain It has been demonstrated to produce a wide range of autonomic effects, electrophysiological effects and action effects consistent with its role as [W. Vale et al., Rec. Prog. Horm. Res. 39: 245 (1983); F. Koob, Persp. Behav. Med. 2:39 (1985); B. De Souza et al., J. Mol. Neurosci. 5: 3189 (1985)]. There is also evidence that CRF plays an important role in integrating responses to physiological, psychological and immunological stressors in the immune system [see, for example, J. et al. E. Black, Physiological Reviews 69: 1 (1989); E. Morley, Life Sci. 41: 527 (1987)].

CRF is associated with psychiatric disorders and nervous system disorders, including depression and neuroanxiety [D. M.M. Nielsen, Life Sci. 78: 909-919; E. Kunzel et al. Psychiatr. Res. 37: 525-533; R. Genlert et al., Eur. J. et al. Pharmacol. 509: 145-153]. The role of CRF has also been postulated in the pathogenesis and pathophysiology of Alzheimer's disease, Huntington's disease, progressive supranuclear palsy and amyotrophic lateral sclerosis. Because they are related to dysfunction of CRF neurons in the central nervous system [for review, see E.C. B. De Souze, Hosp. Practice 23:59 (1988)]. Chronic administration of CRF has been shown to produce dysfunction of the dopamine system, suggesting a role in Parkinson's disease [E. Izzo et al., Pharmacol. Biochem. Behav. 81: 701-708 (2005)]. Other neurological disorders involving CRF include sputum [T. Z. Baram et al., Brain Res. 770. 89-95 (1997)] and migraine [T. C. Theoharides et al., Endocrinology 136: 5745-5750 (1995)]. CRF has been associated with alcohol and drug abuse and related withdrawal symptoms [D. H. Overstreet et al., Pharmacol. Biochem. Behav. 77: 405-413; Shaham et al., Psychopharmacology (Berr) 137: 184-190]. In addition, CRF is associated with various endocrine disorders and cardiovascular diseases such as obesity [E. Timofeva and D. Richard, Neuroendocrinology 66: 327-340 (1997)], metabolic syndrome [AM Ward et al., Metabolism 53: 720. -726 (2004)], congenital adrenal hyperplasia [DP Merke and GB Cutler Jr., Endocrinol. Metab. Clin. North Am. 30: 121-135 (2001)], Cushing's disease [M Labeur et al., Curr. Drug Targets Immunod Endocr. Metabol. Disord. 4: 335-342 (2004)], hypertension [RJ Briscoe et al , Brai. Res.881: 204-207 (2000)], and seizures [S.L Stevens et al , J.Cereb.Blood Flow Metab.23: 1151-1159 play a role in (2003)]], there is evidence that. Gastric disorders (eg, irritable bowel syndrome [Y. Tache et al., Eur J. Surg. Suppl: 16-22 (2002)] and stress-induced gastric ulcer [KE Gabry et al., Mol. Psychiatry 7: 474| -483,433 (2002)]] has been shown to be related to CRF. In addition, CRF has been identified in various areas of human female health, such as premenstrual syndrome [F. Facchinetti et al., Psychosom. Med. 56: 418-422 (1994)], infertility [L. Ghizzoni et al., Endocrinology 138: 4806-4811 (1997)], sexual dysfunction [J. E. Jones et al., Am. J. et al. Physiol. Regul. Integr. Comp. Physiol. 283: R591-597 (2002)], and preterm birth [F. D. Wadhwa et al., Am. J. et al. Obstet. Gynecol. 191: 1063-1069 (2004)]. There is also evidence that CRF plays an important role in the immune system. This is because inflammatory disorders [A. Gravanis and A.M. N. Margioris, Curr. Med. Chem. 12: 1503-1512 (2005)], allergies [L. K. Singh et al., Brain Behav. Immun. 13: 225-239 (1999)], multiple sclerosis and other autoimmune disorders [C. Benou et al. Immunol. 174: 5407-5413 (2005)]. In addition to the above, CRF is a visceral pain [M. Nijsen et al., Neurogastroenterol. Motil. 17: 423-432 (2005)], sleep disorders [T. M.M. Buckley and A.M. F. Schattberg, J.A. Clin. Endocrinol. Metab. 90: 3106-3114 (2005)], pituitary tumors or ectopic pituitary tumors [K. D. Dieterich et al., J. Mol. Clin. Endocrinol. Metab. 83: 3327-3331 (1998)], chronic fatigue syndrome and fibromyalgia [G. Neeck and L.M. J. et al. Croford, Rheum. Dis. Clin. North Am. 26: 989-1002 (2000)].

CRF receptor subtypes (CRF1 and CRF2) have been identified and these are heterogeneously distributed in the brain [D. T.A. Chalmers et al., TIPS 17: 166-72 (1996)], suggesting the possibility of functional diversity [S. C. Heinrichs et al., Regul. Peptides 71:15 (1997)]. For example, widely distributed brain CRF1 receptors are strongly associated with the emotionality associated with exposure to environmental stressors [G. Liebsch et al., Regul. Peptides 59: 229-39 (1995); W. Schulz, PNAS 93: 10477-82 (1996)]. Importantly, the CRF1 receptor, but not the CRF2 receptor, appears to mediate selection of anxiety-like behavior [Heinrichs et al., 1997]. A more discrete septum / hypothalamus distribution [D. T.A. Chalmers et al. Neurosci. 15 (10): 6340-50 (1995)] and the availability of other endogenous ligands [J. Vaughan et al , Nature 378: 287-92 (1995)] suggests a different functional role for the CRF2 receptor [Heinrichs et al., 1997]. For example, a novel CRF family of neuropeptides with a preferential affinity for the CRF2 receptor over the CRF1 receptor may cause an appetite without producing the behavior-activated appearance observed with selective CRF1 agonism. [H. Tezval et al., PNAS 101 (25): 9468-9473 (2004)]. In some cases, CRF2 agonism produces effects similar to those reported for CRF1 antagonists or CRF1 gene deletion [S. C. Heinrichs, Trends in Pharmacological Sciences 20 (8): 311-5 (1999)]. For example, CRF2 agonists have been proposed as anti-obesity agents, but CRF1 antagonists may also be important treatments for obesity [C. Contoreggi et al., Neuroendocrinology 80 (2): 111-23 (2004)].

(Preparation of compounds of the invention)
All of the compounds of the present invention can be chemically prepared, for example, according to the synthetic routes shown below. However, the following discussion is in no way intended to limit the scope of the present invention. For example, the specific synthetic steps for each of the described routes may be combined in different ways to prepare additional compounds of formula I or may be combined in conjunction with steps from different schemes. . The product of each step can be recovered by conventional methods including extraction, evaporation, precipitation, chromatography, filtration, powdering, crystallization and the like. In the schemes below, all substituents are as previously defined unless otherwise stated, and suitable reagents are well known and recognized in the art.

Formation of compounds of formula (6a, b), formula (7), or formula 8 (ac) can be carried out according to the reaction shown in Scheme 1. Suitable compounds of formula (6a, b), formula (7), or formula (8a-c) are those where R^1 and R^2 are as defined for formula I and R^{3a} = H or CH_3, R^{3b} = Br or CH_3 and R^{3c} = H, CH_3, or Br.

In Scheme 1, Step 1, pyrazolo [1,5-a] pyrimidin-7-one of formula (1) uses phosphorus oxychloride and dimethylaniline in an inert solvent (such as toluene) at the reflux temperature of the solvent. To 7-chloro-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine.

In Step 2, the Grignard reagent of formula (3) (X = Cl or Br) is reacted with the chloride of formula (2) at reflux temperature in an inert solvent (such as toluene) to yield the 7-alkyl of formula (4). Give pyrazolopyrimidine.

Alternatively, the 7-alkylpyrazolopyrimidine of formula (4) can be obtained as shown in Step 3, Step 4, and Step 5. In Step 3, ethyl acetoacetate is acylated with an acid halide of formula (4a) in the presence of magnesium chloride to give a diketo-ester of formula (4b). The diketo-ester of formula (4b) is decarboxylated under Krapcho conditions to give the diketone of formula (4c). For example, (4b) is heated in dimethyl sulfoxide at a temperature of about 130-170 ° C. to effect this decarboxylation. In

step 5, the diketone of formula (4c) is cyclized with 3-amino-5-methylpyrazole in a protic solvent (such as methanol, ethanol, or acetic acid) to give a 7-alkylpyrazolopyrimidine of formula (4). give. In preferred conditions, acetic acid is used at a temperature of about 0-60 ° C.

The pyrazolopyrimidine of formula (4) is functionalized in step 6 to an iodopyrazolopyrimidine of formula (5) using an excess of N-iodosuccinimide in acetonitrile.

In Scheme 1, Step 7 or Step 8, iodopyrazolopyrimidine of formula (5) is reacted with thiazole zinc halide in a Negishi cross-coupling reaction to form thiazolylpyrazolopyrimidine of formula (6a) or formula (6b) (Jensen, J .; Skjaerbaek, N .; Vedso, P. Synthesis 2001, 128). The thiazole zinc halide is produced using methods well known to those skilled in the art. For example, in step 7, 2-trimethylsilanyl two thiazole is treated with n- butyllithium, sec- butyllithium or tert- butyllithium, and then lithium $ZnCl_2$ - zinc replacement is made. The organozinc reagent is present in an inert solvent (such as THF) in the presence of a palladium catalyst such as dichloro [1,1'-bis (diphenyl-phosphino) ferrocene] palladium (II) dichloromethane at reflux temperature for about 12-36 hours. Below "coupled with iodopyrazolopyrimidines of formula (5)" to give thiazole pyrazolopyrimidines of formula (6a).

Alternatively, in step 8, thiazole zinc bromide is generated using 5-bromo-4-methylthiazole and zinc metal and is used for Negishi cross coupling essentially as described for step 7 to obtain the formula (6b) Thiazolepyrazolopyrimidine.

in Scheme 1, Step 9, the thiazole of formula (6a, b) is brominated to give the bromothiazole or dibromothiazole of formula (7) where R^{3b} = Br or CH_3 . This thiazole is brominated with 1 or 2 equivalents of N-bromosuccinimide, depending on whether R^{3a} is CH_3 or H, respectively.

The alkyl thiazole of formula (8a-c) is obtained in step 11 from the thiazole of formula (6a, b) or in step 10 from the formula (7) bromothiazole. In step 10, halogen-lithium exchange with n-butyllithium, sec-butyllithium, or t-butyllithium provides a thiazole lithium reagent, which is then subjected to an electrophile (an alkyl such as iodomethyl methyl ether or iodobutane). Reaction with halide). In step 11, the thiazole lithium reagent is generated by deprotonation using n-butyllithium, sec-butyllithium, or tert-butyllithium, followed by an electrophile such as iodomethyl methyl ether or iodobutane. React with.

The thiazole ring system is easily functionalized and that thiazole intermediates such as 2-trimethylsilanyl-thiazole (Dondoni, A. et al., J. Org. Chem. 1988, 53, 1748) can be easily prepared. Those skilled in the art will understand. 5-Bromo-4-methylthiazole is obtained by bromination of 4-methylthiazole with bromine in acetic acid (Collins, IJ et al., WO2003309522, Nov. 13, 2003). . 2,5-Dimethyl-4H-pyrazolo [1,5-a] pyrimidin-7-one (1) is obtained by condensation of ethyl acetoacetate and 5-methyl-2H-pyrazol-3-ylamine in refluxing acetic acid. Easy to prepare.

Formation of the compound of formula (9), formula (10), or formula (11) is carried out according to the reaction shown in Scheme 2. Suitable compounds of formula (9), formula (10), or formula (11) are as R^1 , R^2 , R^6 , and R^b are as defined for formula I, and R^{3b} = Br or CH_3 , R^{4a} = —NR^aR^b or —N-morpholinyl, a compound defined as "het".

In Step 1, the bromothiazole of formula (7) is coupled with a heterocyclic zinc reagent in a Negishi cross-coupling reaction to give a thiazole heterocycle of formula (9). For example, 1-methyl-1,2,4-triazole is treated with n-butyllithium, sec-butyllithium, or tert-butyllithium at about −80 to −65 ° C. and then with zinc chloride, React with bromothiazole of formula (7) in situ. This reaction is carried out in an inert solvent (such as THF) with a palladium catalyst (such as dichloro [1,1'-bis (diphenyl-phosphino) ferrocene] palladium (II) dichloromethane or tetrakis (triphenylphosphine) palladium (0)). Is preferably carried out in the presence of The reaction is warmed to reflux temperature. Alternatively, the heterocyclic zinc reagent is formed from a haloheterocycle (such as 4-iodopyridine) and zinc metal.

In Scheme 2, Step 2, the intermediate 2-formylthiazole is formed by halogen-lithium exchange using n-butyllithium, sec-butyllithium, or t-butyllithium, followed by reaction with N-formylmorpholine. . This formylthiazole is subjected to reductive amination in the presence of an organic amine (such as morpholine) to give the morpholinylmethylthiazole of formula (10). Reductive amination is well known in the art and is usually performed using an inorganic borohydride reagent (such as sodium borohydride or sodium cyanoborohydride). Preferred conditions use sodium triacetoxyborohydride in an inert solvent such as dichloromethane or THF.

In Step 3, the bromothiazole of formula (7) undergoes a substitution reaction with an amine or morpholine of formula —NR^aR^b to give an aminothiazole of formula (11). This reaction is carried out at 70-110 ° C. using an inorganic base (such as cesium carbonate) in an inert solvent (such as THF or dioxane).

Formation of the compound of formula (13), formula (14), or formula (15) can be carried out according to the reaction shown in Scheme 3. Suitable compounds of formula (13), formula (14), or formula (15) are those where R^1 , R^2 , and R^4 are as defined for formula I.

One skilled in the art will recognize that 4-bromothiazole (such as that of formula (12)) is readily manipulated for other functionalizations. For example, in Step 1, the bromide can be dehalogenated with copper (I) chloride to give the thiazole of formula (13), which is then chlorinated with N-chlorosuccinimide to obtain the formula 4-chlorothiazole of (14) is obtained.

In Scheme 3, Step 3, 4-bromothiazole of formula (12) is dissolved in sodium methoxide in the presence of copper (I) iodide in methanol at about 100-120 ° C. in an inert solvent (such as dimethylformamide). To give 4-methoxythiazole of formula (15).

Formation of the compound of formula (17), formula (18), formula (19), formula (20), formula (20a), or formula (21) can be carried out according to the reaction shown in Scheme 4. Suitable compounds of formula (17), formula (18), formula (19), formula (20), formula (20a), or formula (21) are those wherein R^1 , R^2 , R^a , and R^b are of formula A compound as defined for I, wherein R^{4a} is —NR^aR^b or —N-morpholinyl and "het" is indicated.

In Scheme 4, Step 1, iodopyrazolopyrimidine of formula (5) and 5-bromothiazole of formula (16) undergo Negishi cross-coupling to produce dimethylpyrrolylthiazole of formula (17). For example, 5-bromothiazole of formula (16) is treated with n-butyllithium, sec-butyllithium, or tert-butyllithium at about −80 to −65 ° C. and then with zinc chloride. This organozinc reagent is reacted in situ with the iodopyrazolopyrimidine of formula (5). This coupling reaction is preferably carried out in an inert solvent (such as THF) at the reflux temperature in the presence of a palladium catalyst (such as bis (tri-t-butylphosphine) palladium (0)).

In Step 2, the dimethylpyrrolylthiazole of formula (17) is deprotected to give the aminothiazole of formula (19). The dimethylpyrrole is treated with hydroxylamine in acetic acid at a temperature of about 60-100 ° C. for about 4-8 hours, preferably about 6 hours. In step 3, the same conditions as in step 2 are used except that the reaction is continued for about 72 hours to produce thiazoleacetamide of formula (18)

In Scheme 4, Step 4, 2-aminothiazole of formula (19) is converted to 2-bromothiazole of formula (20) using a modified Sandmeyer reaction. Preferred conditions use alkyl nitrite (such as t-butyl nitrite) and copper (II) bromide in acetonitrile at a temperature of about 60-80 ° C.

In Step 5, 2-bromothiazole of formula (20) undergoes a substitution reaction with an amine or morpholine of formula —NR^aR^b to give an aminothiazole of formula (21). The reaction is carried out in an inert solvent (such as methanol, THF, or dioxane) or treated neat with an excess of amine at a temperature of about 70-110 ° C. Alternatively, this reaction is carried out using an amine as a reactant and an excess of triethylamine or an inorganic base (such as cesium carbonate). Various deprotection steps are also envisaged in the synthesis of the aminothiazole of formula (21). For example, if -NR^aR^b possesses additional amine functionality as defined

in Formula I, if necessary or beneficial to carry out the above reaction, tert-butyl ester carbamic acid (BOC) is removed. The selection and use of suitable protecting groups is well known and recognized in the art (see, eg, Protection Groups in Organic Synthesis, Theodora Greene (Wiley-Interscience)).

In Scheme 4, Step 6, the 2-bromothiazole of formula (20) is coupled with a heterocyclic zinc reagent in a Negeshi cross-coupling reaction and is analogous to that described in Scheme 2, Step 1 (20a).) Thiazole heterocycle.

One skilled in the art will recognize that the functionalized thiazole of formula (16) can be prepared by means known in the art. For example, cyclization of thiourea with bromoketones (such as 3-bromo-1,1,1-trifluoropropan-2-one) provides 4-trifluoremethyl-thiazol-2-ylamine. Subsequent bromination and protection of the amine with hexane-2,5-dione gives (16).

Formation of the compound of formula (23) can be carried out according to the reaction shown in Scheme 5. Suitable compounds of formula (23) are those compounds in which R^1 and R^2 are as defined for formula I and R^{4b} is indicated.

An iodopyrazolopyrimidine of formula (5) and for example 4-chloro-2-morpholino-thiazole of formula (22) undergo cross-coupling to produce a pyrazolopyrimidine thiazole of formula (23). For example, these reactants are coupled with a base (such as cesium carbonate) in the presence of copper iodide, palladium acetate, and triphenylphosphine. This coupling reaction is preferably carried out in an inert solvent (such as DMF) at about 100 to 150 ° C. for 4 to 24 hours.

One skilled in the art will recognize that the functionalized thiazole of formula (22) can be prepared by means known in the art. For example, 2-morpholino-thiazole of formula (22) can be obtained by reacting 2,4-dichlorothiazole with morpholine. In addition, 2,5-dichlorothiazole can be all brominated to obtain 2,5-dibromo-4-chlorothiazole. Subsequent bromine-lithium exchange with n-butyllithium and quench with water at -90 ° C. in THF yields 2-bromo-4-chlorothiazole [J. Chem. Soc. Perkin Trans I: Org Bioorg. Chem. (1972-1999), (2): 215-219 (1992)]. 2-Bromo-4-chlorothiazole can also undergo a Negishi cross-coupling reaction with a heterocyclic zinc reagent to give thiazol-2-yltriazole or thiazol-2-ylpyridine.

Formation of the compound of formula (27) can be carried out according to the reaction shown in Scheme 6. Suitable compounds of formula (27) are those wherein R^1 and R^2 are as defined for formula I and "het" is defined as indicated for step 1 or step 3, respectively.

In Scheme 6, Step 1, for example, a heterocyclic thiazole of formula (25) is obtained by reaction of iodopyrazolopyrimidine of formula (5) with bromothiazole of formula (24). This reaction is carried out using palladium catalyst (such as palladium acetate) and tris (2,4-di-tert-butyl-phenyl) -phosphane in the presence of N-butylammonium bromide and base (such as potassium acetate). It is carried out in an active solvent (such as N-methylpyrrolidinone) at a temperature of about 100-150 ° C.

Alternatively, in step 2, the thiazolyl pyrazolopyrimidine of formula (6a) is iodinated to give 2-iodothiazole of formula (26). The thiazole is treated with lithium diisopropylamide for about 1 hour at a temperature of -70 to -80 ° C in an inert solvent (such as THF) and then with N-iodosuccinimide at about the same temperature. This is followed by step 3, in which triazolylthiazole or 4-pyridylthiazole of formula (25) is formed using Negishi cross-coupling conditions similar to those described for Scheme 2, Step 1. .

In Scheme 6, Step 4, the thiazolylpyrazolopyrimidine of formula (25) is brominated to give the bromothiazole of formula (27). This bromination is performed using N-bromosuccinimide in the presence of a small amount of acetic acid in an inert solvent (such as acetonitrile).

The compound of formula (27), where het = 2,5-dimethyl-pyrrol-1-yl, is further converted as described in Scheme 4, Step 2, Step 3, Step 4, and Step 5, A compound can be obtained.

One skilled in the art will recognize that the heterocyclic thiazole of formula (24) can be readily prepared by means known in the art. For example, thiazole-2-carboxylic acid amide can be cyclized with 1,1-dimethoxy-N, N-dimethyl-methanamine to form a triazole, which is then treated with N-methyl-hydrazine to give a triazolyl. Luthiazole is provided, which can then be brominated with N-bromosuccinimide. Literature procedures give 4-thiazol-2-yl-pyridine, which can be brominated to give 4- (5-bromo-thiazol-2-yl) -pyridine. 5-Bromo-2- (2,5-dimethyl-pyrrol-1-yl) -thiazole can be easily obtained by reacting 2-amino-5-bromothiazole with hexane-2,5-dione.

As used herein, "TLC" refers to thin layer chromatography, "HPLC" refers to high performance liquid chromatography, "LC / MS" refers to liquid chromatography / mass spectrometry, and "GC / MS". Refers to gas chromatography / mass spectrometry, "HR-ToF" refers to high-resolution time-of-flight, "APCI" refers to atmospheric pressure chemical ionization, and "δ" refers to the transition from tetramethylsilane to a low magnetic field. ppm, "THF" refers to tetrahydrofuran, "EtOAc" refers to ethyl acetate, "MeOH" refers to methanol, "EtOH" refers to ethanol, and "DMF" refers to dimethylformamide .

Without further elaboration, it is believed that one skilled in the art can fully practice the invention using the foregoing description. The following preparations and examples are provided to further illustrate the present invention. They are intended to illustrate the invention and are not intended to limit the invention in any way. Reagents and starting materials are readily available to those skilled in the art or can be readily synthesized. Examples 1 to 35 provide representative compounds and illustrate their preparation. Examples A-D illustrate various biological tests that can be used to determine the biological properties of the compounds of the present invention. Those skilled in the art will readily recognize appropriate variations from the procedures described in the examples. The names of the compounds of the present invention are those provided by ChemDraw Ultra® version 7.0.1. The salt was named by adding the conjugate acid to the free base.

(Preparation Example 1)
2,5-Dimethyl-4H-pyrazolo [1,5-a] pyrimidin-7-one While maintaining the temperature at 25-28 ° C., ethyl acetoacetate (128 g, 0.98 mol) was replaced with 5-methyl-2H-pyrazole. The solution was added dropwise to an acetic acid solution (500 mL) of -3-ylamine (100 g, 0.95 mol). The mixture was heated at reflux for 10 hours and cooled to room temperature. The reaction was added to tert-butyl methyl ether (5 L) cooled to 5 ° C. keeping the temperature below 10 ° C. Stir at 5 ° C. for 1 hour and filter. The resulting material was dried in vacuo overnight to give a white solid (158 g, 96%).

(Preparation Example 2)
7-chloro-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine 2,5-dimethyl-4H-pyrazolo [1,5-a] pyrimidin-7-one (10.0 g) in toluene (150 mL) , 61.3 mmol) was added N, N-dimethylaniline (9.7 mL, 76.7 mmol). Phosphorus oxychloride (11.2 mL, 122.6 mmol) was added dropwise to the white suspension. Refluxed under an inert atmosphere for 3 hours, cooled to room temperature, and concentrated the reaction to a brown oil using reduced pressure. This oil was dissolved in ethyl acetate (250 mL) and made basic with 1.0 N NaOH. The organic phase was separated and the basic aqueous phase was extracted with ethyl acetate (2 × 100 mL). The organic phases were combined, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to give a brown solid. Use flash chromatography to elute from 80% hexane / 20% (30% THF / hexane) to 0% hexane / 100% (30% THF / hexane) with a step gradient in 20% increments. Was purified to give a pale green solid (6.65 g, 59%). $^{ES / MS}$ m / z (35 Cl) 182.3 (M + 1) +.

(Alternative procedure)
2,5-Dimethylpyrazolo [1,5-a] pyrimidin-7-one (20 g, 122 mmol) was added to 1,4-dioxane (60 mL). The mixture was stirred at 22 ° C. for 10 minutes and then N, N-diethylaniline (20.8 mL, 128 mmol) was added. Stir for an additional 5 minutes and then add phosphorus oxychloride (11.7 mL, 126 mmol) over 15 minutes. The mixture was stirred at 22 ° C. for 15 minutes and then heated to 80-85 ° C. over 35 minutes and the reaction was held at this temperature for 1.5 hours. The cooled reaction mixture was slowly added to an aqueous solution (325 mL) of dipotassium hydrogen phosphate (106.7 g, 612.82 mmol) cooled to 0-5 ° C., keeping the temperature below

5 ° C. during the addition. The mixture was stirred at 22 ° C. and methyl-t-butyl ether (150 mL) was added. The organic layer was separated and the aqueous layer was extracted with methyl-t-butyl ether (2 × 100 mL). The organic portions were combined, dried over sodium sulfate, filtered and the solvent was evaporated. Purification by silica gel chromatography eluting with hexane / ethyl acetate (2/1) gave the title compound as a yellow solid (20.7 g, 88%). ES / MS m / z (^{35}Cl) 182 (M + 1) +.

(Preparation Example 3)
7- (1-Propyl-butyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine
An oven-dried flask equipped with a reflux condenser was charged with anhydrous THF (40 mL), iodine (catalytic amount), magnesium ribbon (1.92 g, 78.9 mmol) and 4-bromoheptane (9.4 mL, 52 mmol). The reaction solution was heated to reflux in an oil bath. The temperature of this reaction solution rose rapidly when the Grignard reaction started. The reaction was stirred at 90 ° C. for a further 4 hours and cooled to room temperature. Magnesium metal is allowed to settle (centrifuged) and the Grignard reagent is added to 7-chloro-2,5-dimethylpyrazolo [1,5-a] pyrimidine (in anhydrous toluene (20 mL) with positive argon pressure (The cannula was transferred to a flask containing 4.80 g, 26 3 mmol). The reaction was refluxed overnight under an inert atmosphere. The reaction was cooled to room temperature and quenched with water. Dilute with ethyl acetate (150 mL) and add water (100 mL) and saturated ammonium chloride (50 mL). The layers were separated and the aqueous phase was extracted with dichloromethane (75 mL). The organic phases were combined, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. Using flash chromatography, the residue produced using 80% hexane / 20% (20% ethyl acetate / hexane) to 0% hexane / 100% (20% ethyl acetate / hexane) with a step gradient in 20% increments. Elution was carried out to obtain yellow crystals (3.06 g, 48%). ES / MS m / z 246.3 (M + 1) $^{+}$.

(Alternative procedure)
A mixture of magnesium shavings (3.5 g, 144 mmol) and catalytic amount of iodine (100 mg) in THF (100 mL) was heated to 65 ° C. under a nitrogen atmosphere. A few drops of neat 4-bromoheptane was added and the mixture was heated until the reaction started. A solution of 4-bromoheptane (17 6 mL, 94.9 mmol) in THF (42 mL) was then added over 2 hours keeping the temperature at 65-70 ° C. The mixture was refluxed for an additional hour and the reaction was cooled to 22 ° C. Prepared Grignard reagent was added to a solution of 7-chloro-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (10.2 g; 53.3 mmol) in THF (60 mL) cooled to 0 ° C. under a nitrogen atmosphere. Added below. The magnesium reagent solution was added using a cannula over 45 minutes keeping the temperature below 10 ° C. The mixture was stirred at 5 ° C. for an additional 30 minutes. To this mixture was added 10% aqueous ammonium chloride (w / w) (125 mL), and the mixture was stirred at 22 ° C. for 30 min. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (2 × 25 mL) The organic layers were combined and dried over sodium sulfate. The mixture was filtered and the solvent was evaporated. The crude product was purified by flash chromatography on silica gel using an eluent of hexane / ethyl acetate (5/1) to give the title compound (8 g, 62%). ES / MS m / z 246 (M + 1) $^{+}$.

(Preparation Example 3a)
7- (1-propyl-butyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (alternative route)
Step 1: 3-Acetyl-3-oxo-4-propyl-heptanoic acid ethyl ester Magnesium chloride (14.63 g, 153.70 mmol) was added to dichloromethane (500 mL), followed by ethyl acetoacetate (19.55 mL, 20.00 g). , 153.79 mmol) were added all at once and stirred at room temperature for 1 hour. The mixture was cooled in an ice-water bath and pyridine (24.86 mL, 24.32 g, 307.39 mmol) was added dropwise. Di-n-propylacetyl chloride (25.00 g, 153.70 mmol) was added dropwise to this white slurry at 0 ° C. under nitrogen. After the addition was complete, the cooling bath was removed, warmed to ambient temperature and stirred for 16 hours. The reaction was quenched with 1N HCl (400 mL) and the bottom layer was separated. The organic portion was dried over magnesium sulfate, filtered and concentrated in vacuo to give a yellow oil (34 g, 86%). This material was used directly in the next step without further purification.

Step 2: 5-propyl-octane-2,4-dione 3-acetyl-3-oxo-4-propyl-heptanoic acid ethyl ester (32.4 g, 126.39 mmol) in dimethyl sulfoxide (100 mL) and water (5 mL) Dissolved in. The solution was heated at 150 ° C. for 6-8 hours or the reaction was followed by GC / MS. The reaction was cooled and extracted with heptane (3 × 100 mL). The combined organic portions were washed with water (100 mL) and brine (100 mL). Concentration in vacuo at 50 ° C. removed most of the heptane. 23.29 g of an oil was obtained and used directly in the next step.

Step 3: 7- (1-propyl-butyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine 5-propyl-octane-2,4-dione (15 g, 81.40 mmol) was added to acetic acid (15 mL).) And cooled in an ice bath. 5-Amino-3-methylpyrazole (7.91 g, 81.40 mmol) was added in portions and stirred at ambient temperature. After 3 hours, the completion of the reaction was checked by GC / MS. GC showed correct regioisomer production by comparison with the reference sample. Excess acetic acid was distilled off. Water (50 mL) was added and extracted with heptane (50 mL). The heptane was washed with brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo to give a crude oil (15.8 g, 79%). ^{1}H NMR ($CDCl_3$): 6.39 (s, 1H); 6.31 (s, 1H); 3.75 (m, 1H); 2.55 (s, 3H); 2.45 (s, 3H); 1.71 (q, 4H); 1.23 (m, 4H); 0.85 (t, 6H).

The following compounds were prepared using either procedure, essentially as described in Preparative Example 3. Grignard reagent was prepared using 3-bromopentane.

(Preparation Example 5)
7- (1-propyl-butyl) -3-iodo-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine 7- (1-propyl-butyl) -2,5-dimethyl-pyrazolo [1,5 -A] Pyrimidine (3.08 g, 12.5 mmol) was dissolved in anhydrous acetonitrile (25 mL), and N-iodosuccinimide (4.2 g, 18.7 mmol) was added 6 times at intervals of 10 minutes (0.70 g each time)) And added. Stir at room temperature over the weekend. Acetonitrile was distilled off and the oil was diluted with dichloromethane (100 mL). Wash with saturated ammonium chloride solution (2 × 50 mL). The organic phase was collected, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure to give a dark red oil. Use flash chromatography to elute from 100% hexane / 0% (20% ethyl acetate / hexane) to 0% hexane / 100% (20% ethyl acetate / hexane) with a step gradient in 50% increments. The oil was purified to give an orange oil (10 97 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): 6.42 (s, 1H), 3.74-3.70 (m, 1H), 2.58 (s, 3H), 2.46 (s, 3H), 1.74-1.68 (m, 4H), 1.28-1.14 (m, 4H), 0.84 (t, J = 7.0 Hz, 6H).

The following compounds were prepared essentially as described in Preparation Example 5.

(Alternative procedure for Preparation 6)
Acetic acid (1 mL) and N-iodosuccinimide (6.7 g, 29.9 mmol) were added to 7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] in acetonitrile (60 mL). To a solution of pyrimidine (6 g, 27.5 mmol) was added in one portion. The mixture was stirred at 22 ° C. for 2 hours. The solvent was evaporated and the residue was taken up in water (50 mL) and methyl-t-butyl ether (100 mL). The organic portion was separated, dried over sodium sulfate, filtered and the solvent was evaporated to give the title compound (9.2 g, 96%). ES / MS m / z 344 (M + 1) $^{+}$.

(Preparation Example 7)
2-Trimethylsilanyl-thiazole In a three-necked flask equipped with a dropping funnel and a thermometer, n-butyllithium (20.4 mL, 51.0 mmol, 2.5 M hexane solution) was mixed with diethyl ether (50 mL). . Cool to −78 ° C. and add dropwise a solution of thiazole (4.25 g, 50.0 mmol) in diethyl ether (50 mL). After the addition was complete, the reaction mixture was stirred at −78 ° C. for 30 minutes and then chlorotrimethylsilane (5.4 g, 50.0 mmol) was added. Stir at −78 ° C. for 1 hour and then warm to room temperature. The reaction was quenched by adding saturated sodium bicarbonate. The aqueous layer was extracted with diethyl ether. The combined organic portions were washed with brine and dried over sodium sulfate. Filtration and concentration under reduced pressure gave a residue. Purification by distillation gave 8.33 g (52-56 ° C./15 mmHg) of the title compound. ^{1}H NMR (400MHz, $CDCl_3$) 8.13 (d, 1H, J = 2.6Hz), 7.54 (d, 1H, J = 2.6Hz), 0.43 (s, 9H).

(Preparation Example 8)
5-Bromo-4-methylthiazole Bromine (9.27 mL, 182 mmol) was added to a solution of 4-methylthiazole (15.0 g, 152 mmol) in acetic acid (30 mL) at 0 ° C. The reaction mixture was slowly warmed to room temperature and stirred overnight. Diluted with dichloromethane and washed with 1N NaOH and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under vacuum. The crude product was purified by silica gel column chromatography eluting with hexane / ethyl acetate (5/1) to give the title compound (9.94 g, 37%). ^{1} H NMR (400 MHz, $CDCl_3$): δ 8.69 (s, 1H), 2.43 (s, 3H).

(Preparation Example 9)
Thiazole-2-carboxylic acid ethyl ester To a mixture of 2-trimethylsilylthiazole (135 g, 858.1 mmol) in toluene (1350 mL) was added a solution of ethyl chloroformate (98.4 mL, 1.03 mol) in toluene (1350 mL). Added over 15 minutes. The reaction was stirred at 22 ° C. for 2 hours. This solution was added onto a 25% (w / w) aqueous solution of sodium carbonate (5 L) and stirred for 30 minutes. The organic layer was separated and the aqueous layer was re-extracted with methylene chloride (2 × 1 L). The organic layers were combined and the solvent was evaporated to give the title compound (134 g, 99%). ES / MS m / z 158 (M + 1) $^+$.

(Preparation Example 10)
Thiazole-2-carboxylic acid amide Thiazole-2-carboxylic acid ethyl ester (150 g, 0.9 mol) was added to a mixture of methanol (75 mL) and 30% aqueous ammonium hydroxide (750 mL) and the mixture was refluxed for 1 hour. Heated. Cool to 22 ° C. and evaporate the methanol under vacuum. The mixture was stirred at room temperature for 30 minutes and the solid was filtered. The isolated solid was dried under vacuum to give the title compound (98 g, 85%). ES / MS m / z 129 (M + 1) $^+$.

(Preparation Example 11)
1-methyl-5-thiazol-2-yl-1H- [1,2,4] triazole 1,1-dimethoxy-N, N-dimethyl-methanamine (240 mL) was cooled to 10 ° C. and thiazole-2-carboxylic acid Acid amide (60 g, 421 mmol) was added in three portions. The mixture was stirred at 10 ° C. for 30 minutes. The mixture was heated to reflux gradually over 45 minutes. The produced methanol was distilled off and the reaction solution was heated at 100 ° C. for 1.5 hours. The mixture was cooled to 60 ° C. and excess 1,1-dimethoxy-N, N-dimethyl-methanamine was removed by vacuum distillation. The residue was cooled to 22 ° C. and hexane (200 mL) was added. This mixture was pulverized for 15 minutes, filtered and the solid was dried to constant weight before being used in the next step.

The solid isolated above (68 g) was added to acetic acid (680 mL) and the mixture was cooled to 10 ° C. N-methyl-hydrazine (27 mL, 509 mmol) was added at a rate to keep the temperature below 15 ° C. The mixture was warmed to 20 ° C. over 30 minutes and then gradually heated to 90 ° C. The mixture was stirred at 90 ° C. for 30 minutes and cooled to 22 ° C. Acetic acid was removed by vacuum distillation. The residue was added to water and adjusted to pH = 8 by adding 25% aqueous sodium hydroxide. The aqueous layer was extracted with methyl-t-butyl ether (3 × 600 mL). The organic layers were combined and the solvent was evaporated. The resulting residue was purified by silica gel chromatography using an eluent of hexane / isopropanol (9/1) to give the title compound (49 g, 70%). ES / MS m / z 167 (M + 1) $^+$

(Preparation Example 12)
5- (5-Bromo-thiazol-2-yl) -1-methyl-1H- [1,2,4] triazole Methyl-5-thiazol-2-yl-1H- [1,2,4] triazole (6 0.55 g; 39.4 mmol) and dimethylformamide (32 mL) were added N-bromosuccinimide (14 g, 78.8 mmol) in three portions over 1 hour. The mixture was stirred at 22 ° C. for 18 hours and then added to water (300 mL) cooled to 0-5 ° C. The aqueous layer was separated and extracted with methyl-t-butyl ether (2 × 200 mL). The organic layers were combined, washed with 7% aqueous sodium hydrogen carbonate solution (100 mL), and dried over sodium sulfate. Filtration and evaporation of the solvent gave the title compound (9.5 g, 93%). $^{ES / MS m / z (79}$ Br / 81 Br) 245/247 (M + 1) +.

(Preparation Example 13)
2,5-Dimethyl-3- [2- (2-methyl-2H- [1,2,4] triazol-3-yl) -thiazol-5-yl] -7- (1-propyl-butyl) -pyrazolo [1,5-a] pyrimidine 5- (5-bromo-thiazol-2-yl) -1-methyl-1H- [1,2,4] triazole (6.5 g, 23.8 mmol) and 2,5- Dimethyl-7- (1-propyl-butyl) -pyrazolo [1,5-a] pyrimidine (6 g, 24.5 mmol) is mixed in N-methylpyrrolidinone (58 mL) and stirred under nitrogen for complete dissolution I let you. Tetra-N-butylammonium bromide (5.47 g, 16.7 mmol) and potassium acetate (11.8 g, 119 mmol) were added and the mixture was heated to 100 ° C. under a nitrogen atmosphere. The hot mixture was degassed by 3 cycles of vacuum / nitrogen purge. Palladium acetate (216 mg, 0.94 mmol) and tris 2,4-di-tert-butyl-phenyl) -phosphane (787 mg, 1.2 mmol) were added. The mixture was heated at 125 ° C. for 4 hours under nitrogen. The mixture was cooled to 22 ° C. and added to water (750 mL). The aqueous layer was extracted with methyl-t-butyl ether (3 × 200 mL), the organic portions were combined and evaporated. The residue was purified by filtration through a silica gel pad eluting with hexane / ethyl acetate (4/1). Fractions containing the product were combined and the solvent was evaporated to give the title compound (7 g, 72%). ES / MS m / z 410 (M + 1) $^+$.

(Preparation Example 14)
2,5-Dimethyl-7- (1-propyl-butyl) -3-thiazol-5-yl-pyrazolo [1,5-a] pyrimidine 2-Trimethylsilanylthiazole (1.1) dissolved in anhydrous THF (30 mL). 765 g, 11.24 mmol) was placed in an oven-dried flask and cooled to −78 ° C. under an inert atmosphere. n-Butyllithium (2.5 M hexane solution, 4.5 mL, 11.24 mmol) was slowly added, and the mixture was stirred at −78 ° C. for 30 minutes. Anhydrous zinc chloride (2.26 g, 16.58 mmol) was added in one portion and stirred at −78 ° C. for 30 minutes. The reaction was warmed to room temperature, stirred for 30 minutes, and 7- (1-propyl-butyl) -3-iodo-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (1.624 g, 5 .18 mmol) and dichloro [1,1'-bis (diphenylphosphino) ferrocene] palladium (II) dichloromethane (0.423 g, 0.518 mmol) were added. Reflux overnight in an oil bath (90 ° C.) under an inert atmosphere. The reaction was cooled to room temperature, quenched with saturated sodium bicarbonate, and diluted with ethyl acetate (150 mL). The layers were separated and the aqueous layer was extracted with ethyl acetate (75 mL). The organic phases were combined, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. This product was generated using flash chromatography eluting with 100% hexane / 0% (30% THF / hexane) to 0% hexane / 100% (30% THF / hexane) with a step gradient in 10% increments. The residue was purified to give a white solid (0.720 g, 42%). ES / MS m / z 329.0 (M + 1) $^+$.

The following compounds were prepared essentially as described in Preparation Example 14.

(Alternative procedure for Preparation 15)
n-Butyllithium (76.5 mL, 191 mmol, 2.5 M hexane solution) was added to a solution of 2-trimethylsilylthiazole (30 g, 191 mmol) in THF (450 mL) at −78 ° C. under nitrogen. The temperature was kept below -74 ° C during the addition. The mixture was stirred at −78 ° C. for 30 minutes, then zinc chloride, dry powder (39.9 g, 286 mmol) was added in one portion and the mixture was warmed to 22 ° C. over 1 hour. 7- (1-Ethyl-propyl) -3-iodo-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (30 g, 87 mmol) and 1,1'-bis (diphenylphosphino) ferrocene palladium (II) Chloride (6.5 g, 8 mmol) was added and the mixture was heated at reflux under nitrogen for 8 hours. The mixture was cooled to 22 ° C. and 10% aqueous ammonium chloride (450 mL) was added. The organic layer was separated and the aqueous layer was washed with methyl-t-butyl ether (2 × 100 mL). The organic portions were combined, dried over sodium sulfate, filtered, and the solvent was evaporated to give the title compound (20.4 g, 78%). ES / MS m / z 301 (M + 1) $^+$.

(Preparation Example 16)
7- (1-Ethyl-propyl) -3- (2-iodo-thiazol-5-yl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine lithium diH-propylamide (150 ml, 62 .4 mmol, 0.6 M THF solution) was prepared as 7- (1-ethyl-propyl) -2,5-dimethyl-3-thiazol-5-yl-pyrazolo [1 in THF (100 mL). , 5-a] pyrimidine (17.8 g, 62.4 mmol) was added at −78 ° C. under a nitrogen atmosphere. The temperature was kept below -74 ° C during the addition. The mixture was stirred at −78 ° C. for 1 hour and then a solution of N-iodosuccinimide (15 g, 63 mmol) in THF (100 mL) was added keeping the temperature below -74 ° C. The reaction was gradually warmed to 22 ° C. and then a 10% aqueous solution of ammonium chloride (300 mL) was added. The organic layer was separated and the aqueous layer was washed with methyl-t-butyl ether (2 × 200 mL). The organic layers were combined, dried over

sodium sulfate, filtered and the solvent was evaporated. The resulting residue was purified by silica gel chromatography eluting with hexane / acetone (5/1) to give the title compound (15 g, 60%). ES / MS m / z 427 (M + 1) $^{+}$.

Example 1

7- (1-Ethyl-propyl) -2,5-dimethyl-3- (4-methyl-thiazol-5-yl) -pyrazolo [1,5-a] pyrimidine Rieke® zinc (in 100 mL THF) 10 g, 13.2 mL, 18.48 mmol) was added to 5-bromo-4-methylthiazole (2.13 g, 18.48 mmol) and heated at reflux for 2 hours. The mixture was cooled to room temperature and the zinc was precipitated by centrifugation Nitrogen gas was blown into 7- (1-ethyl-propyl) -3-iodo-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (900 mg, 2.62 mmol) in anhydrous tetrahydrofuran (10 mL), The organozinc bromide solution was added followed by [1,1'-bis (diphenylphosphino) ferrocene] -dichloropalladium (II) (106 mg, 0.13 mmol). The reaction mixture was stirred at reflux overnight and cooled to room temperature. Ammonium chloride solution was added to the reaction mixture and extracted with dichloromethane. The organic portion was dried over sodium sulfate, filtered and the solvent removed in vacuo. The crude product was purified by silica gel column chromatography eluting with hexane / ethyl acetate (3/1) to give the title compound (552 mg, 79%). ^{1} H NMR (400 MHz, $CDCl_3$): δ 8.80 (s, 1H), 6.48 (s, 1H), 3.63 (m, 1H), 2.57 (s, 3H), 2.43 (S, 3H), 2.40 (s, 3H), 1.85 (m, 4H), 0.90 (t, 6H, J = 7.3 Hz). ES / MS m / z 315 (M + 1) $^{+}$.

(Preparation Example 17)

3- (2,4-Dibromo-thiazol-5-yl) -2,5-dimethyl-7- (1-propyl-butyl) -pyrazolo [1,5-a] pyrimidine 2,5-dimethyl-7- (1-propyl-butyl) -3-thiazol-5-yl-pyrazolo [1,5-a] pyrimidine (3.15 g, 9.59 mmol) was dissolved in acetonitrile (100 mL) and N-bromosuccinimide (4.27 g). , 24.0 mmol) was added in one portion. The mixture was stirred overnight under an inert atmosphere, and the reaction was confirmed to be complete using TLC. Concentrated under reduced pressure and the oil was diluted with dichloromethane (150 mL) and washed with water (75 mL). The organic phase was dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. Step gradient (0-10-15-20-25-30-30) from 100% hexane / 0% (30% THF / hexane) to 0% hexane / 100% (30% THF / hexane) using flash chromatography The resulting oil was purified by eluting with 35-40-45-50-100% 30% THF / hexanes to give yellow crystals (3.70 g, 79%). $^{ES / MS m / z (79}$ Br 81 Br) 486.7 (M + 1) +.

The following compounds were prepared essentially as described in Preparation Example 17 except that dichloromethane was used as the solvent.

* 1.1 equivalents of NBS was used and stirred for 3 days.

Example 4

3- (4-Bromo-2-morpholin-4-yl-thiazol-5-yl) -2,5-dimethyl-7- (1-propyl-butyl) -pyrazolo [1,5-a] pyrimidine

An oven-dried flask was charged with 3- (2,4-dibromothiazol-5-yl) -2,5-dimethyl-7- (1-propyl-butyl) -pyrazolo [1,5-a] pyrimidine (0.973 g, 2.00 mmol), anhydrous dioxane (20 mL), morpholine (0.872 g, 10.0 mmol), and cesium carbonate (1.95 g, 6.00 mmol). Reflux overnight in an oil bath (105 ° C.) under an inert atmosphere. LC / MS was used to confirm that the reaction was complete. Diluted with ethyl acetate (100 mL), washed with water (50 mL), and the aqueous layer was back extracted with ethyl acetate (50 mL). The organic phases were combined, dried over magnesium sulfate, filtered and concentrated under reduced pressure. Using flash chromatography with a step gradient in 10% increments from 100% hexane / 0% (40% dichloromethane / 20% ethyl acetate / 2% 7N ammonia in methanol / 38% hexane) to 0% hexane / 100% (The resulting residue was purified eluting with 40% dichloromethane / 20% ethyl acetate / 2% 7N ammonia in methanol / 38% hexane) to give an off-white solid (0.878 g, 89%). . $^{ES / MS m / z (79}$ Br) 491.7 (M + 1) +.

The following examples were prepared essentially as described in Example 4, using either 2.0 dimethylamine / THF or morpholine as the amine and THF or dioxane as the solvent. The reaction was carried out in a sealed tube or a Schlenk tube.

* 6 equivalents of cesium carbonate were used.

(Example 10)

3- [4-Bromo-2- (2-methyl-2H- [1,2,4] triazol-3-yl) -thiazol-5-yl] -2,5-dimethyl-7- (1-propyl- Butyl) -pyrazolo [1,5-a] pyrimidine

An oven-dried flask was charged with 1-methyl-1,2,4-triazole (0.498, 6.00 mmol) and anhydrous THF (20 mL) and cooled to −78 ° C. under an inert atmosphere. n-Butyllithium (2.5 M hexane solution, 2.4 mL, 6.0 mmol) was slowly added and stirred for 30 minutes. Anhydrous zinc chloride (1.36 g, 10.0 mmol) was added in one portion and stirred at −78 ° C. for 30 minutes. The reaction was warmed to room temperature, stirred for 30 minutes, and 3- (2,4-dibromothiazol-5-yl) -2,5-dimethyl-7- (1-propyl-butyl) -pyrazolo [1, 5-a] pyrimidine (0.973 g, 2.00 mmol) and dichloro [1,1'-bis (diphenylphosphine) ferrocene] palladium (II) dichloromethane (0.163 g, 0.200 mmol) were added. The reaction was refluxed overnight in an oil bath (90 ° C.) under an inert atmosphere. The reaction was cooled to room temperature, quenched with water and diluted with ethyl acetate (100 mL). The layers were separated and the aqueous layer portion was extracted with dichloromethane (50 mL). The organic phases were combined, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. Using flash chromatography with a step gradient in 10% increments from 100% hexane / 0% (10% acetonitrile / 40% THF / 50% hexane) to 0% hexane / 100% (10% acetonitrile / 40% THF / 50 The resulting residue was purified by elution with hexane to give a white solid (0.090 g, 9%). $^{ES / MS m / z (79}$ Br) 487.7 (M + 1) +.

(Alternative preparation example)

2,5-Dimethyl-3- [2- (2-methyl-2H- [1,2,4] triazol-3-yl) -thiazol-5-yl] -7- (1- To a solution of propyl-butyl) -pyrazolo [1,5-a] pyrimidine (5 g, 14.65 mmol), N-bromosuccinimide (2.74 g, 15.4 mmol) was added in one portion and stirred at 22 ° C. for 10 hours. . The solvent was evaporated and the residue was dissolved in a mixture of water (50 mL) and methyl-t-butyl ether (100 mL). The organic layer was separated and the aqueous layer was extracted with additional methyl-t-butyl ether (2 × 50 mL). The organic portions were combined and the solvent was evaporated. The resulting material was purified by filtration through a silica gel pad eluting with hexane / ethyl acetate (3/1). Fractions containing product were combined and the solvent was evaporated. Heptane (25 mL) was added to powder this solid. The solid was filtered and dried under vacuum to give the title compound (5.5 g, 77%). $^{ES / MS m / z (79}$ Br / 81 Br) 488/490 (M + 1) +.

Example 10s

3- [4-Bromo-2- (2-methyl-2H- [1,2,4] triazol-3-yl) -thiazol-5-yl] -2,5-dimethyl-7- (1-propyl- Butyl) -pyrazolo [1,5-a] pyrimidine, hydrochloride

3- [4-Bromo-2- (2-methyl-2H- [1,2,4] triazol-3-yl) -thiazol-5-yl] -2,5-dimethyl-7- (1-propyl- Butyl) -pyrazolo [1,5-a] pyrimidine (750 mg, 1.54 mmol) was dissolved in acetone (5 mL) and 1M HCl diethyl ether solution (1.84 mL, 1.84 mmol) was added. The mixture was stirred at room temperature for 3 hours and concentrated in vacuo. The residue was dissolved in diethyl ether / hexane = 1/1 (5 mL) to crystallize the desired HCl salt (526 mg, 65%). $^{ES / MS m / z (81}$ Br) 490 (M + 1) +; 1 H-NMR (CDCl 3): 8.20 (s, 1H), 6.82 (s, 1H), 4.21 (s, 3H), 3.64 (m, 1H), 2.49 (m, 3H), 2.44 (s, 3H), 1.75 (m, 4H), 1.96 (m, 4H), 0.81 (M, 6H).

The following compounds were prepared essentially as described in Example 10.

* 0.5M zinc chloride in THF was used instead of anhydrous zinc chloride. Stir at 80 ° C. for 3 days.

(Example 12)

3- (4-Bromo-2- (2-methyl-2H- [1,2,4] triazol-3-yl) -thiazol-5-yl) -7- (1-ethyl-propyl) -2,5 -Dimethyl-pyrazolo [1,5-a] pyrimidine

Under a nitrogen atmosphere, n-butyllithium (2.5 M hexane solution, 0.6 mL, 1.5 mmol) was added to 1-methyl-1,2,4-triazole (124.5 mg, 1.5 mmol) in THF (3 mL).) At - 78

° C. and stirred for 30 minutes. Anhydrous zinc chloride (409 mg, 3.0 mmol) was added and stirring was continued for 30 minutes, warmed to room temperature and stirred for 2 hours. Add 3- (2,4-dibromo-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (229 mg, 0.5 mmol) Then tetrakis (triphenylphosphine) palladium (58 mg, 0.05 mmol) was added and refluxed overnight. Cool to room temperature, dilute with ethyl acetate and wash with saturated ammonium chloride. The organic portion was dried over sodium sulfate, filtered and concentrated to give a residue. The crude product was purified by flash chromatography eluting with hexane: ethyl acetate (10: 2.5) to give the title compound as a yellow foam (77 mg). $^{MS\ (APCI)\ m/z\ (}$ 79 Br) 460.4 (M + 1) +.

(Alternative preparation from Example 21)
Acetic acid (1 mL) and N-bromosuccinimide (4.1 g, 22 mmol) were added to 7- (1-ethyl-propyl) -2,5-dimethyl-3- [2- (2-methyl-) in acetonitrile (80 mL). 2H- [1,2,4] triazol-3-yl) -thiazol-5-yl] -pyrazolo [1,5-a] pyrimidine (8 g, 21 mmol) was added. The mixture was stirred at 22 ° C. for 2 hours. The solvent was evaporated and water (50 mL) and methyl-t-butyl ether (100 mL) were added to the resulting residue. The organic portion was separated, dried over sodium sulfate, filtered and the solvent was evaporated. The resulting residue was recrystallized from isopropyl alcohol to give the title compound (8.7 g, 90%). $^{ES\ /\ MS\ (m/z)}$:(79 Br / 81 Br) 460/462 (M + 1) +.

Example 12a
3- (4-Bromo-2- (2-methyl-2H- [1,2,4] triazol-3-yl) -thiazol-5-yl) -7- (1-ethyl-propyl) -2,5 -Dimethyl-pyrazolo [1,5-a] pyrimidine, p-toluenesulfonic acid 3- (4-bromo-2- (2-methyl-2H- [1,2,4] triazol-3-yl) -thiazole- 5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (50 mg, 0.109 mmol) was dissolved in acetone (3 mL). A 0.25M aqueous solution of p-toluenesulfonic acid (434.4 μL, 0.109 mmol) was added and the resulting mixture was evaporated to dryness. Ethyl acetate (12 mL) was added to partially dissolve this solid. Methanol (1 mL) was added to give a clear solution. The solution was concentrated by gentle evaporation until crystals were observed. The crystals were isolated by filtration and dried under vacuum at 25 ° C. to give 50 mg of the title compound.

The stoichiometry of this salt was determined by ion chromatography using the following HPLC conditions: Column: Phenomenex Phenosphere SAX at 30 ° C., 4.6 × 150 mm; Mobile phase: 50% acetonitrile / 50% 0.025M phosphorus Sodium acid buffer (pH = 4.5); flow rate = 1.5 mL / min; detection: UV at 205 nm; injection volume = 5 μL; measurement time = 3 minutes. Calculated theoretical amount: 27.2% tosylate; Found: 28.4% tosylate (average of 3 HPLC measurements).

(Example 13)
(4-Chloro-5- [2,5-dimethyl-7- (1-propyl-butyl) -pyrazolo [1,5-a] pyrimidin-3-yl] -thiazol-2-yl) -dimethylamine oven dried In a prepared flask was (4-bromo-5- [2,5-dimethyl-7- (1-propyl-butyl) -pyrazolo [1,5-a] pyrimidin-3-yl] -thiazol-2-yl-dimethylamine. (0.20, 0.44 mmol) and anhydrous THF (3.0 mL) were charged and cooled to −78 ° C. under inert atmosphere. n-Butyllithium (1.6M hexane solution, 0.42 mL, 0.67 mmol) was added slowly and stirred for 30 minutes. N-chlorosuccinimide (0.120 g, 0.889 mmol) was added in one portion and stirred at −78 ° C. for 30 minutes. The reaction was warmed to room temperature and stirred for 5 hours and the progress was checked using LC / MS. Dilute with ethyl acetate (100 mL), wash with saturated ammonium chloride (50 mL), and back-extract the aqueous layer with dichloromethane (50 mL). The organic phases were combined, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. This was generated using flash chromatography eluting with 100% hexane / 0% (25% THF / hexane) to 0% hexane / 100% (25% THF / hexane) with a step gradient in 10% increments. The residue was purified to give a white solid (0.087 g, 48%). $^{ES\ /\ MS\ m/z\ (35}$ Cl) 406.0 (M + 1) +.

The following examples were prepared essentially as described in Example 13, using the appropriate bromothiazole prepared above.

(Preparation Example 18)
A mixture of thiazolidine-2,4-dione (50 g, 0.43 mol) in 2,4-dichlorothiazole phosphorus oxychloride (240 mL) was cooled to 5 ° C. and pyridine (34 mL, 0.43 mol) was added over 15 minutes. added. The mixture was heated to 125 ° C. for 4 hours and cooled to 22 ° C. Excess phosphorus oxychloride was removed by vacuum distillation and the residue was added to water (1 L) cooled to 5 ° C. This mixture was extracted with methylene chloride (3 × 400 mL). The organic portions were combined and the solvent was evaporated to give the title compound (50 g, 76%). $^{ES\ /\ MS\ m/z\ (35}$ Cl 35 Cl / 35 Cl 37 Cl / 37 Cl 37 Cl) 153/155/157 (M + 1) +

(Preparation Example 19)
4-Chloro-2-morpholino-thiazole To a mixture of 2,4-dichlorothiazole (34 g, 0.22 mol) in acetonitrile (425 mL) was added potassium carbonate (60.9 g, 0.44 mol), followed by morpholine (21. 2 mL, 0.225 mol) was added dropwise over 30 minutes. The mixture was refluxed at 40 ° C. and then cooled to 22 ° C. The mixture was filtered and the filtrate was evaporated. The residue was triturated with i-propyl alcohol (60 mL) at 22 ° C. for 1 hour. The solid was filtered and dried under vacuum to constant weight to give the title compound (34.5 g, 76%). $^{ES\ /\ MS\ m/z\ (35}$ Cl) 205 (M + 1) +.

(Example 16)
3- (4-Chloro-2-morpholin-4-yl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine
Under a nitrogen atmosphere, 3- (4-bromo-2-morpholin-4-yl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a Pyrimidine (116 mg, 0.25 mmol) was dissolved in THF (1.5 mL) and cooled to -78 ° C. n-Butyllithium (0.1 mL, 2.5 M hexane solution, 0.25 mmol) was added, and the mixture was stirred at −78 ° C. for 30 minutes. N-chlorosuccinimide (33.4 mg, 0.25 mmol) was added, stirred for an additional 30 minutes and slowly warmed to room temperature. After stirring overnight, the reaction was quenched by adding a solution of saturated ammonium chloride and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated to give a residue. The crude product was purified by flash chromatography eluting with hexane: dichloromethane: ethyl acetate (5: 5: 2) to give the title compound (54 mg). $^{MS\ (APCI)\ m/z\ (}$ 35 Cl) 420.6 (M + 1) +; 1 H NMR (400MHz, CDCl 3): 6.44 (s, 1H), 3.79 (t, 4H, J = 4. 8Hz), 3.63-3.56 (m, 1H), 3.47 (t, 4H, J = 4.8 Hz), 2.55 (s, 3H), 2.45 (s, 3H), 1 .88-1.75 (m, 4H), 0.87 (t, 6H, J = 7.5 Hz).

(Alternative preparation example from Preparation Example 6)
7- (1-Ethyl-propyl) -3-iodo-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine, (9 g, 26.2 mmol) and 4-chloro-2-morpholino-thiazole (7. 5 g, 36.7 mmol) was mixed in dimethylformamide (90 mL) that had previously been degassed with nitrogen. Cesium carbonate (17.8 g, 55 mmol), copper iodide (250 mg, 1.31 mmol), triphenylphosphine (550 mg, 2.09 mmol) and palladium acetate (117 mg, 0.52 mmol) were added. The mixture was heated to 125 ° C. for 16 hours and cooled to 22 ° C. Water (900 mL) was added and extracted with methyl-t-butyl ether (3 × 200 mL). The organic portions were combined and the solvent was evaporated. Purification by silica gel chromatography eluting with hexane / ethyl acetate (4/1) gave the title compound (6.4 g, 62%). $^{ES\ /\ MS\ m/z\ (35}$ Cl) 420 (M + 1) +.

Example 16a
3- (4-Chloro-2-morpholin-4-yl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine, hydrochloride 3- (4-Chloro-2-morpholin-4-yl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (1. 40 g, 3.33 mmol) was dissolved in acetone (10 mL) at 50 ° C. and cooled to room temperature. Hydrogen chloride (2M diethyl ether solution, 2.0 mL, 4.0 mmol) was added and stirred well in the sonicator. The solution was concentrated slightly and a minimum amount of diethyl ether was added to crystallize the HCl salt. The mixture was cooled in the refrigerator overnight. Additional hydrogen chloride (2M diethyl ether solution, 2.0 mL, 4.0 mmol) was added and cooled in the refrigerator. The crystalline material was filtered and dried to give the title compound (1.15 g, 75%). $^{ES\ /\ MS\ m/z\ (35}$ Cl) 420 (M + 1) +; 1 H NMR (CDCl 3): 9.16 (br, 1H), 6.86 (s, 1H), 3.72 (m, 4H) , 3.49 (m, 1H), 3.39 (m, 4H), 2.48 (s, 3H), 2.38 (s, 3H), 1 .79 (m, 4H), 0.79 (m, 6H).

(Example 17)
3- (4-Bromo-2-butyl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine n- Butyl lithium (2.5 M hexane solution, 0.2 mL, 0.5 mmol) was added to 3-

(2,4-dibromo-thiazol-5-yl) -7- (1-ethyl-propyl) in THF (3 mL). To a solution of -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (230 mg, 0.5 mmol) was added at −78 ° C. After 30 minutes, 1-iodobutane (138 mg, 0.75 mmol) was added and stirring was continued for 1 hour. Warmed to room temperature and stirred for 1 hour. The reaction was quenched by adding saturated ammonium chloride solution and extracted with ethyl acetate. The organic portion was dried over sodium sulfate, filtered and concentrated to give a residue. The crude product was purified by flash chromatography eluting with hexane / ethyl acetate (10 / 1.5) to give the title compound as an orange foam (78 mg). $^{ES / MS}$ m / z (^{81} Br) 437.4 (M + 1) +.

The following examples were prepared essentially as described in Example 17.

(Example 19)
7- (1-Ethyl-propyl) -3- (2-methoxymethyl-thiazol-5-yl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine Under nitrogen atmosphere, n-butyllithium (2.5M hexane solution, 0.4 mL, 1.0 mmol) was added to 7- (1-ethyl-propyl) -2,5-dimethyl-3-thiazol-5-yl-pyrazolo [1, in THF (3 mL). To a solution of 5-a] pyrimidine (300 mg, 1.0 mmol) was added at -78 ° C. Stir for 30 minutes and add 1-iodomethyl methyl ether (205 mg, 1.2 mmol). Stirring was continued for 1 hour, then slowly cooled to room temperature and stirred overnight. The reaction was quenched by adding saturated ammonium chloride solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated in vacuo to give a residue. The crude product was purified by flash chromatography eluting with hexane / ethyl acetate (10/2) to give the title compound as a yellow foam (184 mg). MS (APCI) m / z 345.3 (M + 1) $^{+}$.

(Example 20)
3- (4-Chloro-2-methoxymethyl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine n-butyllithium (2 0.5M hexane solution, 174 mL, 0.43 mmol) was added to 7- (1-ethyl-propyl) -3- (2-methoxymethyl-thiazol-5-yl) -2,5-dimethyl- in THF (3 mL). To a stirred solution of pyrazolo [1,5-a] pyrimidine (150 mg, 0.43 mmol) was added at −78 ° C. Stir for 30 minutes and add N-chlorosuccinimide (87 mg, 0.653 mmol). Stir for 30 minutes, then warm the reaction slowly to room temperature and allow the reaction to continue overnight. The reaction was quenched by adding saturated ammonium chloride solution, extracted with ethyl acetate, dried over sodium sulfate, filtered and concentrated to give a residue. Purification by flash chromatography eluting with hexane / ethyl acetate (10/2) gave the title compound (7 mg). MS (APCI) m / z (< ^{35} > Cl) 379.3 (M + 1) $^{+}$.

(Example 21)
7- (1-Ethyl-propyl) -2,5-dimethyl-3- [2- (2-methyl-2H- [1,2,4] triazol-3-yl) -thiazol-5-yl) -pyrazolo [1,5-a] pyrimidine 3- (4-bromo-2- (2-methyl-2H- [1,2,4] triazol-3-yl) -thiazol-5-yl) -7- (1- Ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (175 mg, 0 38 mmol) and copper (I) chloride (132 mg, 1.33 mmol) were mixed in DMF (5 mL), Heated at 120 ° C. for 24 hours. Cool to room temperature, dilute with ethyl acetate and wash with brine. The organic layer was dried over sodium sulfate, filtered and concentrated under vacuum to a residue. Purification by flash chromatography eluting with hexane then hexane / EtOAc (10 / 1.8) gave a yellow to orange solid (45 mg). ES / MS m / z 382.0 (M + 1) $^{+}$.

(Alternative preparation example)
n-Butyllithium (2.5M hexane solution, 57.6 mL, 144 mmol) was added to a solution of N-methylimiazole (11.95 g, 144 mmol) in THF (600 mL) at −78 ° C. under a nitrogen atmosphere. The temperature was kept below -74 ° C during the addition. Zinc chloride dry powder (26 g, 192 mmol) was added in one portion and the mixture was warmed to 22 ° C. over 1 hour. 7- (1-Ethyl-propyl) -3- (2-iodo-thiazol-5-yl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (12.5 g, 29 mmol) and tetrakis (tri Phenyl) phosphine palladium catalyst (1.15 g, 0.01 mol) was added in one portion and the mixture was heated at reflux under nitrogen for 8 hours. The mixture was cooled to 22 ° C. and water (300 mL) was added. The organic layer was separated and the aqueous layer was extracted with methyl-t-butyl ether (2 × 200 mL). The organic portions were combined, dried over sodium sulfate, filtered and the solvent was evaporated. Purification using hexane / ethyl acetate (4/1) eluting through a silica gel pad afforded the title compound (8 g, 72%). ES / MS m / z 382 (M + 1) $^{+}$.

(Example 22)
3- (4-Chloro-2- (2-methyl-2H- [1,2,4] triazol-3-yl) -thiazol-5-yl) -7- (1-ethyl-propyl) -2,5 -Dimethyl-pyrazolo [1,5-a] pyrimidine 7- (1-ethyl-propyl) -2,5-dimethyl-3- [in dichloromethane (0.5 mL) and acetonitrile (0.5 mL) in a vial. 2- (2-Methyl-2H- [1,2,4] triazol-3-yl) -thiazol-5-yl) -pyrazolo [1,5-a] pyrimidine (20 mg, 0.052 mmol) and N-chlore A mixture of succinimide (7.6 mg, 0.0569 mmol) was stirred at room temperature overnight. Concentration gave a residue. Purification by flash chromatography eluting with hexane then hexane / ethyl acetate (10 / 1.5) gave the title compound (16 mg). $^{ES / MS}$ m / z (^{35} Cl) 416.0 (M + 1) +.

(Example 23)
7- (1-Ethyl-propyl) -3- (4-methoxy-2-morpholin-4-yl-thiazol-5-yl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine Sealed 3- (4-Bromo-2-morpholin-4-yl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl- in methanol (3 mL) in a 4 mL vial A mixture of pyrazolo [1,5-a] pyrimidine (162 mg, 0.35 mmol), sodium methoxide (57 mg, 1.05 mmol) and copper (I) iodide (67 mg, 0.35 mmol) was stirred at 120 ° C. for 15 hours. did. Cool to room temperature, remove the solid by filtration, and concentrate the filtrate in vacuo. The residue was purified by flash chromatography eluting with hexane / THF (10/2). The product was recrystallized from methanol to give the title compound (20 mg). ES / MS m / z 416.0 (M + 1) $^{+}$.

(Preparation Example 20)
4-Bromo-5- [7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl] -thiazole-2-carbaldehyde under nitrogen atmosphere, n -Butyllithium (1.6 M in hexane, 0.312 mL, 0.50 mmol) was added to 3- (2,4-dibromo-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl -A solution of pyrazolo [1,5-a] pyrimidine (230 mg, 0.50 mmol) in THF (2.5 mL) was added at -78 ° C and stirred for 30 minutes. A solution of N-formylmorpholine (58 mg, 0.50 mmol) in THF (0.5 mL) was added. Stir for 1 hour and store the reaction at −20 ° C. overnight. The reaction was warmed to room temperature, diluted with ether and quenched by the addition of 4N HCl (4 mL). The layers were separated and the organic phase was extracted with 4N HCl (2 × 4 mL). The aqueous layers were combined, treated with solid sodium bicarbonate to pH = 8-9 and extracted with diethyl ether. All organic layers were combined, washed with brine, dried over sodium sulfate, filtered and concentrated to a residue. The crude product was purified by flash chromatography eluting with hexane / dichloromethane / ethyl acetate (5/5/1) to give the title compound (154 mg). $^{MS (APCI)}$ m / z (81 Br) 409.0 (M + 1) +.

(Example 24)
3- (4-Bromo-2-morpholin-4-yl-methyl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine 4 -Bromo-5- [7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl] -thiazole-2-carbaldehyde (150 mg, 0.368 mmol) , Morpholine (35 mg, 0.405 mmol) and sodium triacetoxyborohydride (97 mg, 0 46 mmol) were mixed in dichloromethane (3 mL) and methanol (0.5 mL). Stir overnight and add additional morpholine (35 mg, 0.405 mmol) and sodium triacetoxyborohydride (97 mg, 0.46 mmol) and stir for an additional 4 hours. The solvent was removed under vacuum, diluted with dichloromethane and washed with brine. The organic phase was dried over sodium sulfate, filtered and concentrated under vacuum. The resulting material was purified using flash chromatography eluting with dichloromethane: 2M ammonia in methanol (10: 1) to give a mixture. The mixture was purified using a reverse phase column eluting with water / acetonitrile (80/20) to water / acetonitrile (10/90) to give the title compound (20 mg). $^{ES / MS}$ m / z (^{81} Br) 460.0 (M + 1) +.

(Example 25)
3- (4-Bromo-2-pyridin-4-yl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine under nitrogen atmosphere N-butyllithium (1.6M hexane solution, 0.312 mL, 0.5 mmol) was added to 3- (2,4-dibromo-thiazol-5-yl) -7- (1- Ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (229 mg, 0.5 mmol) was added at −78 ° C. After stirring for 30 minutes, anhydrous zinc chloride (264 mg, 1.5 mmol) was added and stirring was continued for 30 minutes. The reaction was warmed to room temperature

and stirred for 1 hour. 4-Iodopyridine (103 mg, 0.5 mmol) was added, followed by 1,1'-bis (diphenylphosphino) ferrocenepalladium (II) chloride (dichloromethane adduct) (0 40.8 mg, 0.05 mmol) It was. The reaction was heated at reflux overnight. Cool to room temperature, dilute with ethyl acetate and wash with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated to give a residue. The crude product was purified by flash chromatography eluting with 2N ammonia in dichloromethane: methanol (10: 0.75) to give a mixture. The mixture was purified using a reverse phase column eluting with water: acetonitrile = 80: 20 to water: acetonitrile = 10: 90 to give the title compound (19 mg). $^{ES / MS\ m / z\ (^{79}Br)}$ 456.0 (M + 1) +.

(Preparation Example 21)
4-trifluoromethyl-thiazol-2-ylamine thioures (4.0 g, 52.3 mmol) and 3-bromo-1,1,1-trifluoropropan-2-one (5.5 mL, 10 g, 52.3 mmol)) Was added to ethanol (100 mL) and heated at 50 ° C. for 2 hours. Cool to room temperature and concentrate to dryness. This residue was dissolved in water and the pH was adjusted to greater than 12 using 2M NaOH. Extracted with diethyl ether (4x). The combined organic extracts were dried over sodium sulfate, filtered and concentrated under vacuum. The resulting material was purified by silica gel chromatography (CH_2Cl_2) to give the title compound (6.9 g, 79%). ES / MS m / z 169 (M + 1) $^{+}$.

(Preparation Example 22)
5-Bromo-4-trifluoromethyl-thiazol-2-ylamine, hydrobromide Bromine (2.0 mL, 6.28 g, 39.3 mmol) was added to 4-trifluoromethyl- in diethyl ether (60 mL). Thiazol-2-ylamine (6.0 g, 35.7 mmol) was added dropwise to the solution cooled in an ice bath. Stir for 1 hour after the end of the addition and then warm to room temperature. The solid was collected by filtration and washed with diethyl ether to give the title compound (10.5 g, 90%). $^{ES / MS\ m / z\ (^{79}Br\ /\ ^{81}Br)}$ 247/249 (M + 1) +.

(Preparation Example 23)
5-Bromo-2- (2,5-dimethyl-pyrrol-1-yl) -4-trifluoromethyl-thiazole hexane-2,5-dione (3.5 mL, 3.4 g, 30.2 mmol) was added to methanol. To a solution of 5-bromo-4-trifluoromethyl-thiazol-2-ylamine hydrobromide (9.0 g, 27.4 mmol) in (60 mL). Stir overnight at room temperature. Phosphate buffer (50 mL, pH = 7) was added. The resulting precipitate was collected by filtration and washed with water. The filter cake was dissolved in CH_2Cl_2 and dried over sodium sulfate. Filtration and concentration in vacuo gave the title compound (8 2 g, 92%). ^{1} H NMR (400 MHz, $CDCl_3$) δ 5.91 (s, 2H), 2.27 (s, 6H).

(Example 26)
3- [2- (2,5-Dimethyl-pyrrol-1-yl) -4-trifluoromethyl-thiazol-5-yl] -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine
A solution of 5-bromo-2- (2,5-dimethyl-pyrrol-1-yl) -4-trifluoromethyl-thiazole (2.2 g, 6.6 mmol) in THF (25 mL) was placed in a dry ice bath. Cooled down. t-Butyllithium (1.7 M pentane solution, 8.5 mL, 14.5 mmol) was added dropwise. Stir for 45 minutes, then add zinc chloride (0.5 M in THF, 14.6 mL, 7.3 mmol) dropwise. Stir for 5 minutes and remove the cooling bath. Stir for 30 minutes, then 7- (1-ethyl-propyl) -3-iodo-2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (1.5 g, 4.4 mmol) and bis (tri-t-Butylphosphine) palladium (0) (450 mg, 0.9 mmol) was added. Refluxed for 24 hours. The reaction was cooled and the mixture was poured into diethyl ether and washed with water (2 ×). The combined aqueous layer was extracted with diethyl ether. The combined organic portions were dried over sodium sulfate, filtered and concentrated under vacuum until dryness. The resulting residue was purified by silica gel chromatography (75-100% CH_2Cl_2 in hexanes) to give the title compound (1.47 g, 72%). $_{HR\text{-}ToF\text{-}MS\ m / z}$ C 23 H 26 F 3 N 5 S + H calcd for $^{+}$ 462.1939, found: 462.1915.

(Example 27)
5- [7- (1-Ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl] -4-trifluoromethyl-thiazol-2-ylamine hydroxylamine (2 mL, 50% aqueous solution) in 3- [2- (2,5-dimethyl-pyrrol-1-yl) -4-trifluoromethyl-thiazol-5-yl] -7- (1-ethyl) in acetic acid (10 mL). -Propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (1.1 g, 2.3 mmol) was added. The reaction was heated to 80 ° C. for 6 hours. Cooled to room temperature. Pour into diethyl ether and wash once with 2M NaOH (2 ×) then water. The organic portion was dried over sodium sulfate, filtered and concentrated under vacuum until dryness. The resulting residue was purified by silica gel chromatography (40% ethyl acetate in hexane) to give the title compound (0.76 g, 87%). ES / MS m / z 384 (M + 1) $^{+}$.

(Preparation Example 24)
3- (2-Bromo-4-trifluoromethyl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine in acetonitrile (20 mL) Of copper (II) bromide (540 mg, 2.4 mmol) and t-butyl nitrite (0.36 mL, 310 mg, 3.0 mmol) were heated to 60 ° C. 5- [7- (1-Ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl] -4-trifluoromethyl-thiazol-2-ylamine (755 mg, 2. 0 mmol) was added as a solid. The reaction was heated to 80 ° C. for 2 hours. The reaction was cooled, poured into diethyl ether and extracted with water (3x). The organic layer was dried over sodium sulfate, filtered and concentrated in vacuo until dryness. The resulting residue was purified by silica gel chromatography with CH_2Cl_2 to give the title compound (0.77 g, 87%). $^{ES / MS\ m / z\ (^{79}Br\ /\ ^{81}Br)}$ 447,449 (M + 1) +.

(Example 28)
{5- [7- (1-Ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl] -4-trifluoromethyl-thiazol-2-yl} -dimethyl- Amine 3- (2-Bromo-4-trifluoromethyl-thiazol-5-yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a in methanol (4 mL) The sealed reaction tube containing pyrimidine (313 mg, 0.7 mmol) and dimethylamine (2M THF solution, 4 mL, 8 mmol) was heated to 80 ° C. for 2 hours. The reaction was cooled and concentrated under vacuum. The residue produced by silica gel chromatography eluting with CH_2Cl_2 containing 0-30% ethyl acetate was purified to give the title compound (0.28 g, 97%). $_{HR\text{-}ToF\text{-}MS\ m / z}$ C 19 H 24 F 3 N 5 calcd 412.1770 for S + $^{H+}$, Found: 412.1783.

The following examples were prepared essentially as described for Example 28 except that the reaction was refluxed for 8 hours.

(Example 30)
N- {5- [7- (1-Ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl] -4-trifluoromethyl-thiazol-2-yl}- Acetamide hydroxylamine (50% aqueous solution, 5 mL) was added 3- [2- (2,5-dimethyl-pyrrol-1-yl) -4-trifluoromethyl-thiazol-5-yl]-in acetic acid (25 mL). 7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (2.4 g, 5.2 mmol) was added to the solution The reaction was heated to 80 ° C. for 72 hours. The reaction was cooled to room temperature, poured into diethyl ether, washed once with 2M NaOH (2 ×) and then water. The organic portion was dried over sodium sulfate, filtered and concentrated to dryness. The resulting residue was purified by column chromatography eluting with 40% ethyl acetate in hexanes to give the title compound (0.28 g, 13%). $_{HR\text{-}ToF\text{-}MS\ m / z}$ C 19 H 22 F 3 N 5 OS + H calcd for $^{+}$ 426.1575, Found: 426.1565.

(Example 31)
{2- {5- [7- (1-Ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl] -4-trifluoromethyl-thiazol-2-ylamino} -Ethyl) -carbamic acid tert-butyl ester
(2-Amino-ethyl) -carbamic acid tert-butyl ester (0.5 mL, 506 mg, 3.2 mmol) was added to 3- (2-bromo-4-trifluoromethyl-thiazole-5- 5 in methanol (1 mL). Yl) -7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidine (100 mg, 0.22 mmol) and triethylamine (0.3 mL, 222 mg, 2.2 mmol) Added to Methanol was evaporated and heated to 80 ° C. overnight. The reaction was cooled to room temperature and concentrated under vacuum. The residue produced by column chromatography eluting with CH_2Cl_2 containing 10-50% ethyl acetate was purified to give the title compound (0.11 g, 97%). ES / MS m / z 527.2 (M + 1) $^{+}$

The following compounds were prepared essentially as described in Example 31, using the appropriate amine.

* Heated at reflux overnight in EtOH. EtOH was evaporated and heated at 110 ° C. for 24 hours.

** Heated at 80 ° C. overnight in EtOH.

*** Prepared HCl salt and recrystallized from EtOAc / hexane.

(Example 33)

N 1 - {5- [7- (1- ethyl-propyl) - 2,5-dimethyl - pyrazolo [1,5-a] pyrimidin-3-yl] -4-trifluoromethyl - thiazol-2-yl} -Ethane-1,2-diamine, hydrochloride {2- {5- [7- (1-ethyl-propyl) -2,5-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl] -4 -Trifluoromethyl-thiazol-2-ylamino) -ethyl) -carbamic acid tert-butyl ester (101 mg, 0.192 mmol) was added to 1 M HCl in methanol (1 mL). The reaction was heated at 70 ° C. overnight. The reaction was cooled and concentrated in vacuo from methanol / ethyl acetate. The resulting residue was triturated with ethyl acetate to give the title compound (64 mg, 78%). ES / MS m / z 427.0 (M + 1) <4> .

The following examples were prepared essentially as described for Example 33.

(Example A)

In Vivo Efficacy Assessment Using in Vitro Binding In order to assess in vivo efficacy, the compounds of the invention were evaluated using in vitro binding. D. R. The compounds were administered to rats by the oral route using the procedure provided in Gehert et al., EJP 509. 145-153 (2005). The binding of ^{125} I-sorbazine to the cerebellum was evaluated in vitro as described by Gehert et al. For example, Example 15 resulted in 65% inhibition at 10 mg / kg.

(Example B)

CRF1 filter binding method The limitation of plasmid-based human CRF1 expression in generating recombinant cell lines with sufficient receptor density to develop binding studies is the Phoenix retrovirus expression system licensed from Stanford. Overcame with Membranes were prepared using the stable HEK-hCRF1 cell line and the binding reaction (200 μL) was set up as follows: 50 μL ^{125} I-sorbazine (0.2 nM final concentration), 50 μL compound and 100 μL. CRF1 membrane (25 μg / reaction). The reaction was incubated at room temperature for 2 hours and stopped by filtering through pretreated FB Millipore glass fiber filter plates (96 wells). The plate was washed twice with ice-cold test buffer (50 mM Tris, 12.5 mM NaCl, 1 mM EDTA, 10 mM $MgCl_2$, 0.05% BSA, pH 7.2), air dried overnight, and in a MicroBeta counter. Counting was performed using 100 μL Microscint40. Non-specific binding (NSB) was measured in the presence of 0.5 μM unlabeled sorvazine. Usually three measurements were taken and the average data points were plotted by Graph Pad Prism.

Using this test, the exemplified compounds of the invention inhibited the binding of ^{125} I-sorbazine (4 nM) with a Ki (inhibition constant) of less than 1 μM in roller / adherent cells. For example, Example 15 showed a Ki of 6.2 nM.

(Example C)

CRF2 filter binding method The limitation of plasmid-based human CRF2 expression in generating recombinant cell lines with sufficient receptor density to develop binding studies is the Phoenix retrovirus expression system licensed from Stanford. Overcame with Membranes were prepared using a stable HEK-hCRF2 cell line and the binding reaction (200 μL) was set up as follows: 50 μL ^{125} I-sorbazine (0.2 nM final concentration), 50 μL compound and 100 μL. CRF2 membrane (25 μg / reaction). The reaction was incubated at room temperature for 2 hours and stopped by filtering through pretreated FB Millipore glass fiber filter plates (96 wells). The plate was washed twice with ice-cold test buffer (50 mM Tris, 12.5 mM NaCl, 1 mM EDTA, 10 mM $MgCl_2$, 0.05% BSA, pH 7.2), air dried overnight, and in a MicroBeta counter. Counting was performed using 100 μL Microscint40. Non-specific binding (NSB) was measured in the presence of 0.5 μM unlabeled sorvazine. Alternatively, compounds were evaluated using a scintillation proximity assay. The test procedure was set up as follows: 50 μL of ^{125} I-sorbazine (0.2 nM final concentration), 50 μL of compound or unlabeled sorvazine (NSB), and 250 μg of wheat germ agglutinin (WGA) SPA beads and 100 μL containing CRF2 membrane (1.5 μg / reaction). Plates were incubated for 4-5 hours at room temperature and centrifuged at 200 xg for 10 minutes. Bound radioactivity was assessed using a Wallac Trilux scintillation counter. Usually, three measurements were taken to assess binding and the average data points were plotted by Graph Pad Prism. Compounds were screened at the initially fixed concentration and when sufficient activity was observed, a concentration-response curve was subsequently generated.

Particularly exemplified compounds of the invention were tested in the CRF2 binding test and showed weak affinity for the CRF2 receptor. For example, Example 15 showed 11% inhibition at a concentration of 50 μM. This result suggests that the compounds of the invention are selective for the CRF1 receptor (rather than CRF2).

(Example D)

Bioavailability and pharmacokinetic properties The volume of distribution (Vdist) relates the amount of drug in the body to the drug concentration in blood or plasma. The volume of distribution refers to the volume of fluid that would be required to contain the entire amount of drug in the body at the same concentration as in blood or plasma: Vdist = amount of drug in the body / drug in blood or plasma Concentration (Goodman and Gilliman literature). For a 10 mg dose and a plasma concentration of 10 mg / L, the distribution volume would be 1 liter. The volume of distribution reflects the extent to which the drug is present in extravascular tissue. A large volume of distribution reflects that certain compounds tend to bind to tissue components rather than plasma protein binding. In the clinical setting, Vdist can be used to determine the loading dose to achieve a steady state concentration.

To test for volume of distribution, male Sprague Dawley rats (N = 3) were dosed with a single intravenous dose of 1 mg / kg. Multiple plasma samples were collected at 0.08-24 hours after administration. The plasma sample was analyzed by LC / MS / MS to determine plasma concentration. Pharmacokinetic calculations in plasma were performed to determine pharmacokinetic parameters including Vdist and plasma clearance (Clp).

The compounds of the present invention preferably have a suitable bioavailability profile. For example, most commercially available CNS drugs and cardiovascular drugs exhibit a Vdist of less than 10 L / Kg for humans. Compared to CRF antagonists, CP154526 (Schulz et al., Proc. Natl. Acad. Sci. (USA), 93: 10477 (1996)) and NBI30775 (Chen et al., Drug Development Research, 65: 216 (2005)) When analyzed separately, the rat Vdist of 114 L / Kg and 76 L / Kg, respectively, is shown. Example 15 of the present invention, when analyzed separately, shows only 7.2 L / Kg rat Vdist after a single intravenous dose of 1 mg / kg.

In addition, plasma clearance (CLp) is a measure of the rate of drug removal from the body. According to intravenous administration and first order rate equations, plasma clearance can be determined using the following formula: CLp = dose / AUC, where AUC is plasma as a function of time from zero to infinity. The total area under the curve representing the drug concentration in the medium). The referenced CRF antagonists CP154526 and NBI27587, when analyzed separately, show rat clearance (CLp) of 83 mL / min / kg and 306 mL / min / kg, respectively, after a single intravenous administration, whereas Example 15 shows only 23.6 mL / min / kg of rat CLp when analyzed separately.

Patent Citations (10)

Publication number	Priority date	Publication date	Assignee	Title
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JP2000302693A *	1999-04-02	2000-10-31	Pfizer Prod Inc	Corticotropin releasing factor antagonist for preventing sudden death
JP2002513382A *	1996-07-24	2002-05-08	デュポン ファーマシューティカルズ カンパニー	Azolotriazines and azolopyrimidines
WO2005063755A1 *	2003-12-22	2005-07-14	Sb Pharmco Puerto Rico Inc	Crf receptor antagonists and methods relating thereto
Family To Family Citations				
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JP3621706B2	1996-08-28	2005-02-16	ファイザー・インク	Substituted 6,5-hetero-bicyclic derivatives
DE60032249T2 *	1999-09-30	2007-07-19	Neurogen Corp., Branford	Amino-substituted pyrazolo [1,5-a] -1,5-pyrimidines and pyrazolo [1,5-a] -1,3,5-triazines
DEP20053608B *	2001-03-13	2005-09-12	Squibb Bristol Myers Co	4-(2-Butylamino)-2,7-Dimethyl-8-(Methyl-6-Methoxypyrid-3-Yl) Pyrazolo-[1,5-A]-1,3,5-Triazine, Its Enantiomers and Pharmaceutically Acceptable Salts as Corticotropin Releasing Factor Receptor Ligands

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Non-Patent Citations (1)

Title
JPN6012062715; CHEN, C. et al.:'Design of 2,5-dimethyl-3-(6-dimethyl-4-methylpyridin-3-yl)-7-dipropylaminopyrazolo[1,5-a]pyrimidine' J. Med. Chem. Vol. 47, No. 19, 2004, p. 4787-4798*

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CN109134481B *	2018-08-07	2021-05-14	中山大学	Substituted pyrrole chromone compound or pharmaceutically acceptable salt thereof, and preparation method and application thereof
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GR1010096B *	2020-07-02	2021-10-08	Uni-Pharma Κλεων Τσετης Φαρμακευτικα Εργαστηρια Αβεε,	Pyrazolo[1,5-a]pyrimidines as autotaxin inhibitors
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Priority And Related Applications

Priority Applications (3)

Application	Priority date	Filing date	Title
US82626406P	2006-09-20	2006-09-20	*US Provisional Application*
US60/826,264		2006-09-20	
PCT/US2007/078605	2006-09-20	2007-09-17	Thiazole pyrazolopyrimidines as crf1 receptor antagonists

Legal Events

Date	Code	Title	Description
2010-09-10	A521	Written amendment	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20100909
2010-09-10	A621	Written request for application examination	**Free format text**: JAPANESE INTERMEDIATE CODE: A621 **Effective date**: 20100909
2012-11-27	TRDD	Decision of grant or rejection written	
2012-12-05	A01	Written decision to grant a patent or to grant a registration (utility model)	**Free format text**: JAPANESE INTERMEDIATE CODE: A01 **Effective date**: 20121204
2012-12-20	A61	First payment of annual fees (during grant procedure)	**Free format text**: JAPANESE INTERMEDIATE CODE: A61 **Effective date**: 20121213
2012-12-21	R150	Certificate of patent or registration of utility model	**Ref document number**: 5161226 **Country of ref document**: JP **Free format text**: JAPANESE INTERMEDIATE CODE: R150

			Free format text: JAPANESE INTERMEDIATE CODE: R150
2012-12-27	FPAY	Renewal fee payment (event date is renewal date of database)	**Free format text:** PAYMENT UNTIL: 20121221 **Year of fee payment:** 3
2015-12-08	R250	Receipt of annual fees	**Free format text:** JAPANESE INTERMEDIATE CODE: R250
2016-12-06	R250	Receipt of annual fees	**Free format text:** JAPANESE INTERMEDIATE CODE: R250
2017-12-12	R250	Receipt of annual fees	**Free format text:** JAPANESE INTERMEDIATE CODE: R250
2018-12-18	R250	Receipt of annual fees	**Free format text:** JAPANESE INTERMEDIATE CODE: R250
2019-12-17	R250	Receipt of annual fees	**Free format text:** JAPANESE INTERMEDIATE CODE: R250
2020-12-09	R250	Receipt of annual fees	**Free format text:** JAPANESE INTERMEDIATE CODE: R250

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Name	Image	Sections	Count	Query match
receptor antagonist		title,abstract,description	4	0.000
CRHR1		title,description	33	0.000
CRHR1		title,description	33	0.000
compounds		claims,abstract,description	147	0.000
Metabolic Syndrome		claims,abstract,description	10	0.000
pharmaceutical composition		claims,abstract,description	8	0.000
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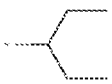
sodium chloride		claims,description	44	0.000
salts		claims,description	42	0.000
pentan-3-yl group		claims,description	12	0.000
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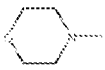
morpholin-4-yl group		claims,description	10	0.000
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Anxiety		claims,description	7	0.000
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Chronic fatigue syndrome		claims,description	7	0.000
Hypersensitivity		claims,description	7	0.000
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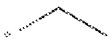			
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Congenital Adrenal Hyperplasia	claims,description	6	0.000
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Epilepsy	claims,description	6	0.000
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Infertility	claims,description	6	0.000
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Migraine Disorders	claims,description	6	0.000
Premature baby	claims,description	6	0.000
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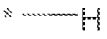			
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	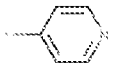			
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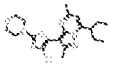
tildacerfont		claims,description	3	0.000
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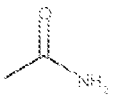
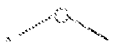
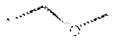
acetamide		claims,description	2	0.000
methoxy group		claims,description	2	0.000
methoxymethyl group		claims,description	2	0.000
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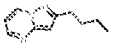
N1=CC=CN2N=C(CCCC)C=C21		claims	1	0.000
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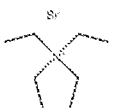
Tetraethylammonium bromide		claims	1	0.000
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disease		abstract,description	9	0.000
Nervous System Disease		abstract,description	6	0.000
neuroendocrine		abstract,description	3	0.000

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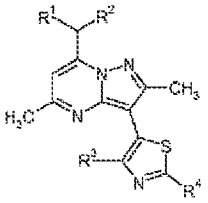
(71)出願人　594197872
イーライ　リリー　アンド　カンパニー
アメリカ合衆国　インディアナ州　４６２８５　インディアナポリス　リリー　コーポレイト　センター　（番地なし）
(74)代理人　100068526
弁理士　田村　恭生
(74)代理人　100100158
弁理士　鮫島　睦
(74)代理人　100138900
弁理士　新田　昌宏
(74)代理人　100076521
弁理士　坪井　有四郎

最終頁に続く

(54)【発明の名称】ＣＲＦ１受容体アンタゴニストとしてのチアゾールピラゾロピリミジン

(57)【要約】
本発明は、式Ｉの化合物、その医薬組成物、ならびに精神障害および神経内分泌系障害、神経系疾患およびメタボリックシンドロームの治療における副腎皮質刺激ホルモン放出因子１（ＣＲＦ１）受容体アンタゴニストとしてのその使用に関する。



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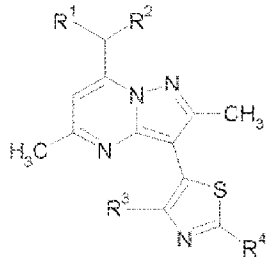
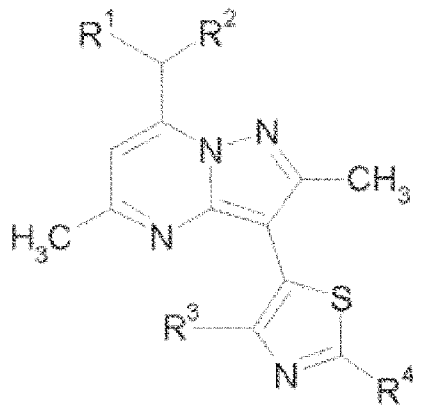


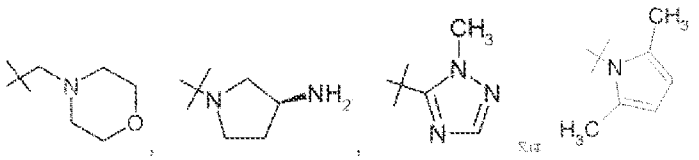
式Ｉ

【特許請求の範囲】
【請求項1】
式Ｉの化合物
【化1】



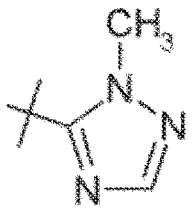
式Ｉ

（式中、
R^1 およびR^2 は独立に、エチルまたはｎ－プロピルであり、
R^3 は水素、Ｃｌ、Ｂｒ、メチル、トリフルオロメチルまたはメトキシであり、
R^4 は水素、Ｂｒ、$R^a R^b N$－、メトキシメチル、ｎ－ブチル、アセトアミド、ピリジン－４－イル、モルホリン－４－イル、
【化2】



であり、
R^a およびR^b は独立に、水素、C_1－C_3 アルキル、$H_2NCH_2CH_2$－、$(CH_3)_3COC(O)NHCH_2CH_2$－、または$CH_3CH_2CH_2NHCH_2CH_2$－である）
またはその薬理学的に許容できる塩。
【請求項2】
R^3 がＣｌ、Ｂｒ、メチルまたはトリフルオロメチルである、請求項１に記載の化合物またはその薬理学的に許容できる塩。
【請求項3】
R^3 がＣｌまたはＢｒである、請求項１または請求項２に記載の化合物またはその薬理学的に許容できる塩。
【請求項4】
R^4 が$R^a R^b N$－、ピリジン－４－イル、モルホリン－４－イル、または

【化3】

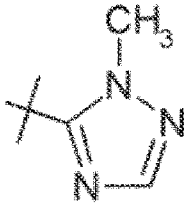


である、請求項１から請求項３のいずれか１項に記載の化合物またはその薬理学的に許容できる塩。

【請求項5】

R^4 がモルホリン－４－イルまたは

【化4】



である、請求項１から請求項４のいずれか１項に記載の化合物またはその薬理学的に許容できる塩。

【請求項6】

R^4 がR^a R^b Ｎ－であり、R^a およびR^b が独立にC_1－C_3 アルキルである、請求項１から請求項４のいずれか１項に記載の化合物またはその薬理学的に許容できる塩。

【請求項7】

３－［４－ブロモ－２－（２－メチル－２Ｈ－［１，２，４］トリアゾール－３－イル）－チアゾール－５－イル］－２，５－ジメチル－７－（１－プロピル－ブチル）－ピラゾロ［１，５－ａ］ピリミジンである、請求項１に記載の化合物またはその薬理学的に許容できる塩。

【請求項8】

３－（４－ブロモ－２－（２－メチル－２Ｈ－［１，２，４］トリアゾール－３－イル）－チアゾール－５－イル）－７－（１－エチル－プロピル）－２，５－ジメチル－ピラゾロ［１，５－ａ］ピリミジンである、請求項１に記載の化合物またはその薬理学的に許容できる塩。

【請求項9】

３－（４－クロロ－２－モルホリン－４－イル－チアゾール－５－イル）－７－（１－エチル－プロピル）－２，５－ジメチル－ピラゾロ［１，５－ａ］ピリミジンである、請求項１に記載の化合物またはその薬理学的に許容できる塩。

【請求項10】

請求項１から請求項９のいずれか１項に記載の化合物またはその薬理学的に許容できる塩と、薬理学的に許容できる賦形剤とを含む、医薬組成物。

【請求項11】

患者のうつ病、不安症、アルコール濫用または薬物濫用、肥満、高血圧、メタボリックシンドローム、過敏性大腸症候群、癲癇、発作、睡眠障害、アレルギー、片頭痛、月経前症候群、不妊性、性的機能不全、先天性副腎過形成、クッシング病、早産、ストレスにより誘発される胃潰瘍、炎症性障害、下垂体または異所性下垂体由来の腫瘍、慢性疲労症候群、線維筋痛、内臓痛または多発性硬化症を治療する方法であって、それを必要とする患者に治療上有効量の請求項１から請求項９のいずれか１項に記載の化合物またはその薬理

学的に許容できる塩を投与することを含む、方法。
【請求項12】
患者の不安症またはうつ病を治療する方法であって、それを必要とする患者に治療上有効量の請求項１から請求項９のいずれか１項に記載の化合物またはその薬理学的に許容できる塩を投与することを含む、方法。
【請求項13】
患者のアルコール濫用または薬物濫用を治療する方法であって、それを必要とする患者に治療上有効量の請求項１から請求項９のいずれか１項に記載の化合物またはその薬理学的に許容できる塩を投与することを含む、方法。
【請求項14】
治療における使用のための、請求項１から請求項９のいずれか１項に記載の化合物またはその薬理学的に許容できる塩。
【請求項15】
うつ病、不安症、アルコール濫用または薬物濫用、肥満、高血圧、メタボリックシンドローム、過敏性大腸症候群、癲癇、発作、睡眠障害、アレルギー、片頭痛、月経前症候群、不妊性、性的機能不全、先天性副腎過形成、クッシング病、早産、ストレスにより誘発される胃潰瘍、炎症性障害、下垂体または異所性下垂体由来の腫瘍、慢性疲労症候群、線維筋痛、内臓痛または多発性硬化症の治療において使用するための、請求項１から請求項９のいずれか１項に記載の化合物またはその薬理学的に許容できる塩。
【請求項16】
不安症またはうつ病の治療において使用するための、請求項１から請求項９のいずれか１項に記載の化合物またはその薬理学的に許容できる塩。
【請求項17】
アルコール濫用または薬物濫用の治療において使用するための、請求項１から請求項９のいずれか１項に記載の化合物またはその薬理学的に許容できる塩。

【発明の詳細な説明】
【技術分野】
【0001】
本発明は、精神障害および神経内分泌系障害、神経系疾患およびメタボリックシンドロームの治療におけるＣＲＦ１受容体アンタゴニストとしての新規なチアゾールピラゾロピリミジン化合物、その医薬組成物およびそれらの使用に関する。
【背景技術】
【0002】
副腎皮質刺激ホルモン放出因子（ＣＲＦ）は、脳下垂体前葉からのプロオピオメラノコルチン（ＰＯＭＣ）由来のペプチド分泌の主要な生理的調節因子である４１アミノ酸のペプチドである。下垂体でのその内分泌の役割に加えて、ＣＲＦの免疫組織化学的局在から、このホルモンが中枢神経系の幅広い視床下部外分布を有し、脳での神経伝達物質または神経修飾物質としての役割と整合した広い範囲の自律神経的効果、電子生理的効果および行為への効果を生じることが証明された。免疫系における生理的、心理的、および免疫学的ストレス因子に対する反応を統合する面でＣＲＦが重要な役割を果たすという証拠もある。
【0003】
ＣＲＦは、うつ病および神経不安症を含めた精神障害および神経系疾患、ならびにアルツハイマー病、ハンチントン病、進行性核上麻痺、筋萎縮性側索硬化症、パーキンソン病、癲癇、片頭痛、アルコール濫用および薬物濫用および関連する禁断症状、肥満、メタボリックシンドローム、先天性副腎過形成、クッシング病、高血圧、発作、過敏性大腸症候群、ストレスにより誘発される胃潰瘍、月経前症候群、性的機能不全、早産、炎症性障害、アレルギー、多発性硬化症、内臓痛、睡眠障害、下垂体部腫瘍または異所性下垂体由来の腫瘍、慢性疲労症候群および線維筋痛に関連付けられてきた。

【0004】
ＣＲＦ受容体亜型（ＣＲＦ１およびＣＲＦ２）が確認されており、これらは脳内に不均一に分布しており、これは機能的多様性の可能性を示唆している。例えば、広く分布した脳ＣＲＦ１受容体は、環境ストレス因子への曝露に付随する情動性に強く関係する。重要なことは、ＣＲＦ２受容体ではなくＣＲＦ１受容体が、不安惹起様行動の選択を媒介するように見えることである。より離散的な中隔／視床下部分布および別の内在性リガンドの入手可能性は、ＣＲＦ２受容体の異なる機能的な役割を示唆する。例えば、ＣＲＦ１受容体よりもＣＲＦ２受容体に対して優先的な親和性を有する新規なＣＲＦ族の神経ペプチドが、選択的なＣＲＦ１アゴニズムで観察される行動活性化の外観を生成することのなく食欲を抑制することが報告されている。他の場合には、ＣＲＦ２アゴニズムは、ＣＲＦ１アンタゴニストまたはＣＲＦ１遺伝子欠失について報告されている効果に類似の効果をもたらす。例えば、ＣＲＦ２アゴニストが抗肥満薬として提唱されてきたが、ＣＲＦ１アンタゴニストも同様に肥満に対する重要な治療法であるかも知れない。
【0005】
ＣＲＦアンタゴニストとして有用な特定のピロロ［２，３－ｄ］ピリミジン、ピロロ［３，２－ｄ］ピリミジン、ピラゾロ［１，５－ａ］ピリミジン、１，２，３－トリアゾロ［４，５－ｂ］ピリジン、およびピラゾロ［１，５－ａ］－１，３，５－トリアジンが特許文献１、特許文献２、特許文献３、および特許文献４に記載されている。

【国際調査報告】

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/078605

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D487/04 A61K31/505 A61P29/00 A61P25/22 A61P25/24
A61P25/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	GEHLERT ET AL: "3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-b]pyridazine: a novel brain-penetrant, orally available corticotropin-releasing factor receptor 1 antagonist with efficacy in animal models of alcoholism." THE JOURNAL OF NEUROSCIENCE, vol. 27, no. 10, 7 March 2007 (2007-03-07), pages 2718-2726, XP002465426 figure 1; table 1	1-17
Y	WO 01/23388 A (NEUROGEN CORP [US]; PFIZER [US]; DARROW JAMES W [US]; LOMBAERT STEPHAN) 5 April 2001 (2001-04-05) page 69; example 95 page 13, line 3	1-17

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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 January 2008	31/01/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gettins, Marc

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/078605

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/072202 A (BRISTOL MYERS SQUIBB PHARMA CO [US]) 19 September 2002 (2002-09-19) claims 1,16	1-17
A	HE LIQI ET AL: "4-(1,3-Dimethoxyprop-2-ylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)pyrazolo[1,5-a]-1,3,5-triazine: A Potent, Orally Bioavailable CRF1 Receptor Antagonist" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 43, 2000, pages 449-456, XP002196777 ISSN: 0022-2623 table 1	1-17
A	WO 2005/063755 A (SB PHARMCO INC [US]; NEUROCRINE BIOSCIENCES INC [US]; LUO ZHIYONG [US]) 14 July 2005 (2005-07-14) claims 1,24	1-17
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/078605

Box No. II Observations where certain claims were found unsearchable (Continuation of item 3 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 11-13 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2007/078605

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INTERNATIONAL SEARCH REPORT

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International application No
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Ａ６１Ｐ	3/04	(2006.01)	Ａ６１Ｐ	25/36	
Ａ６１Ｐ	9/12	(2006.01)	Ａ６１Ｐ	3/04	
Ａ６１Ｐ	1/00	(2006.01)	Ａ６１Ｐ	9/12	
Ａ６１Ｐ	25/08	(2006.01)	Ａ６１Ｐ	1/00	
Ａ６１Ｐ	25/20	(2006.01)	Ａ６１Ｐ	25/08	
Ａ６１Ｐ	37/08	(2006.01)	Ａ６１Ｐ	25/20	
Ａ６１Ｐ	25/06	(2006.01)	Ａ６１Ｐ	37/08	
Ａ６１Ｐ	25/00	(2006.01)	Ａ６１Ｐ	25/06	
Ａ６１Ｐ	15/08	(2006.01)	Ａ６１Ｐ	25/00	
Ａ６１Ｐ	15/10	(2006.01)	Ａ６１Ｐ	15/08	
Ａ６１Ｐ	13/12	(2006.01)	Ａ６１Ｐ	15/10	
Ａ６１Ｐ	15/06	(2006.01)	Ａ６１Ｐ	13/12	
Ａ６１Ｐ	1/04	(2006.01)	Ａ６１Ｐ	15/06	
Ａ６１Ｐ	29/00	(2006.01)	Ａ６１Ｐ	1/04	
Ａ６１Ｐ	35/00	(2006.01)	Ａ６１Ｐ	29/00	
Ａ６１Ｐ	21/00	(2006.01)	Ａ６１Ｐ	35/00	
			Ａ６１Ｐ	21/00	

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(72)発明者　チャオゲン・チェン
アメリカ合衆国４６０３２インディアナ州カーメル、タイタン・ラン１０５３２番
(72)発明者　チャフィク・アムドウチ・アムドウチ
アメリカ合衆国４６０３２インディアナ州カーメル、ツイン・オークス・ドライブ１４４６９番
(72)発明者　エリック・ジェイムズ・ヘンプル
アメリカ合衆国４６２０２インディアナ州インディアナポリス、ノース・ニュー・ジャージー・ストリート１３３２番
(72)発明者　フィリップ・アーサー・ヒップスカインド
アメリカ合衆国４６１６３インディアナ州ニュー・パレスタイン、キャビン・コート４２５５番
(72)発明者　ジェイソン・ケネス・マイヤーズ
アメリカ合衆国４６２５６インディアナ州インディアナポリス、スプリングウォーター・サークル８０２１番
(72)発明者　高▲桑▼　貴子
アメリカ合衆国４６２３７インディアナ州インディアナポリス、ジャーマンダー・レイン７６５３番
(72)発明者　ジェイムズ・リー・トス
アメリカ合衆国４６１４８インディアナ州ナイツタウン、サウス６２５ウエスト４６４２番

Ｆターム(参考)　4C050 AA01 BB05 CC08 DD10 EE03 FF02 GG01 HH04
4C086 AA01 AA02 AA03 CB06 MA01 MA04 NA14 ZA02 ZA05 ZA06
ZA08 ZA12 ZA42 ZA66 ZA68 ZA70 ZA81 ZA94 ZB11 ZB13
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(71) Applicant *(for all designated States except US)*: **VELACOR THERAPEUTICS PTY LTD** [AU/AU]; 205-211 Grattan Street, Parkville, Victoria 3052 (AU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **HOVENS, Christopher** [AU/AU]; 86 Warrigal Road, Surrey Hills, Victoria 3127 (AU). **COSTELLO, Anthony** [AU/AU]; Apartment 141, 85 Rouse Street, Port Melbourne, Victoria 3207 (AU). **CORCORAN, Niall** [IE/AU]; 13 Sturt Street, Flemington, Victoria 3031 (AU).

(74) Agents: **MORRIS, Kathryn, B** et al.; Davies Collison Cave, 1 Nicholson Street, Melbourne, Victoria 3000 (AU).

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(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2007/109853 A1

(54) Title: INORGANIC SELENIUM FOR TREATMENT OF BENIGN TUMORS

(57) Abstract: The invention relates to the use of selenate or its pharmaceutically acceptable salts, in nutritional or supranutritional amounts, in methods and compositions for inhibiting the growth of benign tumor cells and treating or preventing benign tumors, particularly benign tumors in which the Akt signaling pathway is activated or in which Bcl 2 levels are elevated. Methods of preventing malignant conversion of benign tumors and benign tumor cells to malignant tumors or malignant tumor cells is also described.

"INORGANIC SELENIUM FOR TREATMENT OF BENIGN TUMORS"

FIELD OF THE INVENTION

[0001] This invention relates generally to the use of selenate or its pharmaceutically acceptable salts, especially in supranutritional amounts, in methods and compositions for inhibiting the growth or proliferation of benign tumor cells. The present invention also relates to the use of selenate or a pharmaceutically acceptable salt thereof in combination with at least one other therapy for treating or preventing benign tumors or for inhibiting the growth or proliferation of benign tumor cells. In certain embodiments, the methods of the invention are useful for treating or preventing benign tumors, especially benign tumors in which the Akt signaling pathway is activated or benign tumors in which Bcl-2 levels are elevated. Additionally, the present invention relates to the use of selenate or a pharmaceutically acceptable salt thereof for preventing malignant conversion of benign tumor cells or benign tumors to malignant tumor cells or malignant tumors.

BACKGROUND OF THE INVENTION

[0002] Although many benign tumors are asymptomatic and harmless, some benign tumors occur in positions that cause symptoms such as pain and in the brain or spine symptoms such as loss of mobility, loss of sight etc. Some benign tumors may cause clinical symptoms that are bothersome to a patient, for example, lower urinary tract symptoms (LUTS) resulting from benign prostatic hyperplasia. Some benign tumors may grow to a size that causes damage to tissues, organs or nerves in its vicinity, whilst others may cause serious illness or death if they occur near vital organs or press against vital organs. Some 13,000 deaths per year in the US are attributed to benign tumors and many more benign tumors require treatment. Furthermore, some benign tumors are known to have a high propensity to undergo malignant conversion to produce malignant or cancerous tumors.

[0003] There has been much interest in the use of selenium compounds as cancer-preventive agents. Studies spanning the last 20 years have documented their cancer-preventive action in tumors of the mammary gland (Ip, C. 1981, *Cancer Res.* 41:4386-4390), colon (Reddy *et al.* 1981, *Cancer Res.* 47:5901-5904), lung and prostate (Clark *et al.* 1996, *Jama* 276:1957-1963). Both phase II and III clinical trials for prostate cancer prevention using an organic selenomethionine are currently underway (Nelson *et al.* 1999, *Semin. Urol. Oncol.*, 17(2):91-6).

[0004] Selenium compounds used in chemoprevention studies can broadly be classified into inorganic and organic selenium forms. The typical form of inorganic selenium, sodium selenite, (Na_2SeO_3) is relatively toxic, causing single- and double-strand break DNA

damage, whilst the typical organic selenium entity, selenomethionine (SeMet) is relatively non-toxic and non-DNA-damaging (Lu *et al.* 1995, *Biochem. Pharmacol.* 50(2):213-9; Sinha *et al.* 1996, *Cancer Lett.* 107(2):277-84; Stewart *et al.* 1999, *Free Radic. Biol. Med.* 26(1-2):42-8).

[0005] Organic selenomethionine, the major constituent of dietary selenium, is incorporated into cellular selenoproteins, such as thioredoxin reductase and glutathione peroxidase *via* a series of complex intermediates (Allan *et al.* 1999, *Annu. Rev. Nutr.* 19:1-16). Since these selenocysteine-containing selenoenzymes, such as the antioxidant glutathionine peroxidase and the redox-regulatory thioredoxins, are involved in cellular responses to mutagenic oxidative stress, it has long been proposed that supranutritional levels of selenium intake boosts anticarcinogenic cellular activity by promoting a cellular reducing environment (Allan *et al.* 1999, *supra*).

[0006] It is, however, becoming increasingly clear that the nature of the anti-tumorigenic action of selenium compounds is dependent on the chemical form in which the element is administered (Ip, C. 1998, *J. Nutr.* 128:1845-1854). Organic selenium in the form of methylseleninic acid induces G1 arrest, as well as DNA fragmentation and caspase mediated cleavage of PARP, which are two markers of apoptosis, in DU145 prostate cancer cells (Jiang *et al.* 2001, *Cancer Res.* 61:3062-3070).

[0007] In contrast, selenite induces S-phase arrest and apoptotic DNA fragmentation, which is independent of caspase function (Jiang *et al.* 2002, *Mol. Cancer Ther.* 1:1059-1066). Differences have also been reported for the inorganic selenium compounds, with selenite and selenate inhibiting lymphocyte growth *via* different mechanisms (Spyrou *et al.* 1996, *Cancer Res.* 56:4407-4412). These differences may be explained in part by variations in metabolism and bioavailability between *in vitro* cell culture and *in vivo* studies (Dong *et al.* 2003, *Cancer Res.* 63:52-59).

[0008] The role of the PI3K/Akt pathway in uncontrolled cell proliferation is increasingly recognized. Akt is a serine/threonine kinase that is activated in response to membrane receptor stimulated PI3K phosphorylation. Akt, in turn, regulates the activity of several proteins involved in the control of apoptosis, including the Forkhead (FKHR) transcription factors, Bad, Caspase 9, GSK-3β and mTOR (Vivanco, I. and Sawyers, C.L. 2002, *Nat. Rev. Cancer* 2:289-501). Akt overactivity may occur in benign tumors resulting in lack of apoptosis. Furthermore, higher than normal levels of Bcl-2 proteins may occur in benign tumors, including those that may be susceptible to malignant conversion.

[0009] Radiotherapy resistance in tumor cells has also been linked with upregulation of the PI3K/Akt pathway (Soderlund *et al.* 2005, *Int. J. Oncol.*, 26:25-32; Zhan *et al.* 2004, *Histol. Histopathol.*, 19:915-923; Tanno *et al.* 2004, *Cancer Res.*, 64:3486-3490; Li *et*

al. 2004, *Oncogene*, 23:4594-4602; McKenna *et al.*, 2003, *Genes Chromosomes Cancer*, 28:330-338; Liang *et al.* 2003, *Mol. Cancer Ther.* 2:353-360).

[0010] Interest in the clinical use of selenium-containing compounds as a chemopreventive agent has become widespread following the publication of Clark *et al.* (1996, *supra*). These results have spurred a number of human clinical trials using supranutritional selenomethionine as a chemopreventive agent for prostate cancer (Meuillet *et al.* 2004, *J. Cell Biochem.* 91:443-458).

[0011] However, it has been noted that there are marked differences in tumor progression *in vivo*, depending upon the selenium compound administered. For instance, inorganic sodium selenate significantly reduces tumor progression in comparison to organic selenomethionine, methylselenocysteine or selenized yeast (Corcoran *et al.* 2004, *J. Urol.*, 171:907-910). In addition, certain selenium compounds, such as sodium selenite have been found to be toxic to cells when used at certain doses (Jiang *et al.* 2002, *supra*).

SUMMARY OF THE INVENTION

[0012] The present invention is predicated in part on the discovery that a specific type of inorganic selenium compound, namely selenate, significantly inhibits benign tumor cell proliferation, especially when used at high or supranutritional amounts, as compared to other selenium compounds. It has also been found that selenate and its pharmaceutically acceptable salts have an inhibitory effect on benign tumor cells, especially those in which the Akt signaling pathway is activated or those in which Bcl-2 activity is elevated, and have a strong synergistic inhibitory effect on benign tumor cell growth when used in combination with at least one of a cytostatic agent, a cytotoxic agent and a radiotherapy that is optionally administered with a radiosensitizing agent. Further, it has been found that selenate and its pharmaceutically acceptable salts have an inhibitory effect on malignant conversion of benign tumor cells to malignant tumor cells.

[0013] Accordingly, in one aspect, the present invention provides methods for inhibiting the growth or proliferation of benign tumor cells. These methods generally comprise exposing the benign tumor cells to an effective amount of selenate or a pharmaceutically acceptable salt thereof. In some embodiments, the benign tumor cells are tumor cells in which the Akt-signaling pathway is activated. The activation of the Akt signaling pathway involves activation of at least one member selected from Akt, mTOR, GSK-3β and FKHR. In illustrative examples of this type, the activation of the Akt signaling pathway involves phosphorylation of Akt (e.g., phosphorylation of the Thr 308 and Ser 473 residues of Akt). In other embodiments, the activation of the Akt signaling pathway involves inactivation of PTEN. In some embodiments, the Akt signaling pathway is over-activated. In some embodiments, the benign tumor cells have an abnormally increased Bcl-2 activity. In some embodiments, the amount of selenate or its pharmaceutically acceptable salt, to which the tumor cells are exposed, is a nutritional amount. In other embodiments, the amount is a supranutritional amount. Suitably, the tumor cells are selected from benign adenoma tumor cells, angiofibroma tumor cells, hemangioma tumor cells, leiomyoma tumor cells (fibroid), benign chorangioma tumor cells, cystadenoma tumor cells, dermoid tumor cells, desmoid tumor cells, fibroadenoma tumor cells, fibroma tumor cells, benign ganglioneuroma tumor cells, lipoma tumor cells, meningioma tumor cells, myxoma tumor cells, neurofibroma tumor cells, nevus tumor cells, osteochondroma tumor cells, pheochromocytoma tumor cells, polyposis tumor cells, schwannoma tumor cells, benign teratoma tumor cells, benign thymoma tumor cells and Brenner tumor cells. In specific embodiments, the tumor cells are prostatic intraepithelial neoplasia (PIN) tumor cells, benign prostatic hyperplastic (hypertrophic) tumor cells, ductal carcinoma in situ (DCIS) tumor cells, meningioma tumor cells or benign colonic tumor cells (colon polyps).

[0014] In another aspect, the present invention provides methods for preventing malignant conversion of a benign tumor cell to a malignant tumor cell. These methods generally comprise administering to the subject a therapeutically effective amount of selenate or a pharmaceutically acceptable salt thereof. In some embodiments, the therapeutically effective amount is a nutritional amount. In other embodiments, the therapeutically effective amount is a supranutritional amount. In specific embodiments, the benign tumor cell is a benign colonic or rectal tumor cell, a high grade prostatic intraepithelial neoplasia tumor cell, an atypical small acinar proliferative tumor cell or a ductal carcinoma in situ (DCIS) tumor cell.

[0015] In a further aspect, the present invention provides a use of selenate or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for treating or preventing a benign tumor.

[0016] In still a further aspect, the present invention provides a use of selenate or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for preventing malignant conversion of benign tumors to malignant tumors.

[0017] In some embodiments of the methods and uses broadly described above, the selenate or its pharmaceutically acceptable salt is administered in combination with at least one of a cytostatic agent, a cytotoxic agent or a radiotherapy that is optionally administered with a radiosensitizing agent. In other embodiments, the selenate is formulated in a composition with at least one cytostatic agent or cytotoxic agent. In still other embodiments, the selenate is formulated in a composition with a radiosensitizing agent for use in combination with radiotherapy.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: graphically represents the growth inhibitory (cytostatic) effect of sodium selenate at 25 μM, 50 μM and 100 μM on prostate stromal cells with time.

Figure 2: graphically represents the growth inhibitory effect of sodium selenate at 25 μM, 50 μM and 100 μM on the proliferation of prostate stromal cells at 4 days (A), 11 days (B) and 18 days (C).

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

[0018] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

[0019] The articles "a" and "an" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0020] As used herein, the term "about" refers to a quantity, level, value, dimension, size, or amount that varies by as much as 30%, 20%, or 10% to a reference quantity, level, value, dimension, size, or amount.

[0021] As used herein, the term "Akt signaling pathway activation-inhibiting amount" in the context of treating or preventing a benign tumor or inhibiting the growth of benign tumor cells is meant the administration of an amount or series of doses of selenate, which is effective in antagonizing the Akt signaling pathway, including preventing or reducing activation of Akt by preventing or reducing the expression of Akt or an upstream member of the pathway, or by reducing the level or functional activity of an expression product of the *Akt* gene or of an upstream gene member of the pathway, or by preventing or reducing phosphorylation of Akt. The amount will vary depending on the health and physical condition of the individual to be treated, the taxonomic group of the individual to be treated, the formulation of the composition, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall within a relatively broad range that can be determined by routine trials. In specific embodiments, an Akt signaling pathway activation-inhibiting amount is a nutritional or supranutritional amount of selenate.

[0022] As used herein, the term "Bcl-2 activity-reducing amount" in the context of treating or preventing a benign tumor or inhibiting the growth of benign tumor cells is meant the administration of an amount or series of doses of selenate, which is effective in reducing the levels of Bcl-2 activity in benign tumor cells or benign tumors, including downregulating Bcl-2 transcription and modulation of activity of Bcl-2. The amount will vary depending on the health and physical condition of the individual to be treated, the taxonomic group of the individual to be treated, the formulation of the composition, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall within a relatively broad range that

can be determined by routine trials. In specific embodiments, a Bcl-2 activity-reducing amount is a nutritional or supranutritional amount of selenate.

[0023] By "benign tumor in which Bcl-2 activity is elevated" is meant a benign tumor which has levels of Bcl-2 protein that are above levels found in normal cells. Normal levels of Bcl-2 protein found in normal cells are very low and generally not detectable. Benign tumors in which Bcl-2 activity is elevated may undergo malignant conversion to malignant tumors in which Bcl-2 activity is elevated. Examples of such benign tumors include colonic polyps and DCIS which may convert to colon cancer or breast cancer respectively.

[0024] As used herein, the term "benign tumor" refers to abnormal growth or proliferation cells in a localised manner. Benign tumors do not invade surrounding tissue or metastasize to other organs. Benign tumors may be asymptomatic, and discovered, for example, by screening, or they may be symptomatic and require treatment. Benign tumors although causing no immediate threat to the subject, may grow and press on other tissues or organs resulting in symptoms of the tumor, for example a meningioma in the brain may grow to a size where it damages the brain or causes malfunction in the brain. In some cases, a benign tumor may undergo malignant conversion to give a malignant tumor or cancer. Illustrative examples of benign tumors include, but are not limited to benign adenomas, angiofibromas, hemangiomas, leiomyomas, benign chorangiomas, cystadenomas, dermoid tumors, desmoid tumors, fibroadenomas, fibromas, benign ganglioneuromas, lipomas, meningiomas, myxomas, neurofibromas, nevus tumors, osteochondromas, pheochromocytomas, polyps such as colonic polyps, schwannomas, benign teratomas, benign thyomas and Brenner tumors. Other types of benign tumors include prostatic intraepithelial neoplasia, benign prostatic hyperplastic (hypertrophic) tumors and ductal carcinoma in situ.

[0025] The phrases "benign tumor in which the Akt pathway is over-activated," "benign tumor in which Akt is over-activated" and the like refer to a benign tumor in which not only Akt is overexpressed, but the kinase activity of Akt is positively enhanced by phosphorylation of the Thr 308 and Ser 473 residues. Akt is often over-activated in drug-resistant or refractory tumors. Examples of benign tumors in which Akt is over-activated or constitutively activated include, but are not limited to, prostatic intraepithelial neoplasia and breast ductal carcinoma in situ.

[0026] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0027] As used herein the term "cytostatic agent" refers to a substance that can inhibit cell proliferation or cell division without necessarily killing the cell. Suitably, the cytostatic agent inhibits the proliferation of tumor cells.

[0028] The term "cytotoxic agent" or "cytotoxic therapy" as used herein refers to a substance or therapy that is harmful to cells and ultimately causes cell death. In some embodiments, the cytotoxic agent harms rapidly dividing cells such as tumor cells and causes tumor cell death, especially tumor cell death while not causing damage to or causing less damage to non-tumor cells. An example of a cytotoxic therapy is radiotherapy.

[0029] As used herein the terms "drug resistant" and "refractory" refer to a tumor cell which is unresponsive or partially unresponsive to treatments normally used to treat the cancer or kill the tumor cell.

[0030] As used herein the term "in combination with" refers to the treatment of cancer or exposure of a tumor cell to at least two agents such that their effects on the tumor cell occur, at least in part, over the same time period. Administration of at least two agents may occur simultaneously in a single composition, or each agent may be simultaneously or sequentially administered in separate compositions.

[0031] The phrase "inhibiting growth of tumor cells" is taken to mean that tumor cell growth is ceased or reduced and cell proliferation or cell division is ceased or reduced. This is also known as cytostasis. The growth of tumor cells can be measured in terms of weight or volume or cell number or cellular metabolic activity, i.e. MTT assay.

[0032] The term "malignant conversion" as used herein refers to the conversion of benign tumors or benign tumor cells into malignant tumors or malignant tumor cells. Once malignant conversion has occurred, the tumor or tumor cells may invade other tissues or metastasize to other organs or tissues, and the subject has a cancerous tumor. Examples of benign tumors that undergo malignant conversion include colonic polyps and DCIS which may be converted to colon cancer or breast cancer respectively.

[0033] As used herein, the term "nutritional amount" includes an amount of selenium that provides an average daily intake. In the US, the average daily intake is 80-120 μg/day.

[0034] By "pharmaceutically acceptable carrier" it is meant a solid or liquid filler, diluent or encapsulating substance that may be safely used in topical, local or systemic administration.

[0035] The term "pharmaceutically acceptable salt" as used herein in relation to selenate refers to metal ion salts which are toxicologically safe for human and animal administration. For example, suitable metal ion salts of selenate include, but are not limited to,

sodium, potassium, lithium, magnesium, calcium, iron, nickel, zinc, ammonium and alkylammonium salts. A preferred salt of selenate is the sodium salt, Na_2SeO_4.

[0036] The term "radiotherapy" as used herein refers to the treatment or exposure of a tumor or tumor cells to high energy radiation. The effectiveness of radiotherapy may be enhanced by selenate or its pharmaceutically acceptable salt. Furthermore, radiotherapy may be further enhanced by administration of radiosensitizing agent. Illustrative examples of radiosensitizing agents include but are not limited to efaproxiral, etanidazole, fluosol, misonidazole, nimorazole, temoporfin and tirapazamine.

[0037] The terms "subject" or "individual" or "patient", used interchangeably herein, refer to any subject, particularly a vertebrate subject and more particularly a mammalian subject, for whom prophylaxis or treatment is desired. Suitable vertebrate animals that fall within the scope of the invention include, but are not limited to, primates, avians, livestock animals (e.g., pigs, sheep, cows, horses, donkeys), laboratory test animals (e.g., rabbits, mice, rats, guinea pigs, hamsters), companion animals (e.g., cats and dogs) and captive wild animals (e.g., foxes, deer, dingoes). A preferred subject is a human in need of treatment or prophylaxis of a benign tumor or may be at risk of a benign tumor undergoing malignant conversion to a malignant tumor. However, it will be understood that the aforementioned terms do not imply that symptoms are present.

[0038] The term "supranutritional" as used herein, refers to an amount which is greater than the amount considered as a nutritional requirement. The average daily intake of selenium in the US is 80-120 μg/day. A supranutritional amount of selenium provides selenium to a subject at above the recommended daily allowance. For example, a supranutritional amount of selenium may be 3 μg/kg to 20 mg/kg per day, 0.015 mg/kg to 20 mg/kg, 0.15 mg/kg to 20.0 mg/kg, 0.1 mg/kg to 14 mg/kg, 0.1 mg/kg to 13 mg/kg, 0.1 mg/kg to 12 mg/kg, 0.1 mg/kg to 10 mg/kg, 0.1 mg/kg to 9 mg/kg, 0.1 mg/kg to 8 mg/kg, 0.1 mg/kg to 7 mg/kg, 0.1 mg/kg to 6 mg/kg, 0.15 mg/kg to 5 mg/kg, 0.15 mg/kg to 4 mg/kg, 0.15 mg/kg to 3 mg/kg, 0.15 mg/kg to 2 mg/kg, 0.15 mg/kg to 1 mg/kg, especially 0.07 mg/kg to 6.5 mg/kg or 0.1 mg/kg to 14 mg/kg or 0.15 mg/kg to 5 mg/kg per day, and more especially 0.07 mg/kg to 2 mg/kg per day.

[0039] As used herein, the term "effective amount" in the context of treating or preventing a benign tumor or inhibiting the growth of benign tumor cells is meant the administration of an amount of selenate or a pharmaceutically acceptable salt thereof, either in a single dose or as part of a series of doses, that is effective for inhibiting the growth and/or proliferation of benign tumor cells or for causing benign tumor cell death or for preventing metastatic conversion of a benign tumor to a malignant tumor. The effective amount will vary depending on the health and physical condition of the individual to be treated, the taxonomic group of the individual to be treated, and the formulation of the composition, the assessment of

the medical situations and other relevant factors. It is expected that the amount will fall within a relatively broad range that can be determined through routine trials. In specific embodiments, an effective amount is a nutritional or supranutritional amount. In some embodiments, an effective amount is an Akt-signaling pathway activation inhibiting amount or a Bcl-2 activity-reducing amount.

2: Methods for inhibiting tumor cell growth and proliferation and for preventing malignant conversion

[0040] The present invention is predicated in part on the determination that selenate, as opposed to other forms of selenium such as selenite, is effective in inhibiting the growth or proliferation of benign tumor cells, especially those in which the Akt signaling pathway is activated or in which Bcl-2 activity is elevated. Accordingly, in one aspect, the present invention provides methods for inhibiting the growth or proliferation of benign tumor cells, wherein the methods generally comprise exposing the tumor cells to an effective amount, such as an Akt signaling pathway activation-inhibiting amount or a Bcl-2 activity-reducing amount of selenate or a pharmaceutically acceptable salt thereof. In some embodiments of the invention, the amount of selenate or its pharmaceutically acceptable salt thereof is a nutritional amount. In other embodiments, the amount of selenate or its pharmaceutically acceptable salt is a supranutritional amount, which is generally from about 0.015 mg/kg to 20.0 mg/kg, usually from about 0.07 mg/kg to 6.5 mg/kg or 0.1 mg/kg to 14 mg/kg or from about 0.15 mg/kg to 5 mg/kg, especially 0.07 mg/kg to 2 mg/kg per day.

[0041] The present invention can be used effectively against various types of tumor cells. Illustrative examples of benign tumors that may be treated or prevented include, but are not limited to benign adenomas, angiofibromas, hemangiomas, leiomyomas, benign chorangiomas, cystadenomas, dermoid tumors, desmoid tumors, fibroadenomas, fibromas, benign ganglioneuromas, lipomas, meningiomas, myxomas, neurofibromas, nevus tumors, osteochondromas, pheochromocytomas, polyps, schwannomas, benign teratomas, benign thyomas and Brenner tumors. In some embodiments, the tumor is a prostatic intraepithelial neoplasia, colonic polyps, atypical small acinar proliferative tumor cells, benign prostatic hyperplastic (hypertrophic) tumor cells (benign prostatic hyperplasia) or a ductal carcinoma in situ. In a related aspect, the invention provides methods for treating or preventing a benign tumor in which Akt is activated, wherein the methods generally comprise administering to a subject in need thereof an Akt signaling pathway activation-inhibiting amount of selenate or a pharmaceutically acceptable salt thereof. In a further related aspect, the invention provides methods for treating or preventing a benign tumor in which Bcl-2 activity is elevated, wherein the methods generally comprise administering to a subject in need thereof a Bcl-2 activity-reducing amount of selenate or a pharmaceutically acceptable salt thereof. In some

embodiments, the amount of selenate or pharmaceutically acceptable salt thereof is a nutritional amount. In other embodiments, the amount is a supranutritional amount as broadly defined above.

[0042] In some embodiments, the tumor cell is drug resistant. In some embodiments, the tumor cell has an overactive Akt pathway.

[0043] In certain embodiments, the selenate or its pharmaceutically acceptable salt is administered in combination with at least one cytostatic agent or cytotoxic agent. Non-limiting examples of cytostatic agents are selected from: (1) kinase inhibitors, illustrative examples of which include Iressa®, Gleevec, Tarceva™, (Erlotinib HCl), BAY-43-9006, inhibitors of the split kinase domain receptor tyrosine kinase subgroup (e.g., PTK787/ZK 222584 and SU11248); (2) receptor kinase targeted antibodies, which include, but are not limited to, Trastuzumab (Herceptin®), Cetuximab (Erbitux®), Bevacizumab (Avastin™), Rituximab (ritusan®), Pertuzumab (Omnitarg™); (3) mTOR pathway inhibitors, illustrative examples of which include rapamycin and CCI-778; (4) Apo2L/Trail, anti-angiogenic agents such as but not limited to endostatin, combrestatin, angiostatin, thrombospondin and vascular endothelial growth inhibitor (VEGI); (5) antineoplastic immunotherapy vaccines, representative examples of which include activated T-cells, non-specific immune boosting agents (i.e., interferons, interleukins); (6) hormonal antineoplastic agents, non-limiting examples of which include Nilutamide, Cyproterone acetate, Anastrozole, Exemestane, Tamoxifen, Raloxifene, Bicalutamide, Aminoglutethimide, Leuprorelin acetate, Toremifene citrate, Letrozole, Flutamide, Megestrol acetate and Goserelin acetate; (7) gonadal hormones such as but not limited to Cyproterone acetate and Medoxyprogesterone acetate; (8) antimetabolites, illustrative examples of which include Cytarabine, Fluorouracil, Gemcitabine, Topotecan, Hydroxyurea, Thioguanine, Methotrexate, Colaspase, Raltitrexed and Capicitabine; (9) anabolic agents, such as but not limited to, Nandrolone; (10) adrenal steroid hormones, illustrative examples of which include Methylprednisolone acetate, Dexamethasone, Hydrocortisone, Prednisolone and Prednisone.

[0044] The methods of the present invention may be employed in combination with other known treatments for benign tumors, for instance but not limited to, surgery, chemotherapy and radiotherapy. In some embodiments, the selenate or its pharmaceutically acceptable salt is used in combination with radiotherapies, such as but not limited to, conformal external beam radiotherapy (50-100 Grey given as fractions over 4-8 weeks), either single shot or fractionated, high dose rate brachytherapy, permanent interstitial brachytherapy, systemic radio-isotopes (e.g., Strontium 89). In some embodiments the radiotherapy may be administered in combination with a radiosensitizing agent. Illustrative examples of radiosensitizing agents include but are not limited to efaproxiral, etanidazole, fluosol, misonidazole, nimorazole,

temoporfin and tirapazamine. In other embodiments, selenate or its pharmaceutically acceptable salt is used in combination with a tumorectomy.

[0045] In some embodiments, the cytostatic agent is a nucleic acid molecule, suitably an antisense or siRNA recombinant nucleic acid molecule. In other embodiments, the cytostatic agent is a peptide or polypeptide. In still other embodiments, the cytostatic agent is small molecule. The cytostatic agent may be a cytotoxic agent that is suitably modified to enhance uptake or delivery of the agent. Non-limiting examples of such modified cytotoxic agents include, but are not limited to, pegylated or albumin-labelled cytotoxic drugs.

[0046] In embodiments where the benign tumor is benign prostatic hypertrophy (benign prostatic hyperplasia) or prostatic intraepithelial neoplasia, the selenate or pharmaceutically acceptable salt thereof may be administered in combination with 5-alpha reductase inhibitors such as finasteride or dutasteride; alpha adrenergic receptor blockers such as terazosin (Hytrin), doxazosin (cardura), alfuzosin, tamsulosin (flomax) and Prazosin (Minipress); selective estrogen receptor modulators (SERMs) such as toremifene citrate (Acapodene), tamoxifen and raloxifen; selective androgen receptor modulators (SARMs) such as prostarine; or phytotherapeutics such as those derived from the American dwarf palm tree (saw palmetto or *Serenoa repens*), African plum tree (Pygeum africanum), pumpkin seeds, rye pollen extracts, South African star grass roots, stinging nettle roots and the purple cone flower.

[0047] In embodiments where the benign tumor is colonic polyps, the selenate or pharmaceutically acceptable salt thereof may be administered in combination with non steroidal anti inflammatory drugs such as aspirin, ibuprofen, naproxene; or Cox-2 inhibitors such as sulindac, celocoxib (celebrex), refocoxib (vioxx) and lumiracoxib.

[0048] In embodiments where the benign tumor is ductal carcinoma in situ the selenate or pharmaceutically acceptable salt thereof may be administered in combination with selective estrogen receptor modulators such as tamoxifen, raloxifen and toremifene citrate.

[0049] In embodiments where the benign tumor is meningioma, the selenate or pharmaceutically acceptable salt thereof may be administered in combination with progesterone receptor antagonists such as mifepristone; immune modulating agents such as interferon; chemotherapy such as hydroxyurea and external beam radiation.

[0050] Certain embodiments of the present invention are directed to methods for treating or preventing a benign tumor in a subject, which methods generally comprise administering to the subject a therapeutically effective amount of selenate or a pharmaceutically acceptable salt thereof. To practice these methods, the person managing the subject can determine the effective dosage form of selenate or its pharmaceutically acceptable salts for the particular condition and circumstances of the subject. A therapeutically effective amount of

selenate is one that is effective for the treatment or prevention of the benign tumor, including the prevention of incurring a symptom (e.g., proliferation of tumor cells), holding in check such symptoms, and/or treating existing symptoms associated with the tumor (e.g., pain, tenderness, headaches, menstrual irregularity, urinary retention, cosmetic effect, shortness of breath, altered bowel habit, malena, haematemesis, focal neurological deficit). In some embodiments, the therapeutically effective amount is a nutritional amount. In other embodiments, the amount is a supranutritional amount of selenate or its pharmaceutically acceptable salt. In specific embodiments, the selenate is suitably in the form of the salt, sodium selenate (Na_2SeO_4).

[0051] Modes of administration, amounts of selenate administered, and selenate formulations, for use in the methods of the present invention, are discussed below. The tumor to be treated may be determined by measuring one or more diagnostic parameters indicative of the course of the disease, compared to a suitable control. In the case of a human subject, a "suitable control" may be the individual before treatment, or may be a human (e.g., an age-matched or similar control) treated with a placebo. In accordance with the present invention, the treatment or prevention of a benign tumor includes and encompasses without limitation: (i) preventing a benign tumor in a subject who may be predisposed to the benign tumor but has not yet been diagnosed with the benign tumor and, accordingly, the treatment constitutes prophylactic treatment for the benign tumor; (ii) inhibiting tumorigenesis, i.e., arresting the development of the benign tumor; (iii) relieving symptoms resulting from the benign tumor; or (iv) preventing malignant conversion of a benign tumor.

[0052] The methods of the present invention are suitable for treating an individual who has been diagnosed with a benign tumor, who is suspected of having a benign tumor, who is known to be susceptible and who is considered likely to develop a benign tumor, who is considered likely to develop a recurrence of a previously treated benign tumor, or who has a benign tumor which may undergo malignant conversion to a malignant tumor.

[0053] In specific embodiments of the above methods, the benign tumor is a high grade prostatic intraepithelial neoplasia (HGPIN), colonic polyp or ductal carcinoma in situ (DCIS) that may undergo malignant conversion to prostate cancer, colon cancer or breast cancer respectively. In other embodiments, the tumor to be treated may be a meningioma.

[0054] Exemplary subjects for treatment with the methods of the invention are vertebrates, especially mammals. In certain embodiments, the subject is selected from the group consisting of humans, sheep, cattle, horses, bovine, pigs, dogs and cats. A preferred subject is a human.

[0055] The selenate or pharmaceutically acceptable salt may be formulated by following any number of techniques known in the art of drug delivery. Selenate or its pharmaceutically acceptable salts may of course be administered by a number of means keeping

in mind that all formulations are not suitable for every route of administration. Selenate or its pharmaceutically acceptable salts can be administered in solid or liquid form. The application may be oral, rectal, nasal, topical (including buccal and sublingual), or by inhalation. Selenate or its pharmaceutically acceptable salts may be administered together with conventional pharmaceutical acceptable adjuvant, carriers and/or diluents.

[0056] The solid forms of application comprise tablets, capsules, powders, pills, pastilles, suppositories and granular forms of administration. They may also include carriers or additives, such as flavors, dyes, diluents, softeners, binders, preservatives, lasting agents and/or enclosing materials. Liquid forms of administration include solutions, suspensions and emulsions. These may also be offered together with the above-mentioned additives.

[0057] Solutions and suspensions of selenate or pharmaceutically acceptable salt thereof, assuming a suitable viscosity for ease of use, may be injected. Suspensions too viscous for injection may be implanted using devices designed for such purposes, if necessary. Sustained release forms are generally administered *via* parenteral or enteric means. Parenteral administration is another route of administration of the selenate or a pharmaceutically acceptable salt thereof used to practice the invention. "Parenteral" includes formulations suitable for injection and for nasal, vaginal, rectal, and buccal administration.

[0058] The administration of selenate or its pharmaceutically acceptable salts may involve an oral prolonged dose formulation. Oral dose formulations are preferably administered once daily to three times daily in the form of a sustained release capsule or tablet, or alternatively as an aqueous based solution. Selenate or its pharmaceutically acceptable salt may be administered intravenously either daily, continuously, once a week or three times a week.

[0059] The administration of selenate or its pharmaceutically acceptable salts may include daily administration, preferably once daily in the form of a sustained release capsule or tablet, or once daily as an aqueous solution.

[0060] Combinations of selenate or its pharmaceutically acceptable salt and at least one cytostatic agent or a cytotoxic agent or a radiosensitizing agent may be administered in solid or liquid form in a single formulation or composition or in separate formulations or compositions. In some embodiments, the selenate or its pharmaceutically acceptable salt and the cytostatic agent(s) or cytotoxic agent(s) or radiosensitizing agent(s) are administered orally as a single tablet or capsule or separate tablets or capsules. In other embodiments, the selenate or its pharmaceutically acceptable salt and the cytostatic agent(s) or cytotoxic agent(s) or radiosensitizing agent(s), are administered intravenously in a single composition or separate compositions.

[0061] The present invention also provides pharmaceutical compositions for treating or preventing a benign tumor or preventing a benign tumor undergoing malignant conversion, generally comprising a supranutritional amount, suitably from about 0.5 mg to about 1.0 g of selenate or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier. In some embodiments, the selenate or its pharmaceutically acceptable salt is in an amount of about 5.0 mg to about 700 mg or 5 mg to 450 mg. In illustrative examples, the selenate or its pharmaceutically acceptable salt is an amount of about 0.07 mg to 6.5 mg, 5 mg to 450 mg or 7.5 mg to 250 mg, especially 50 mg to 200 mg, for example, 50 mg to 100 mg or 100 mg to 150 mg for a single or divided daily dose. The pharmaceutical composition may be for treating a benign tumor in which Akt is activated or over-activated or a benign tumor in which Bcl-2 activity is elevated.

[0062] In some embodiments, the pharmaceutical compositions further comprise at least one cytostatic agent or cytotoxic agent. In other embodiments, the pharmaceutical compositions may further comprise a radiosensitizing agent for use with radiotherapy. In other embodiments, the pharmaceutical composition may further comprise a second agent that is suitable for treating a benign tumor, such as 5-α-reductase inhibitors, α-adrenergic receptor blockers, selective estrogen receptor modulators, selective androgen receptor modulators, phytotherapeutics, anti-inflammatory drugs, Cox-2 inhibitors, progesterone receptor antagonists, immune modulating agents or chemotherapy.

[0063] The pharmaceutical composition of the present invention may include any additional components that are non-immunogenic and biocompatible with selenate, as well as capable of bioabsorption, biodegradation, elimination as an intact molecule. The formulation may be supplied in a ready-to-use form or may be supplied as a sterile powder or liquid requiring vehicle addition prior to administration. If sterility is desired, the formulation may be made under sterile conditions, the individual components of the mixture may be sterile, or the formulation may be sterile filtered prior to use. Such a solution can also contain appropriate pharmaceutically acceptable carriers, such as but not limited to buffers, salts, excipients, preservatives, etc.

[0064] In some embodiments, sustained release oral formulations are used for administering selenate or its pharmaceutically acceptable salt in the methods of the invention. These formulations generally comprise selenate or its pharmaceutically acceptable salt having decreased solubility in order to delay absorption into the bloodstream. In addition, these formulations may include other components, agents, carriers, etc., which may also serve to delay absorption of the selenate or its pharmaceutically acceptable salt. Microencapsulation, polymeric entrapment systems, and osmotic pumps, which may or may not be bioerodible, may

also be used to allow delayed or controlled diffusion of the selenate or a pharmaceutically acceptable salt thereof from a capsule or matrix.

[0065] The selenate or its pharmaceutically acceptable salts can be used solus or as part of another agent. Accordingly, the present invention also contemplates an agent that comprises selenate or a pharmaceutically acceptable salt thereof for the treatment of a benign tumor, especially one in which Akt is activated or in which Bcl-2 activity is elevated, or for the prevention of malignant conversion of a benign tumor to a malignant tumor.

[0066] In order that the nature of the present invention may be more clearly understood and put into practical effect, particular preferred embodiments thereof will now be described with reference to the following non-limited examples.

EXAMPLES

EXAMPLE 1

Isolation of primary benign tumor cell lines

[0067] Primary meningioma tissue samples will be obtained from the Royal Melbourne Hospital under a protocol approved by the Institutional Review Board. Primary cultures of meningiomas will be established according to established protocols (Friend KE, Radinsky R, McCutcheon IE. Growth hormone receptor expression and function in meningiomas: effect of a specific receptor antagonist. *J Neurosurg* 1999; 91:93-9).

[0068] Briefly, tumor tissue will be collected at the time of resection from patients with benign, or atypical, meningioma as determined by neuropathologic review using WHO 2000 criteria. Tumor fragments will be immediately transferred to the laboratory for further processing, carefully minced into small pieces, and treated with 0.4% trypsin/EDTA to dissociate the cells. The cells will be washed with PBS supplemented with 200 units/mL penicillin and 200 µg/mL streptomycin and transferred to 100-mm^2 tissue culture dishes containing DMEM supplemented with 10% FCS, 100 units/mL penicillin, and 100 µg/mL streptomycin. The cells will be grown in humidified incubators at 37°C with 5% CO_2 for 5 days, with periodic medium changes and then allowed to reach confluence. Low-passage cells (<7 passages) will used for proliferation experiments.

EXAMPLE 2

Cell proliferation assays

Benign tumor growth curve

[0069] Benign meningioma tumor growth curves will be analysed by allowing 2 x 10^5 tumor cells. The cells will be allowed to attach overnight in culture dishes. After 16 hours the medium will be changed to include sodium selenate in the presence of serum at the indicated concentrations and allowed to grow until specified time points. Supernatants and cells will then be harvested, combined and viable cells assessed by Trypan Blue exclusion assay. Experiments will be performed in triplicate.

MTT Growth Assay

[0070] MTT Growth Assays will be performed by plating 1 x 10^3 meningioma tumor cells per well in a 96 well plate and allowing the cells to attach overnight. At 16 hours the medium will be replaced with fresh media containing sodium selenate (5-250 µM). For experiments determining the additional effect of PI3K inhibition on sodium selenate growth

inhibition, LY294002 (Promega, Madison, WI, USA) will be added at a concentration of 10-50 μM with the sodium selenate. The controls for this experiment will then receive an equal volume of LY294002 diluent (DMSO). MTT growth assays will then be performed according to the manufacturer's protocol (Sigma). Experiments will be performed in triplicate.

5

EXAMPLE 3

The effect of sodium selenate on the growth/proliferation or survival of benign tumor cells. Primary human prostate stromal cell lines as a model for human benign prostatic hyperplasia (BPH)

10 **MATERIALS AND METHODS**

[0071] Cell Culture. The prostate stromal (PrS) cell line was obtained from Cambrex Bio Science (Walkersville, MC, USA, www.cambrex.com) and was routinely cultured in Stromal Cell Medium BulletKit (Clonetics SCGM BulletKit, CC-3205) purchased from Cambrex. Complete growth medium was prepared from the BulletKit containing stromal cell
15 basal medium supplemented with 0.1% human fibroblast growth factor B, 0.1% insulin, 5% fetal bovine serum and 0.1% gentamicin sulphate amphotericin-B according to supplier specification. Cells were cultured at 37°C in 5% CO_2.

[0072] ***In Vitro* – growth proliferation study**. 4 x 10^3 PrS cells were plated per well in a Falcon 6-well plate and were allowed to attach overnight at 5%CO_2/ 37°C in complete
20 growth medium. The medium was replaced with fresh complete growth medium containing various concentration of sodium selenate (25, 50 and 100 μM) and were allowed to grow until specified time points (day 4, 11 and 18). Cells were trypsinised according to supplier specification and viable cells were assessed by trypan blue exclusion assay at the specified time points indicated above. Experiments were performed in triplicate per selenate treatment dose.

25 **[0073] Statistical analysis**. Data are presented as mean ± SEM. unless otherwise indicated. Differences between treatment groups were analyzed using Fisher's least significant difference test with significance assumed at $p<0.01$, ANOVA. All statistical analysis was performed using STATGRAPHICS statistical software (Virginia, USA).

[0074] The results are shown in Table 1 and Figures 1 and 2.

Table 1. Growth inhibitory (cytostatic) effect of sodium selenate on prostate stromal cell growth over time.

Treatment Groups	No. of days in culture	No. of viable cells (mean)
Complete growth medium (control)	1	40000.0
25 μM Selenate	1	40000.0
50 μM Selenate	1	40000.0
100 μM Selenate	1	40000.0
Complete growth medium (control)	4	44666.7
25 μM Selenate	4	48000.0
50 μM Selenate	4	15666.7
100 μM Selenate	4	11666.7
Complete growth medium (control)	11	163333.0
25 μM Selenate	11	61333.3
50 μM Selenate	11	19333.3
100 μM Selenate	11	4000.0
Complete growth medium (control)	18	480000.0
25 μM Selenate	18	81333.3
50 μM Selenate	18	7666.7
100 μM Selenate	18	2962.7

[0075] The results show that 25 μM selenate effectively impedes prostate stromal cell growth after 11 days in culture. Higher concentrations of selenate, 50 μM and 100 μM are very effective at controlling prostate stromal cell growth.

[0076] The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

[0077] The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

[0078] Throughout the specification the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. Those of skill in the art will therefore appreciate that, in light of the instant disclosure, various modifications and changes can be made in the particular embodiments exemplified without departing from the scope of the present invention. All such modifications and changes are intended to be included within the scope of the appended claims.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of inhibiting the growth or proliferation of benign tumor cells comprising exposing the benign tumor cells to an effective amount of selenate or a pharmaceutically acceptable salt thereof.

2. A method according to claim 1, wherein the benign tumor cells are tumor cells in which the Akt-signaling pathway is activated or over-activated.

3. A method according to claim 1, wherein the benign tumor cells have abnormally increased Bcl-2 activity.

4. A method according to claim 1, wherein the benign tumor cells are selected from benign adenoma tumor cells, angiofibroma tumor cells, hemangioma tumor cells, leiomyoma tumor cells (fibroid), benign chorangioma tumor cells, cystadenoma tumor cells, dermoid tumor cells, desmoid tumor cells, fibroadenoma tumor cells, fibroma tumor cells, benign ganglioneuroma tumor cells, lipoma tumor cells, meningioma tumor cells, myxoma tumor cells, neurofibroma tumor cells, nevus tumor cells, osteochondroma tumor cells, pheochromocytoma tumor cells, polyposis tumor cells, schwannoma tumor cells, benign teratoma tumor cells, benign thymoma tumor cells and Brenner tumor cells.

5. A method according to claim 1, wherein the benign tumor cells are selected from prostatic intraepithelial neoplasia (PIN) tumor cells, benign prostatic hyperplastic (hypertrophic) tumor cells, ductal carcinoma in situ (DCIS) tumor cells, meningioma tumor cells or benign colonic tumor cells (colon polyps).

6. A method of treating or preventing a benign tumor in a subject comprising administering to the subject an effective amount of selenate or a pharmaceutically acceptable salt thereof.

7. A method according to claim 6, wherein the effective amount of selenate or a pharmaceutically acceptable salt thereof is a nutritional amount.

8. A method according to claim 6, wherein the effective amount of selenate or a pharmaceutically acceptable salt thereof is a supranutritional amount.

9. A method according to claim 6, wherein the benign tumor is selected from benign adenomas, angiofibromas, hemangiomas, leiomyomas, benign chorangiomas, cystadenomas, dermoid tumors, desmoid tumors, fibroadenomas, fibromas, benign ganglioneuromas, lipomas, meningiomas, myxomas, neurofibromas, nevus tumors, osteochondromas, pheochromocytomas, polyps, schwannomas, benign teratomas, benign thyomas and Brenner tumors.

10. A method according to claim 6, wherein the benign tumor is selected from prostatic intraepithelial neoplasia, colonic polyps, atypical small acinar proliferative tumor cells, benign prostatic hyperplastic (hypertrophic) tumor cells or a ductal carcinoma in situ.

11. A method according to claim 6, further comprising administration in combination with another known treatment for benign tumors.

12. A method according to claim 11, wherein the other known treatments for benign tumors are selected from surgery, radiotherapy and chemotherapy.

13. A method according to claim 6, further comprising administering at least one cytotoxic or cytostatic agent or a radiosensitizing agent or a second agent used to treat benign tumors.

14. A method according to claim 13, wherein the cytostatic agent is selected from kinase inhibitors, receptor kinase targeted antibodies, mTOR pathway inhibitors, Apo2L/Trail antiangiogenic agents, antineoplastic immunotherapy vaccines, hormonal antineoplastic agents, gonadal hormones, antimetabolites, anabolic agents, adrenal steroid hormones and microtubule-stabilizing agents.

15. A method according to claim 13, wherein the radiosensitizing agent is selected from efaproxiral, etanidazole, fluosol, misonidazole, nimorazole, temoporfin and tirapazamine.

16. A method according to claim 13, wherein the second agent used to treat benign tumors is selected from 5-α-reductase inhibitors, α-adrenergic receptor blockers, selective estrogen receptor modulators, selective androgen receptor modulators, phytotherapeutics, non-steroidal anti-inflammatory drugs, Cox-2 inhibitors, progesterone receptor antagonists, immune modulating agents and chemotherapy.

17. A method of preventing benign tumor cells or a benign tumor undergoing malignant conversion to malignant tumor cells or a malignant tumor in a subject, comprising administering to the subject an effective amount of selenate or a pharmaceutically acceptable salt thereof.

18. A method according to claim 17, wherein the effective amount of selenate or a pharmaceutically acceptable salt thereof is a nutritional amount.

19. A method according to claim 17, wherein the effective amount of selenate or a pharmaceutically acceptable salt thereof is a supranutritional amount.

20. A method according to claim 17, wherein the benign tumor cells are benign colonic or rectal tumor cells, high grade prostatic intraepithelial neoplasia tumor cells, atypical small acinar proliferative tumor cells or ductal carcinoma in situ tumor cells.

21. A method according to claim 17, wherein the benign tumor is a high grade prostatic intraepithelial neoplasia, a colonic polyp or a ductal carcinoma in situ.

22. A pharmaceutical composition comprising selenate or a pharmaceutically acceptable salt thereof and at least one of a cytostatic agent, a cytotoxic agent, a radiosensitizing agent or a second agent used to treat benign tumors and a pharmaceutically acceptable carrier.

23. Use of selenate or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for treating or preventing a benign tumor.

24. Use of selenate or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for preventing benign tumor cells or a benign tumor undergoing malignant conversion to malignant tumor cells or a malignant tumor.

FIGURE 1

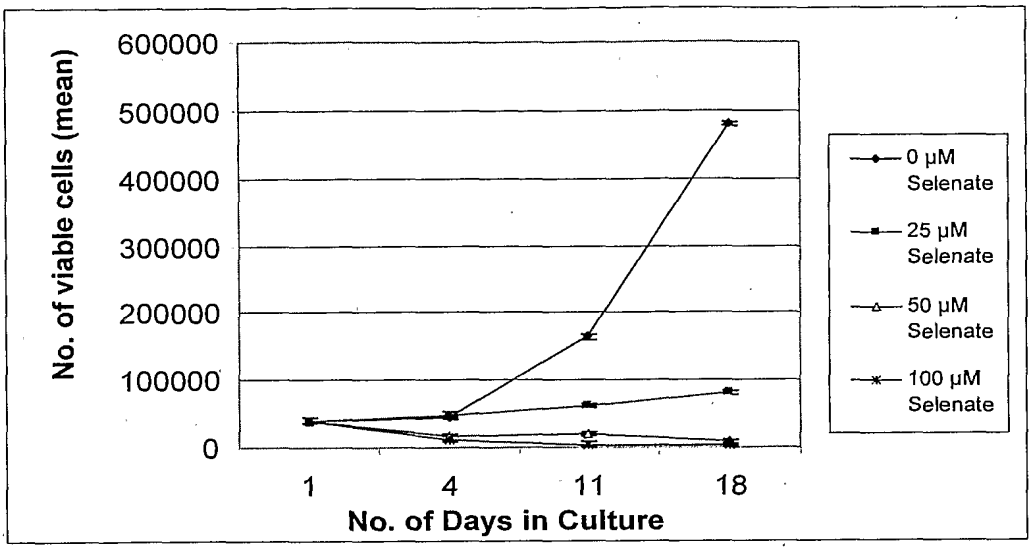
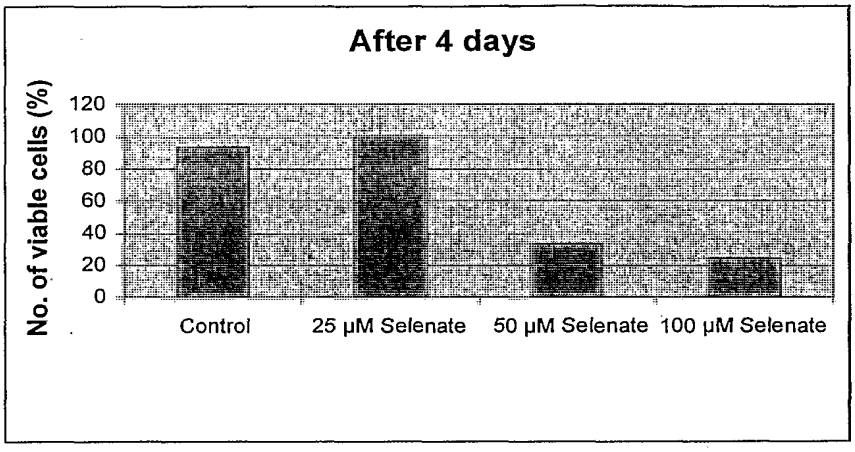
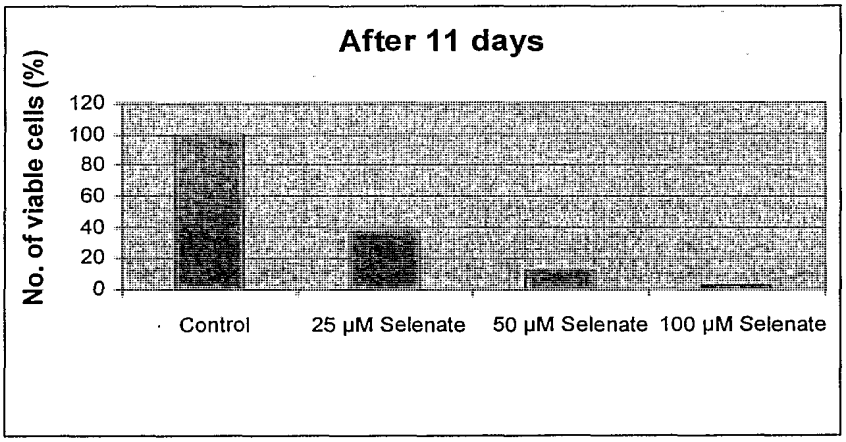

600000
500000
400000
300000
200000
100000
0
No. of viable cells (mean)
1
4
11
18
No. of Days in Culture
0 µM Selenate
25 µM Selenate
50 µM Selenate
100 µM Selenate

FIGURE 2

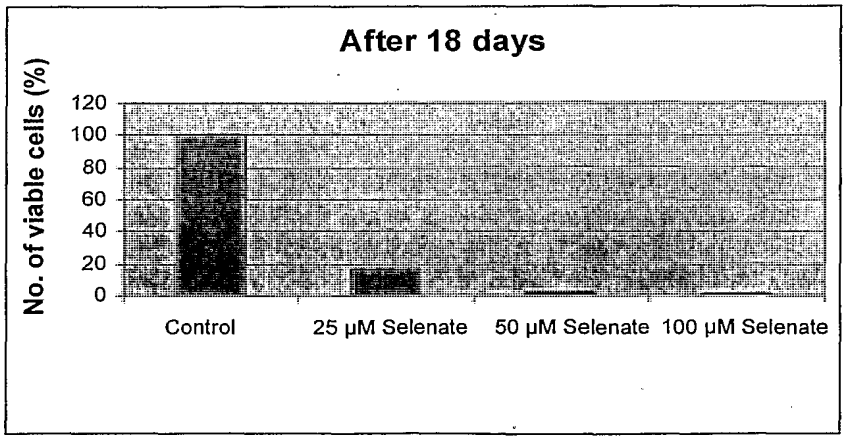
A


After 4 days
No. of viable cells (%)
120
100
80
60
40
20
0
Control
25 µM Selenate
50 µM Selenate
100 µM Selenate

B


After 11 days
No. of viable cells (%)
120
100
80
60
40
20
0
Control
25 µM Selenate
50 µM Selenate
100 µM Selenate

C


After 18 days
No. of viable cells (%)
120
100
80
60
40
20
0
Control
25 µM Selenate
50 µM Selenate
100 µM Selenate

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2007/000393

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61K 33/04 (2006.01) ***A61P 35/00*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPIDS and Medline. Keywords: selenate, cancer, tumour, tumor, neoplasm, benign and related terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO2005/115472 A2 (Abbott Laboratories), 8 December 2005 see page 9 line 31, page 13 line 5 to page 14 line 25, page 27 line 16 to page 30 line 25, page 31 line 4 to page 34 line 30.	1-4, 6-9, 11-14, 17-19, 22-24
Y	see page 9 line 31, page 13 line 5 to page 14 line 25, page 27 line 16 to page 30 line 25, page 31 line 4 to page 34 line 30.	1-24
X	DAVIS, C.D. ET AL The Chemical Form of Selenium Influences 3,2'-Dimethyl-4-aminobiphenyl-DNA Adduct Formation in Rat Colon. Journal of Nutrition, 1999, volume 129, pages 63-69 see abstract and page 63, right hand column, 2nd paragraph.	6-10, 17-21, 23-24
Y	see abstract and page 63, right hand column, 2nd paragraph.	1-24

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
08 May 2007

Date of mailing of the international search report 11 MAY 2007

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaustralia.gov.au
Facsimile No. (02) 6285 3929

Authorized officer
Steven Chew
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No : (02) 6283 2248

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO2005/048925 A2 (The Board of Trustees of the Leland Stanford Junior University), 2 June 2005 see claim 1 and paragraphs 0025, 0044, 0049, 0051, 0070 and 0080.	11-16, 22
Y	CLARK, L.C. ET AL Plasma Selenium Concentration Predicts the Prevalence of Colorectal Adenomatous Polyps. Cancer Epidemiology, Biomarkers and Prevention, January/February 1993, volume 2, pages 41-46 (see abstract)	1-24
P,X	WO2006/032074 A1 (University of Melbourne), 30 March 2006 (see whole document)	1-24
A	WO2001/054688 A1 (Merck and Co Inc.), 2 August 2001	
A	CORCORAN, N.M. ET AL Inorganic Selenium Retards Progression of Experimental Hormone Refractory Prostate Cancer. Journal of Urology, February 2004, volume 171, pages 907-910	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU2007/000393

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	2005115472	CA	2564592	US	2006034944		
WO	2005048925						
WO	2006032074	AU	2005287854				
WO	0154688	AU	36533/01	CA	2396593	EP	1253921
		US	6486204	US	2001047022		

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicant *(for all designated States except US)*: **ELI LILLY AND COMPANY** [US/US]; Lilly Corporate Center, Indianapolis, Indiana 46285 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **CHEN, Zhaogen** [US/US]; 735 Canal Court #B, Indianapolis, Indiana 46202 (US). **HAMDOUCHI, Chafiq, Hamdouchi** [ES/US]; 14469 Twin Oaks Drive, Carmel, Indiana 46032 (US). **HEMBRE, Erik, James** [US/US]; 1332 North New Jersey Street, Indianapolis, Indiana 46202 (US). **HIPSKIND, Philip, Arthur** [US/US]; 4255 Cabin Court, New Palestine, Indiana 46163 (US). **MYERS, Jason, Kenneth** [US/US]; 8021 Springwater Circle, Indianapolis, Indiana 46256 (US). **TAKAKUWA, Takako** [JP/US]; 7653 Germander Lane, Indianapolis, Indiana 46237 (US). **TOTH, James, Lee** [US/US]; 4642 South 625 West, Knightstown, Indiana 46148 (US).

(74) Agents: **HOSTETTLER, Danica** et al.; Eli Lilly and Company, Patent Division, P.O. Box 6288, Indianapolis, Indiana 46206-6288 (US).

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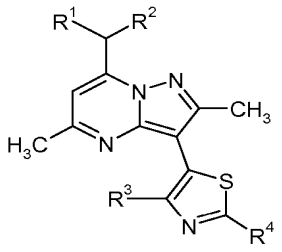
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(54) Title: THIAZOLE PYRAZOLOPYRIMIDINES AS CRF1 RECEPTOR ANTAGONISTS



I

(57) Abstract: The present invention relates to compounds of Formula (I), pharmaceutical compositions thereof, and use thereof as corticotropin releasing factor 1 (CRF1) receptor antagonists in the treatment of psychiatric and neuroendocrine disorders, neurological diseases, and metabolic syndrome.

WO 2008/036579 A1

THIAZOLE PYRAZOLOPYRIMIDINES AS CRF1 RECEPTOR ANTAGONISTS

FIELD OF THE INVENTION

This invention relates to novel thiazole pyrazolopyrimidine compounds, pharmaceutical compositions thereof, and use thereof as CRF1 receptor antagonists in the treatment of psychiatric and neuroendocrine disorders, neurological diseases, and metabolic syndrome.

BACKGROUND OF THE INVENTION

Corticotropin releasing factor (CRF) is a 41 amino acid peptide that is the primary physiological regulator of proopiomelanocortin (POMC) derived peptide secretion from the anterior pituitary gland. In addition to its endocrine role at the pituitary gland, immunohistochemical localization of CRF has demonstrated that the hormone has a broad extrahypothalamic distribution in the central nervous system and produces a wide spectrum of autonomic, electrophysiological and behavioral effects consistent with a neurotransmitter or neuromodulator role in the brain. There is also evidence that CRF plays a significant role in integrating the response in the immune system to physiological, psychological, and immunological stressors.

CRF has been implicated in psychiatric disorders and neurological diseases including depression and anxiety, as well as the following: Alzheimer's disease, Huntington's disease, progressive supranuclear palsy, amyotrophic lateral sclerosis, Parkinson's disease, epilepsy, migraine, alcohol and substance abuse and associated withdrawal symptoms, obesity, metabolic syndrome, congenital adrenal hyperplasia, Cushing's disease, hypertension, stroke, irritable bowel syndrome, stress-induced gastric ulceration, premenstrual syndrome, sexual dysfunction, premature labor, inflammatory disorders, allergies, multiple sclerosis, visceral pain, sleep disorders, pituitary tumors or ectopic pituitary-derived tumors, chronic fatigue syndrome and fibromyalgia.

CRF receptor subtypes, CRF1 and CRF2, have been identified and are distributed heterogeneously within the brain thereby suggesting potential functional diversity. For example, widely distributed brain CRF1 receptors are strongly implicated in emotionality accompanying exposure to environmental stressors. Significantly, CRF1, not CRF2, receptors appear to mediate select anxiogenic like behaviors. A more discrete

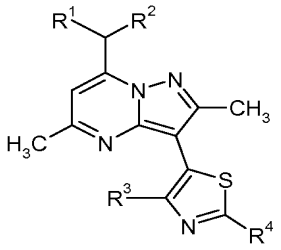
septal/hypothalmic distribution and the availability of alternative endogenous ligands suggest a different functional role for the CRF2 receptor. For example, a novel CRF-family neuropeptide with preferential affinity for CRF2 relative to CRF1 receptors is reported to suppress appetite without producing the profile of behavioral activation observed with selective CRF1 agonism. In other cases, CRF2 agonism produces similar effects to those reported for CRF1 antagonists or CRF1 gene deletion. For example, while CRF2 agonists have been proposed as antiobesity agents, CRF1 antagonists may be an important treatment for obesity as well.

Certain pyrrolo[2,3-*d*]pyrimidines, pyrrolo[3,2-*d*]pyrimidines, pyrazolo[1,5-*a*]pyrimidines, 1,2,3-triazolo[4,5-*b*]pyridines, and pyrazolo[1,5-*a*]-1,3,5-triazines, useful as CRF antagonists, are described in WO 94/13676, WO 97/29109, WO 98/08847, and WO 98/03510.

The present invention provides novel thiazole pyrazolopyrimidines useful as CRF1 receptor antagonists. In view of the above, it is desirable to discover new efficacious and selective antagonists of CRF1 as potentially valuable therapeutic agents for the treatment of psychiatric and neuroendocrine disorders, neurological diseases, and metabolic syndrome. Further, since a majority of commercial CNS and cardiovascular drugs exhibit unfavorable bioavailability and pharmacokinetic profiles, it is also desirable to discover new compounds with favorable bioavailability and pharmacokinetic profiles relative to known CRF antagonists such as CP154526 and NBI30775.

SUMMARY OF THE INVENTION

In one embodiment, the present invention provides a compound of Formula I

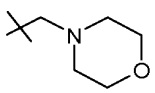
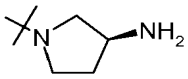
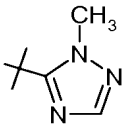
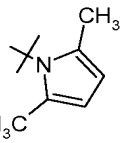


Formula I

wherein:

R^1 and R^2 are independently ethyl or *n*-propyl;

R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl or methoxy;

R^4 is hydrogen, Br, R^aR^bN-, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl,  ,  ,  or  ;

R^a and R^b are independently hydrogen, C_1-C_3alkyl, $H_2NCH_2CH_2$-, $(CH_3)_3COC(O)NHCH_2CH_2$-, or $CH_3CH_2CH_2NHCH_2CH_2$-;

or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention provides a pharmaceutical composition comprising: a compound of Formula I, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

In another embodiment, the present invention relates to a method of treating depression, anxiety, alcohol or substance abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, stroke, sleep disorders, allergy, migraine, premenstrual syndrome, infertility, sexual dysfunction, congenital adrenal hyperplasia, Cushing's disease, premature labor, stress-induced gastric ulceration, inflammatory disorders, pituitary or ectopic pituitary-derived tumors, chronic fatigue syndrome, fibromyalgia, visceral pain or multiple sclerosis in a patient, comprising: administering to a patient in need thereof a therapeutically effective amount of a compound of Formula I, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to use of a compound of Formula I, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for the treatment of depression, anxiety, alcohol or substance abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, stroke, sleep disorders, allergy, migraine, premenstrual syndrome, infertility, sexual dysfunction, congenital adrenal hyperplasia, Cushing's disease, premature labor, stress-induced gastric ulceration, inflammatory disorders, pituitary or ectopic pituitary-derived tumors, chronic fatigue syndrome, fibromyalgia, visceral pain or multiple sclerosis.

In another embodiment, the present invention relates to a compound of Formula I, or a pharmaceutically acceptable salt thereof, for use in the treatment of depression,

anxiety, alcohol or substance abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, stroke, sleep disorders, allergy, migraine, premenstrual syndrome, infertility, sexual dysfunction, congenital adrenal hyperplasia, Cushing's disease, premature labor, stress-induced gastric ulceration, inflammatory disorders, pituitary or ectopic pituitary-derived tumors, chronic fatigue syndrome, fibromyalgia, visceral pain or multiple sclerosis.

In another embodiment, the present invention relates to a compound of Formula I, or a pharmaceutically acceptable salt thereof, for use in therapy.

DETAILED DESCRIPTION OF THE INVENTION

As used above, and throughout the description of the invention, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

"Alkyl" means a saturated aliphatic hydrocarbon group, which may be straight or branched, having 1 to 5 carbon atoms in the chain.

"Pharmaceutically acceptable excipient" refers to a pharmaceutically acceptable formulation carrier, solution, or additive to enhance the formulation characteristics. Such excipients must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof and are well known to the skilled artisan (see e.g. *Remingtons Pharmaceutical Sciences*, 19th Edition, Mack Publishing Company, 1995).

"Pharmaceutically acceptable salts" refers to the relatively non-toxic, inorganic and organic acid addition salts, and base addition salts, of compounds of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the compounds. In particular, acid addition salts can be prepared by separately reacting the purified compound in its free base form with a suitable organic or inorganic acid and isolating the salt thus formed (see e.g. *Remingtons Pharmaceutical Sciences*, 19th Edition, Mack Publishing Company, 1995).

"Therapeutically effective amount" or "effective amount" means the amount of the compound of formula I of the present invention or pharmaceutical composition containing a compound of formula I of the present invention that will elicit the biological or medical response of or desired therapeutic effect on a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.

The terms "treatment," "treat," "treating," and the like, are meant to include both slowing and reversing the progression of a disorder. These terms also include alleviating, ameliorating, attenuating, eliminating, or reducing one or more symptoms of a disorder or condition, even if the disorder or condition is not actually eliminated and even if progression of the disorder or condition is not itself slowed or reversed. The term "treatment" and like terms also include preventive (e.g., prophylactic) and palliative treatment. Prevention of the disease is manifested by a prolonging or delaying of the onset of the symptoms of the disease.

The symbol "——" in a molecular structure indicates the position of attachment for that particular substituent.

When any variable occurs more than one time in any constituent or in formula I, its definition on each occurrence is independent of its definition at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds. In choosing compounds of the present invention, one of ordinary skill in the art will recognize that the various substituents, i.e. R^1, R^2, etc., are to be chosen in conformity with well- known principles of chemical structure connectivity.

Under standard nomenclature used throughout this disclosure, the terminal portion of the designated side chain is described first, followed by the adjacent functionality toward the point of attachment. For example, an arylcarbonylaminoalkyl substituent is equivalent to aryl-C(O)-NH-alkyl-.

The present invention contemplates the following embodiments, which can alternatively be further combined:

(a) A compound of Formula I wherein R^1 and R^2 are ethyl;

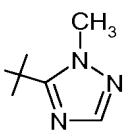
(b) A compound of Formula I wherein R^1 and R^2 are *n*-propyl;

(c) A compound of Formula I wherein R^3 is Cl, Br, methyl or trifluoromethyl;

(d) A compound of Formula I wherein R^3 is Cl;

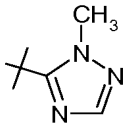
(e) A compound of Formula I wherein R^3 is Br;

(f) A compound of Formula I wherein R^4 is R^aR^bN-, pyridin-4-yl, morpholin-4-yl,



or ;

(g) A compound of Formula I wherein R^4 is morpholin-4-yl;

(h) A compound of Formula I wherein R^4 is  ;

(i) A compound of Formula I wherein R^a and R^b are independently C_1-C_3alkyl;

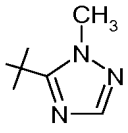
(j) A compound of Formula I wherein R^1 and R^2 are ethyl, R^3 is Cl, and R^4 is morpholin-4-yl;

(k) A compound of Formula I wherein R^1 and R^2 are *n*-propyl, R^3 is Cl, and R^4 is morpholin-4-yl;

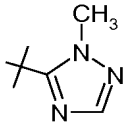
(l) A compound of Formula I wherein R^1 and R^2 are ethyl, R^3 is Br, and R^4 is morpholin-4-yl;

(m) A compound of Formula I wherein R^1 and R^2 are *n*-propyl, R^3 is Br, and R^4 is morpholin-4-yl;

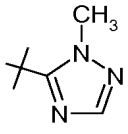
(n) A compound of Formula I wherein R^1 and R^2 are ethyl, R^3 is Cl, and R^4 is

 ;

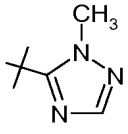
(o) A compound of Formula I wherein R^1 and R^2 are *n*-propyl, R^3 is Cl, and R^4 is

 ;

(p) A compound of Formula I wherein R^1 and R^2 are ethyl, R^3 is Br, and R^4 is

 ;

(q) A compound of Formula I wherein R^1 and R^2 are *n*-propyl, R^3 is Br, and R^4 is

 ;

(r) Use of a compound of Formula I , or a pharmaceutically acceptable salt thereof, for treating depression or anxiety;

(s) Use of a compound of Formula I, or a pharmaceutically acceptable salt thereof, for treating alcohol or substance abuse;

(t) Use of a comound of Formula I, or a pharmaceutically acceptable salt thereof, for treating alcohol or substance abuse and associated withdrawal symptoms;

(u) A compound of Formula I, or a pharmaceutically acceptable salt thereof, exhibiting a Ki value for CRF1 binding of ≤ 500 nM;

(v) A compound of Formula I, or a pharmaceutically acceptable salt thereof, exhibiting a Ki value for CRF1 binding of ≤ 50 nM;

(w) A compound of Formula I, or a pharmaceutically acceptable salt thereof, exhibiting a Ki value for CRF1 binding of ≤ 5 nM;

(x) A compound of Formula I, or a pharmaceutically acceptable salt thereof, exhibiting a Ki value for CRF1 binding of ≤ 500 nM and selectively binding to CRF1 (i.e., lower Ki) relative to CRF2;

(y) A compound of Formula I, or a pharmaceutically acceptable salt thereof, exhibiting a Ki value for CRF1 binding of ≤ 50 nM and selectively binding to CRF1 (i.e., lower Ki) relative to CRF2;

(z) A compound of Formula I, or a pharmaceutically acceptable salt thereof, exhibiting a Ki value for CRF1 binding of ≤ 5 nM and selectively binding to CRF1 (i.e., lower Ki) relative to CRF2; and/or

(aa) Particular exemplified compounds with superior bioavailability and pharmacokinetic profiles relative to some known CRF antagonists (e.g., CP154526 and NBI30775), such as Example 15.

The compounds of the present invention are preferably formulated as pharmaceutical compositions administered by a variety of routes. Preferably, such compositions are for oral administration. Such pharmaceutical compositions and processes for preparing same are well known in the art (see, e.g., *Remington: The Science and Practice of Pharmacy*, A. Gennaro, et al., eds., 19th ed., Mack Publishing Co., 1995).

The compounds of Formula I are generally effective over a wide dosage range. For example, dosages per day normally fall within the range of about 0.0001 to about 30

mg/kg of body weight. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect, and therefore the above dosage range is not intended to limit the scope of the invention in any way. It will be understood that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound or compounds administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms.

The compounds of Formula I are CRF-1 antagonists and, as such, are useful for treating a condition which is treatable by reducing CRF1 receptor tone or stimulation. Corticotropin releasing factor (CRF), a 41 amino acid peptide that is the primary physiological regulator of proopiomelanocortin (POMC) derived peptide secretion from the anterior pituitary gland [J. Rivier et al., *Proc. Natl. Acad. Sci* (*USA*) 80:4851 (1983); W. Vale et al., *Science* 213:1394 (1981)], has been linked to a number of medical conditions. For example, in addition to its endocrine role at the pituitary gland, immunohistochemical localization of CRF has demonstrated that the hormone has a broad extrahypothalamic distribution in the central nervous system and produces a wide spectrum of autonomic, electrophysiological and behavioral effects consistent with a neurotransmitter or neuromodulator role in the brain [W. Vale et al., *Rec. Prog. Horm. Res.* 39:245 (1983); G. F. Koob, *Persp. Behav. Med.* 2:39 (1985); E. B. De Souza et al., *J. Neurosci.* 5:3189 (1985)]. There is also evidence that CRF plays a significant role in integrating the response in the immune system to physiological, psychological, and immunological stressors [see, e.g., J. E. Blalock, *Physiological Reviews* 69:1 (1989); J. E. Morley, *Life Sci.* 41:527 (1987)].

CRF is involved in psychiatric disorders and neurological diseases including depression and anxiety [D. M. Nielsen, *Life Sci.* 78:909-919; H. E. Kunzel et al., *J. Psychiatr. Res.* 37:525-533; D. R. Gehlert et al., *Eur. J. Pharmacol.* 509:145-153]. A role for CRF has also been postulated in the etiology and pathophysiology of Alzheimer's disease, Huntington's disease, progressive supranuclear palsy and amyotrophic lateral sclerosis, as they relate to the dysfunction of CRF neurons in the central nervous system [for a review, see: E. B. De Souze, *Hosp. Practice* 23:59 (1988)]. Chronic administration of CRF has been shown to produce impairment of the dopamine system suggesting a role

in Parkinson's disease [E. Izzo et al., *Pharmacol. Biochem. Behav.* 81:701-708 (2005)]. Other neurological disorders in which CRF is involved include epilepsy [T. Z. Baram et al., *Brain Res.* 770:89-95 (1997)] and migraine [T. C. Theoharides et al., *Endocrinology* 136:5745-5750 (1995)]. CRF has been implicated in alcohol and substance abuse and associated withdrawal symptoms [D. H. Overstreet et al., *Pharmacol. Biochem. Behav.* 77:405-413; Y. Shaham et al., *Psychopharmacology (Berl)* 137:184-190]. Moreover, there is evidence that CRF has a role in various endocrine disorders and cardiovascular diseases such as obesity [E. Timofeeva and D. Richard, *Neuroendocrinology* 66:327-340 (1997)], metabolic syndrome [A. M. Ward et al., *Metabolism* 53:720-726(2004)], congenital adrenal hyperplasia [D. P. Merke and G. B. Cutler Jr., *Endocrinol. Metab. Clin. North Am.* 30:121-135 (2001)], Cushing's disease [M. Labeur et al., *Curr. Drug Targets Immune Endocr. Metabol. Disord.* 4:335-342 (2004)], hypertension [R. J. Briscoe, et al., *Brain Res.* 881:204-207 (2000)], and stroke [S. L. Stevens et al., *J. Cereb. Blood Flow Metab.* 23:1151-1159 (2003)]. Gastric disturbances such as irritable bowel syndrome [Y. Tache et al., *Eur J. Surg.* Suppl:16-22 (2002)] and stress-induced gastric ulceration [K. E. Gabry et al., *Mol. Psychiatry* 7:474-483, 433 (2002)] have been shown to be related to CRF. In addition, there is indication that CRF has a role in various areas of human female health, for example, premenstrual syndrome [F. Facchinetti et al., *Psychosom. Med.* 56:418-422 (1994)], infertility [L. Ghizzoni et al., *Endocrinology* 138:4806-4811 (1997)], sexual dysfunction [J. E. Jones et al., *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 283:R591-597 (2002)], and premature labor [P. D. Wadhwa et al., *Am. J. Obstet. Gynecol.* 191:1063-1069 (2004)]. There is also evidence that CRF has a significant role in the immune system indicating therapeutic potential for treating inflammatory disorders [A. Gravanis and A. N. Margioris, *Curr. Med. Chem.* 12:1503-1512 (2005)], allergies [L. K. Singh et. al., *Brain Behav. Immun.* 13:225-239 (1999)], multiple sclerosis and other autoimmune disorders [C. Benou et al., *J. Immunol.* 174:5407-5413 (2005)]. In addition to the preceding, CRF has been implicated in visceral pain [M. Nijsen et al., *Neurogastroenterol. Motil.* 17:423-432 (2005)], sleep disorders [T. M. Buckley and A. F. Schatzberg, *J. Clin. Endocrinol. Metab.* 90:3106-3114(2005)], pituitary tumors or ectopic pituitary-derived tumors [K. D. Dieterich et al., *J. Clin. Endocrinol. Metab.* 83:3327-3331 (1998)], chronic fatigue syndrome and

fibromyalgia [G. Neeck and L. J. Crofford, *Rheum. Dis. Clin. North Am.* 26:989-1002 (2000)].

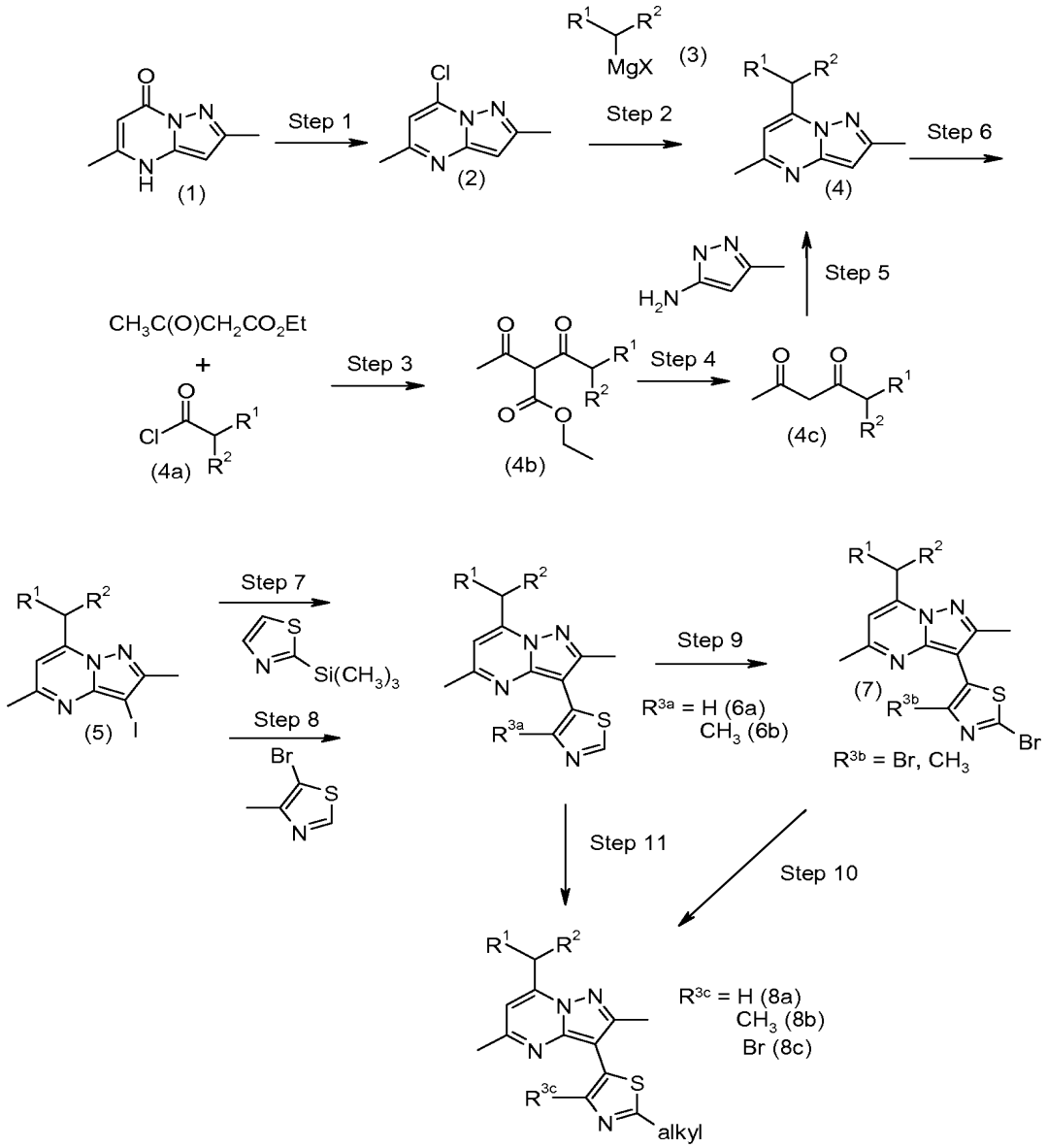
CRF receptor subtypes, CRF1 and CRF2, have been identified and are distributed heterogeneously within the brain [D. T. Chalmers et al., *TIPS* 17:166-72 (1996)] thereby suggesting potential functional diversity [S. C. Heinrichs et al., *Regul. Peptides* 71:15 (1997)]. For example, widely distributed brain CRF1 receptors are strongly implicated in emotionality accompanying exposure to environmental stressors [G. Liebsch et al., *Regul. Peptides* 59: 229-39 (1995); D. W. Schulz, *PNAS* 93: 10477-82 (1996)]. Significantly, CRF1, not CRF2, receptors appear to mediate select anxiogenic like behaviors [Heinrichs et al., 1997]. A more discrete septal/hypothalmic distribution [D. T. Chalmers et al., *J. Neurosci.* 15(10): 6340-50 (1995)] and the availability of alternative endogenous ligands [J. Vaughan et al., *Nature* 378: 287-92 (1995)] suggest a different functional role for the CRF2 receptor [Heinrichs et al., 1997]. For example, a novel CRF-family neuropeptide with preferential affinity for CRF2 relative to CRF1 receptors is reported to suppress appetite without producing the profile of behavioral activation observed with selective CRF1 agonism (H. Tezval et al., *PNAS* 101(25): 9468-9473 (2004)]. In some cases, CRF2 agonism produces similar effects to those reported for CRF1 antagonists or CRF1 gene deletion [S. C. Heinrichs, *Trends in Pharmacological Sciences* 20(8):311-5 (1999)]. For example, while CRF2 agonists have been proposed as antiobesity agents, CRF1 antagonists may be an important treatment for obesity as well [C. Contoreggi et al., *Neuroendocrinology* 80(2):111-23 (2004)].

PREPARING COMPOUNDS OF THE INVENTION

All of the compounds of the present invention can be chemically prepared, for example, by following the synthetic routes set forth below. However, the following discussion is not intended to be limiting to the scope of the present invention in any way. For example, the specific synthetic steps for each of the routes described may be combined in different ways, or in conjunction with steps from different schemes, to prepare additional compounds of Formula I. The products of each step can be recovered by conventional methods including extraction, evaporation, precipitation, chromatography, filtration, trituration, crystallization, and the like. In the Schemes below

all substituents, unless otherwise indicated, are as previously defined and suitable reagents are well known and appreciated in the art.

Scheme 1



Formation of a compound of formula (6a,b), (7), or 8(a-c) can be carried out in accordance with reactions as depicted in Scheme 1. An appropriate compound of formula

(6a,b), (7), or (8a-c) is one in which R^1 and $R^2$are as defined for formula I and R^{3a} = H or CH_3, R^{3b} = Br or CH_3 and R^{3c} = H, CH_3, or Br.

In Scheme 1, Step 1, the pyrazolo[1,5-a]pyrimidine-7-one of formula (1) is converted to 7-chloro-2,5-dimethyl- pyrazolo[1,5-a]pyrimidine using phosphorous oxychloride and dimethylaniline in an inert solvent, such as toluene, at the reflux temperature of the solvent.

In Step 2, a Grignard reagent of formula (3) (X = Cl or Br) is reacted with the chloride of formula (2), in an inert solvent such as toluene, at reflux temperature to provide the 7-alkyl pyrazolopyrimidine of formula (4).

Alternatively, a 7-alkyl pyrazolopyrimidine of formula (4) can be obtained as shown in Steps 3, 4, and 5. In Step 3 ethyl acetoacetate is acylated with an acid halide of formula (4a) in the presence of magnesium chloride to provide a diketo-ester of formula (4b). The diketo-ester of formula (4b) is decarboxylated under Krapcho conditions to provide the diketone of formula (4c). For example, (4b) is heated in dimethyl sulfoxide at a temperature of about 130 to 170 °C to perform the decarboxylation. In Step 5, the diketone of formula (4c) is cyclized with 3-amino-5-methylpyrazole to give the 7-alkyl pyrazolopyrimidine of formula (4) in a protic solvent such as methanol, ethanol, or acetic acid. Preferred conditions use acetic acid at a temperature of about 0 to 60 °C.

The pyrazolopyrimidine of formula (4) is functionalized to an iodo pyrazolopyrimidine of formula (5) in Step 6 using an excess of N-iodosuccinimide in acetonitrile.

In Scheme 1, Step 7 or 8, the iodo pyrazolopyrimidine of formula (5) is reacted with a thiazole zinc halide in a Negishi cross-coupling reaction to provide a thiazolyl pyrazolopyrimidine of formula (6a) or (6b) (Jensen, J.; Skjaerbaek, N.; Vedso, P. *Synthesis* 2001, 128). The thiazole zinc halide is generated using methods well known to those skilled in the art. For example, in Step 7, 2-trimethylsilanylthiazole is treated with *n*-, *sec*-, or *tert*-butyl lithium, followed by lithium-zinc exchange with $ZnCl_2$. The organozinc reagent is coupled with the iodo pyrazolopyrimidine of formula (5) in the presence of a palladium catalyst, for example, dichloro[1,1'-bis(diphenyl-phosphino)ferrocene]palladium (II) dichloromethane, in an inert solvent, such as THF, at reflux temperature for about 12 to 36 hours, to provide a thiazole pyrazolopyrimidine of formula (6a).

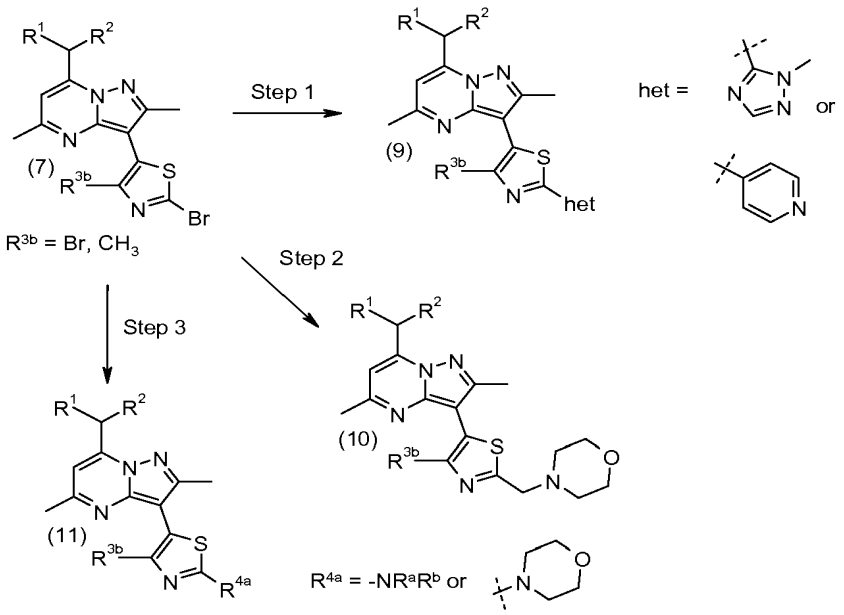
Alternatively, in Step 8, a thiazole zinc bromide is generated using 5-bromo-4-methylthiazole and zinc metal, and used in the Negishi cross-coupling essentially as described for Step 7, to provide a thiazole pyrazolopyrimidine of formula (6b).

In Scheme 1, Step 9, a thiazole of formula (6a,b) is brominated to give a bromo or dibromo thiazole of formula (7), wherein R^{3b} = Br or CH_3. The thiazole is brominated with either 1 or 2 eq of N-bromosuccinimide, depending on whether R^{3a} is CH_3 or H, respectively.

An alkyl thiazole of formula (8a-c) is obtained from either a thiazole of formula (6a,b) in Step 11, or from a bromothiazole of formula (7) in Step 10. In Step 10, halogen-lithium exchange with *n*-, *sec*-, or *t*-butyl lithium provides a thiazole lithium reagent, which is subsequently reacted with electrophiles, such as alkylhalides, like iodomethyl methylether or iodobutane. In Step 11, the thiazole lithium reagent is formed via deprotonation using *n*-, *sec*-, or *tert*-butyl lithium and then subsequently reacted with an electrophile, like iodomethyl methylether or iodobutane.

It will be appreciated by one skilled in the art that the thiazole ring system is readily functionalized and that thiazole intermediates such as 2-trimethylsilanyl-thiazole (Dondoni, A.; et. al. *J. Org. Chem.* 1988, *53*, 1748) can be readily prepared. 5-Bromo-4-methylthiazole is obtained by bromination of 4-methylthiazole with bromine in acetic acid (Collins, I. J., et. al. WO2003093252, 13 Nov 2003). 2,5-Dimethyl-4*H*-pyrazolo[1,5-a]pyrimidine-7-one (1) is readily prepared by condensation of ethyl acetoacetate and 5-methyl-2*H*-pyrazol-3-ylamine in refluxing acetic acid.

Scheme 2



Formation of a compound of formula (9), (10), or (11) can be carried out in accordance with reactions as depicted in Scheme 2. An appropriate compound of formula (9), (10), or (11) is one in which R^1, R^2, R^a, and R^b are as defined for formula I and R^{3b} = Br or CH_3, and R^{4a} = -NR^aR^b or –N-morpholinyl and "het" is defined as depicted.

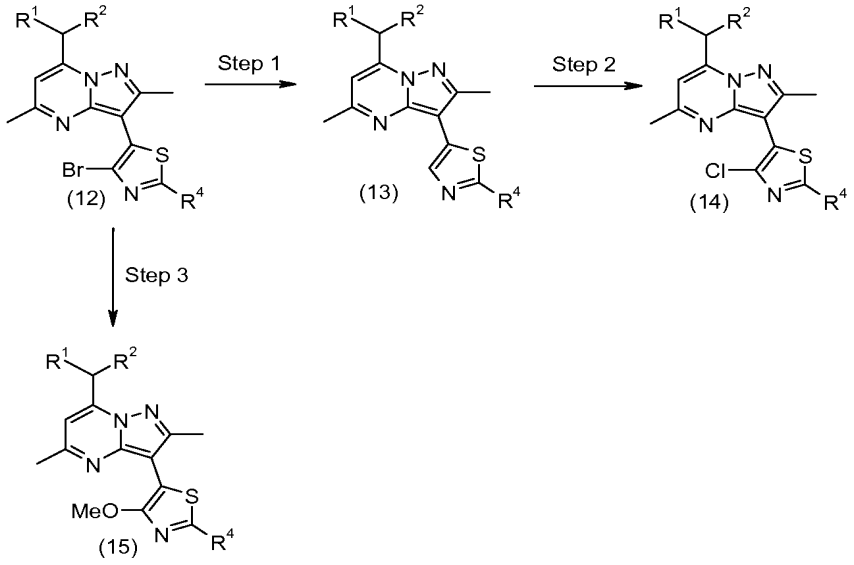
In Step 1, a bromothiazole of formula (7) is coupled with a heterocyclic zinc reagent in a Negishi cross-coupling reaction to provide a thiazole heterocycle of formula (9). For example, 1-methyl-1,2,4-triazole is treated with *n*-, *sec*-, or *tert*-butyl lithium, followed by zinc chloride, at about -80 to -65 °C, and reacted *in situ* with a bromothiazole of formula (7). The reaction is performed preferentially in an inert solvent, such as THF, in the presence of a palladium catalyst, such as dichloro[1,1'-bis(diphenyl-phosphino)ferrocene]palladium (II) dichloromethane or tetrakis(triphenylphosphine)palladium (0). The reaction is warmed to reflux temperature. Alternatively, the heterocyclic zinc reagent is formed from a haloheterocycle, such as 4-iodopyridine and zinc metal.

In Scheme 2, Step 2, an intermediate 2-formyl thiazole is formed via halogen-lithium exchange using *n*-, *sec*-, or *t*-butyl lithium followed by reaction with N-formyl morpholine. The formyl thiazole is subjected to a reductive amination in the presence of an organic amine, such as morpholine, to provide a morpholinyl methyl thiazole of formula (10). Reductive aminations are well-known in the art typically using an

inorganic borohydride reagent such as sodium borohydride or sodium cyanoborohydride. Preferred conditions use sodium triacetoxyborohydride in an inert solvent such as dichloromethane or THF.

In Step 3, a bromothiazole of formula (7) undergoes a displacement reaction with an amine of formula $-NR^aR^b$ or with morpholine to provide an aminothiazole of formula (11). The reaction is preformed in an inert solvent, such as THF or dioxane, using an inorganic base, such as cesium carbonate at 70 to 110 °C.

Scheme 3



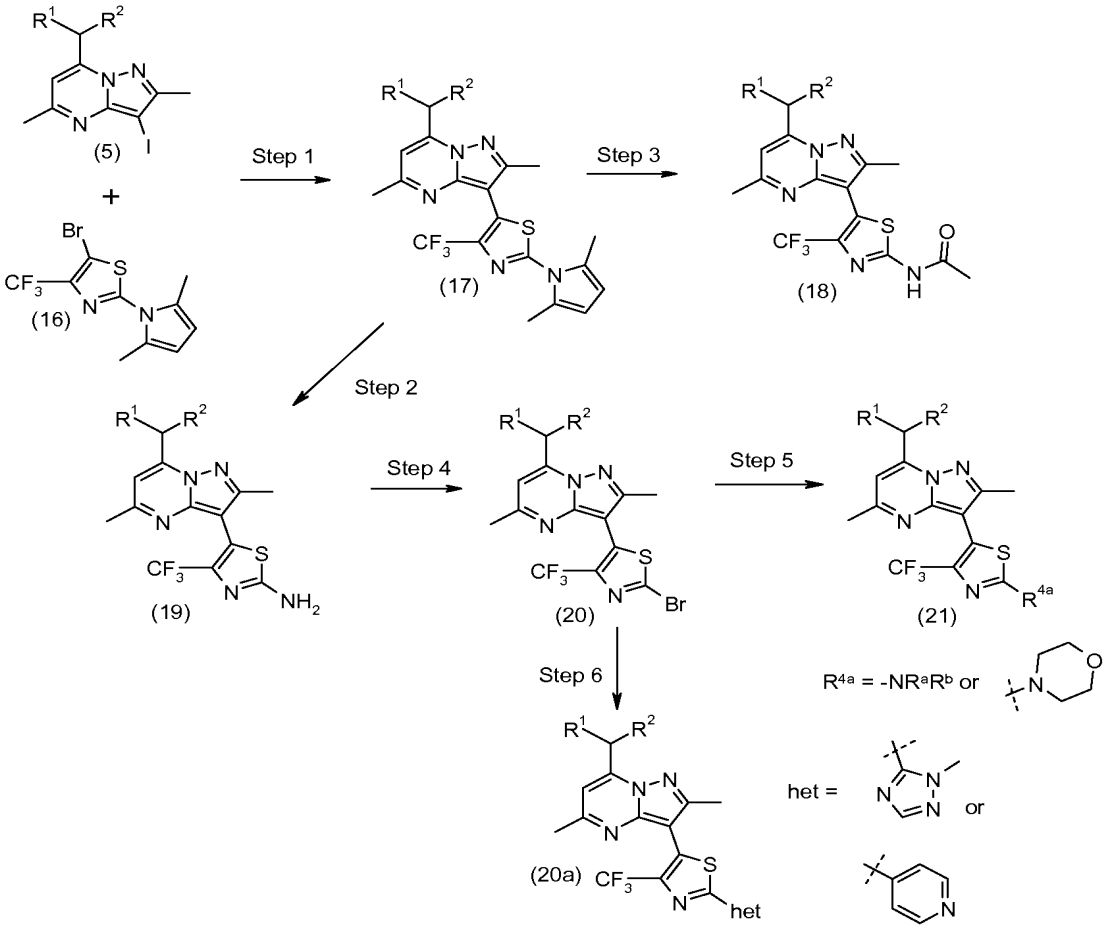
Formation of a compound of formula (13), (14), or (15) can be carried out in accordance with reactions as depicted in Scheme 3. An appropriate compound of formula (13), (14), or (15) is one in which R^1, R^2, and R^4 are as defined for formula I.

It will be recognized by one skilled in the art that a 4-bromothiazole, such as that of formula (12) is readily manipulated to other functionality. For example, in Step 1, the bromide can be dehalogenated with cuprous chloride to give a thiazole of formula (13) which is subsequently chlorinated with N-chlorosuccinimide to provide a 4-chlorothiazole of formula (14).

In Scheme 3, Step 3, a 4-bromothiazole of formula (12) is displaced with sodium methoxide in the presence of copper (I) iodide in methanol at about 100 to 120 °C in an

inert solvent such as dimethylformamide, to provide a 4-methoxy thiazole of formula (15).

Scheme 4



Formation of a compound of formula (17), (18), (19), (20), (20a), or (21) can be carried out in accordance with reactions as depicted in Scheme 4. An appropriate compound of formula (17), (18), (19), (20), (20a), or (21) is one in which R^1, R^2, R^a, and R^b are as defined for formula I and R^{4a} is $-NR^aR^b$ or –N-morpholinyl and "het" is defined as depicted.

In Scheme 4, Step 1, an iodopyrazolopyrimidine of formula (5) and a 5-bromothiazole of formula (16) undergo a Negishi cross-coupling to form a dimethylpyrrolylthiazole of formula (17). For example, the 5-bromothiazole of formula

(16) is treated with *n*-, *sec*-, or *tert*-butyl lithium and then with zinc chloride at about -80 to -65 °C. The organozinc reagent is reacted *in situ* with an iodopyrazolopyrimidine of formula (5). The coupling reaction is performed preferentially in an inert solvent, such as THF, at reflux temperature, in the presence of a palladium catalyst, such as bis(tri-*t*-butylphosphine)palladium (0).

In Step 2, a dimethylpyrrolylthiazole of formula (17) is deprotected to provide an aminothiazole of formula (19). The dimethylpyrrole is treated with hydroxylamine in acetic acid at a temperature of about 60 to 100 °C, for about 4 to 8 h, preferably for about 6 h. In Step 3, to form the thiazole acetamide of formula (18), the same conditions are used as in Step 2, with the exception that the reaction is continued for about 72 h.

In Scheme 4, Step 4, a 2-aminothiazole of formula (19) is converted to a 2-bromothiazole of formula (20) using a modified Sandmeyer reaction. Preferred conditions use an alkylnitrite, such as *t*-butylnitrite, and copper (II) bromide, in acetonitrile at a temperature of about 60 to 80 °C.

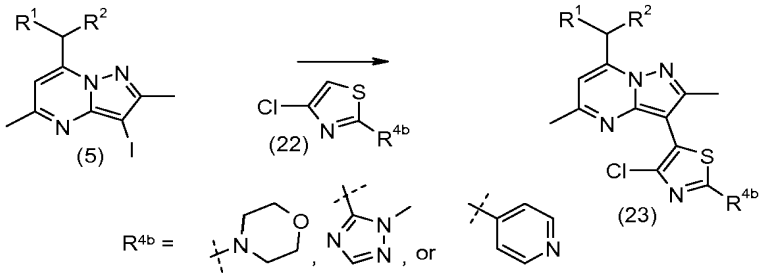
In Step 5, a 2-bromothiazole of formula (20) undergoes a displacement reaction with an amine of formula $-NR^aR^b$ or with morpholine to provide an aminothiazole of formula (21). The reaction is performed in an inert solvent, such as methanol, THF, or dioxane, or is treated neat, with an excess of the amine at a temperature of about 70 to 110 °C. Alternatively the reaction is performed with the reacting amine and an excess of triethylamine, or an inorganic base, such as cesium carbonate. Also contemplated in the synthesis of aminothiazoles of formula (21) are various deprotection steps, such as removal of a *tert*-butyl ester carbamic acid (BOC), as may be required or beneficial for carrying out the reactions above wherein $-NR^aR^b$ carries additional amine functionality as defined for formula I. The selection and use of suitable protecting groups is well known and appreciated in the art (see for example, *Protecting Groups in Organic Synthesis*, Theodora Greene (Wiley-Interscience)).

In Scheme 4, Step 6, a 2-bromothiazole of formula (20) is coupled with a heterocyclic zinc reagent in a Negishi cross-coupling reaction to provide a thiazole heterocycle of formula (20a) in a manner similar to that as described for Scheme 2, Step 1.

It will be appreciated by the skilled artisan that a functionalized thiazole of formula (16) can be prepared by means known in the art. For example, cyclization of

thiourea with a bromoketone, such as 3-bromo-1,1,1-trifluoropropan-2-one, provides 4-trifluoromethyl-thiazole-2-ylamine. Subsequent bromination and protection of the amine using hexane-2,5-dione provides (16).

Scheme 5

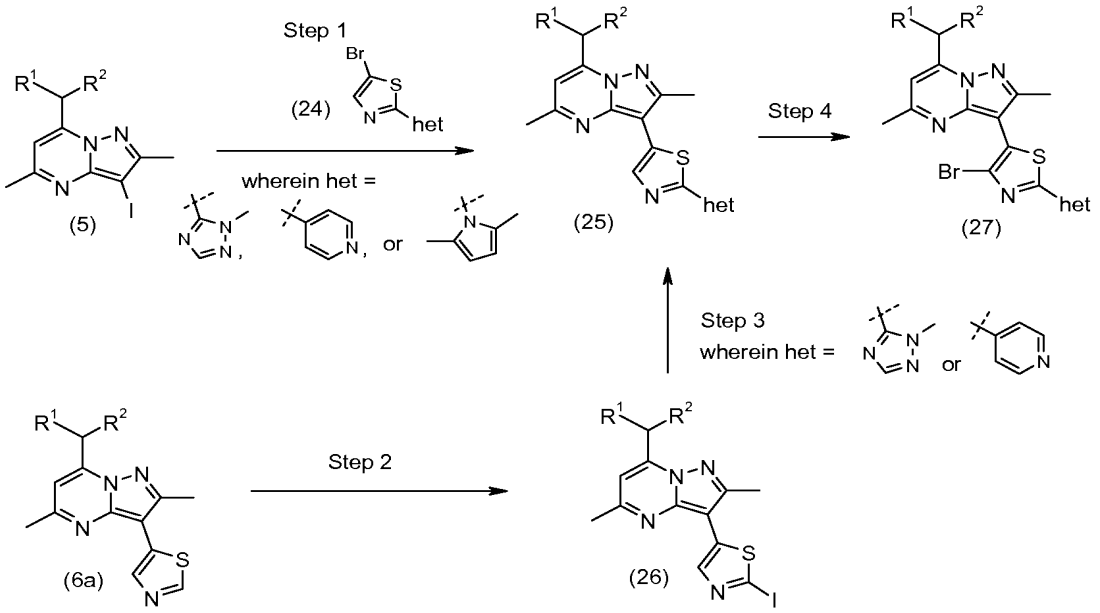


Formation of a compound of formula (23) can be carried out in accordance with reactions as depicted in Scheme 5. An appropriate compound of formula (23) is one in which R^1 and R^2 are as defined for formula I and R^{4b} is defined as depicted.

An iodopyrazolopyrimidine of formula (5) and a 4-chloro-2-morpholino-thiazole, for example, of formula (22) undergo cross-coupling to form a pyrazolopyrimidine thiazole of formula (23). For example, the reactants are coupled in the presence of copper iodide, palladium acetate, and triphenylphosphine, with a base such as cesium carbonate. The coupling reaction is performed preferentially in an inert solvent, such as DMF, at about 100 – 150 °C for 4 – 24 h.

It will be appreciated by the skilled artisan that a functionalized thiazole of formula (22) can be prepared by means known in the art. For example, 2,4-dichlorothiazole can be reacted with morpholine to give the 2-morpholino-thiazole of formula (22). 2,4-Dichlorothiazole can also be exhaustively brominated to provide 2,5-dibromo-4-chlorothiazole. Subsequent bromine-lithium exchange with *n*-butyl lithium in THF at -90 °C and quench with water provides 2-bromo-4-chlorothiazole [*J. Chem. Soc. Perkin Trans I: Org Bioorg. Chem.* (1972-1999), (2):215-219 (1992)]. 2-Bromo-4-chlorothiazole can be subjected to a Negishi cross-coupling reaction with a heterocyclic zinc reagent to obtain the thiazol-2-yl triazole or pyridine.

Scheme 6



Formation of a compound of formula (27) can be carried out in accordance with reactions as depicted in Scheme 6. An appropriate compound of formula (27) is one in which R^1 and R^2 are as defined for formula I and "het" is defined as depicted for Step 1 or Step 3, respectively.

In Scheme 6, Step 1, a heterocyclic thiazole, for example, of formula (25) is obtained by reaction of an iodopyrazolopyrimidine of formula (5) with a bromothiazole of formula (24). The reaction is performed in the presence of N-butylammonium bromide and a base, such as potassium acetate with a palladium catalyst such as palladium acetate with tris(2,4-di-*tert*-butyl-phenyl)-phosphane, in an inert solvent such as N-methylpyrrolidinone at a temperature of about 100 – 150 °C.

Alternatively, in Step 2, a thiazolyl pyrazolopyrimidine of formula (6a) is iodinated to provide a 2-iodothiazole of formula (26). The thiazole is treated with lithium diisopropylamide at a temperature of -70 to -80 °C for about one hour and then treated with N-iodosuccinimide at about the same temperature in an inert solvent, such as THF. This is followed by Step 3, wherein a triazolyl or 4-pyridyl thiazole of formula (25) is formed using Negishi cross-coupling conditions similar to those described for Scheme 2, Step 1.

In Scheme 6, Step 4, a thiazolyl pyrazolopyrimidine of formula (25) is brominated to give a bromothiazole of formula (27). The bromination is effected using N-

bromosuccinimide in the presence of a small amount of acetic acid in an inert solvent, such as acetonitrile.

A compound of formula (27), wherein het = 2,5-dimethyl-pyrrol-1-yl, can be further elaborated to obtain compounds of the invention as described in Scheme 4, Steps 2, 3, 4, and 5.

It will be appreciated by the skilled artisan that heterocycle thiazoles of formula (24) can be readily prepared by means known in the art. For example, thiazole-2-carboxylic acid amide can be cyclized to the triazole with 1,1-dimethoxy-N,N-dimethyl-methanamine, followed by N-methyl-hydrazine to obtain the triazolyl thiazole, which can be subsequently brominated with N-bromosuccinimide. Literature procedures afford 4-thiazol-2-yl-pyridine which can be brominated to provide 4-(5-bromo-thiazol-2-yl)-pyridine. 5-Bromo-2-(2,5-dimethyl-pyrrol-1-yl)-thiazole can be readily obtained by reaction of 2-amino-5-bromothiazole with hexane-2,5-dione.

As used herein, "TLC" refers to thin layer chromatography; "HPLC" refers to high performance liquid chromatography; "LC/MS" refers to liquid chromatography/mass spectrometry; "GC/MS" refers to gas chromatography/mass spectrometry"; "HR-ToF' refers to high resolution time-of-flight; "APCI" refers to atmospheric pressure chemical ionization; "δ" refers to part per million down-field from tetramethylsilane; "THF" refers to tetrahydrofuran; "EtOAc" refers to ethyl acetate; "MeOH" refers to methanol; "EtOH" refers to ethanol; "DMF" refers to dimethylformamide.

EXAMPLES

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, practice the present invention to its fullest extent. The following preparations and examples are provided to describe the invention in further detail. They are intended to illustrate and not to limit the invention in any way whatsoever. The reagents and starting materials are readily available to, or may be readily synthesized by, one of ordinary skill in the art. Examples 1- 35 provide representative compounds and illustrate the preparation thereof. Examples A-D illustrates various biological assays that can be used for determining the biological properties of the compounds of the inventions. Those skilled in the art will promptly recognize appropriate variations from the procedures described in the examples. The names of the compounds of the present

invention are provided by ChemDraw Ultra® version 7.0.1. Salts are named as the free base plus the conjugate acid.

Preparation 1

2,5-Dimethyl-4H-pyrazolo[1,5-*a*]pyrimidin-7-one

Add ethyl acetoacetate (128 g, 0.98 mol) dropwise to an acetic acid solution (500 mL) of 5-methyl-2*H*-pyrazol-3-ylamine (100 g, 0.95 mol), keeping the temperature at 25-28 °C. Heat the mixture at reflux for 10 h and then cool to room temperature. Add the reaction to *tert*-butyl methyl ether (5 L) cooled to 5 °C, keeping the temperature below 10 °C. Stir for 1 h at 5 °C, and filter. Dry the resulting material *in vacuo* overnight to provide a white solid (158 g, 96%).

Preparation 2

7-Chloro-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

To a suspension of 2,5-dimethyl-4*H*-pyrazolo[1,5-*a*]pyrimidin-7-one (10.0 g, 61.3 mmol) in toluene (150 mL) add *N,N*-dimethylaniline (9.7 mL, 76.7 mmol). Add phosphorus oxychloride (11.2 mL, 122.6 mmol) dropwise to this white suspension. Reflux for 3 h under an inert atmosphere, cool to room temperature, and concentrate the reaction to a brown oil using reduced pressure. Dissolve the oil in ethyl acetate (250 mL) and basify with 1.0 N NaOH. Separate the organic phase and extract the basic aqueous phase with ethyl acetate (2 × 100 mL). Combine the organic phases, dry over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure to yield a brown solid. Purify the material using flash chromatography, eluting with 80% hexane/20% (30% THF/hexane) to 0% hexane/100% (30% THF/hexane) in a step gradient of 20% increments to provide a light green solid (6.65 g, 59%). ES/MS m/z (^{35}Cl) 182.3 $(M+1)^+$.

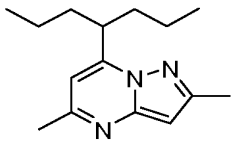
Alternate Procedure:

Add 2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-one (20 g, 122 mmol) to 1,4-dioxane (60 mL). Stir the mixture at 22 °C for 10 min and then add N,N−diethylaniline (20.8 mL, 128 mmol). Stir for an additional 5 min and then add phosphorus oxychloride (11.7 mL, 126 mmol) over 15 min. Stir the mixture at 22 °C for 15 min, then heat to 80-85 °C over 35 min and hold the reaction at this temperature for 1.5 h. Add the cooled reaction mixture slowly to a solution of potassium phosphate dibasic (106.7 g, 612.82

mmol) in water (325 mL) cooled to 0-5 °C, keeping the temperature below 5 °C during the addition. Stir the mixture at 22 °C and then add methyl-*t*-butyl ether (150 mL). Separate the organic layer and extract the aqueous layer with methyl-*t*-butyl ether (2 × 100 mL). Combine the organic portions, dry over sodium sulfate, filter, and evaporate the solvent. Purify by silica gel chromatography, eluting with hexanes/ethyl acetate (2/1) to provide the title compound as a yellow solid (20.7 g, 88%). ES/MS m/z (^{35}Cl) 182 $(M+1)^+$.

Preparation 3

7-(1-Propyl-butyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine



Charge an oven dried flask equipped with a reflux condenser, with anhydrous THF (40 mL), iodine (catalytic amount), magnesium ribbon (1.92 g, 78.9 mmol) and 4-bromoheptane (9.4 mL, 52 mmol). Heat the reaction to reflux in an oil bath. The temperature of the reaction spikes as the Grignard reaction is initiated. Stir the reaction an additional 4 h at 90 °C and cool to room temperature. Allow the magnesium metal to settle out (can also be centrifuged) and canulate off the Grignard reagent under positive argon pressure into a flask charged with 7-chloro-2,5-dimethylpyrazolo [1,5-*a*]pyrimidine (4.80 g, 26.3 mmol) in anhydrous toluene (20 mL). Reflux the reaction under an inert atmosphere overnight. Cool the reaction to room temperature and quench with water. Dilute with ethyl acetate (150 mL), then add water (100 mL) and saturated ammonium chloride (50 mL). Separate and extract the aqueous phase with dichloromethane (75 mL). Combine the organic phases, dry over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure. Purify the resulting residue using flash chromatography, eluting with 80% hexane/20% (20% ethyl acetate/hexane) to 0% hexane/100% (20% ethyl acetate/hexane) in a step gradient of 20% increments to give yellow crystals (3.08 g, 48%). ES/MS m/z 246.3 $(M+1)^+$.

Alternate Procedure:

Heat a mixture of magnesium turnings (3.5 g, 144 mmol) and a catalytic amount of iodine (100 mg) in THF (100 mL) to 65 °C under a nitrogen atmosphere. Add a few drops of neat 4-bromoheptane and heat the mixture until the reaction starts. Then add a solution of 4-bromoheptane (17.6 mL, 94.9 mmol) in THF (42 mL) keeping the temperature at 65–70 °C over 2 h. Reflux the mixture for an additional hour and then cool the reaction to 22 °C. Add the prepared Grignard reagent to a solution of 7-chloro-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (10.2 g; 53.3 mmol) in THF (60 mL) cooled to 0 °C under a nitrogen atmosphere. Add the magnesium reagent solution via cannula over 45 min while keeping the temperature below 10 °C. Then stir the mixture for an additional 30 min at 5 °C. Add to this mixture a 10% aqueous ammonium chloride solution (wt/wt) (125 mL) and stir at 22 °C for 30 min. Separate the organic layer and extract the aqueous layer with ethyl acetate (2 × 25 mL). Combine the organic layers and dry over sodium sulfate. Filter the mixture and evaporate the solvent. Purify the crude material by silica gel flash chromatography using an eluent of hexanes/ethyl acetate (5/1) to provide the title compound (8 g, 62%). ES/MS m/z 246 $(M+1)^+$.

Preparation 3a

7-(1-Propyl-butyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

Alternate Route

Step 1: 3-Acetyl-3-oxo-4-propyl-heptanoic acid ethyl ester

Add magnesium chloride (14.63 g, 153.70 mmol) to dichloromethane (500 mL) followed by addition of ethyl acetoacetate (19.55 mL, 20.00 g, 153.79 mmol) all at once and stir at room temperature for one hour. Cool the mixture in an ice water bath and and add pyridine (24.86 mL, 24.32 g, 307.39 mmol) dropwise. Add di-*n*-propylacetyl chloride (25.00 g, 153.70 mmoles) dropwise at 0 °C under nitrogen to the white slurry. After the addition is complete remove the cooling bath, warm to ambient temperature, and stir for 16 h. Quench the reaction with 1 N HCl (400 mL) and separate the bottom layer. Dry the organic portion over magnesium sulfate, filter, and concentrate under vacuum to give a yellow oil (34 g, 86%). Use the material directly in the next step without further purification.

Step 2: 5-Propyl-octane-2,4-dione

Dissolve 3-acetyl-3-oxo-4-propyl-heptanoic acid ethyl ester (32.4 g, 126.39 mmoles) in dimethyl sulfoxide (100 mL) and water (5 mL). Heat the solution at 150 °C

for 6 to 8 h or follow the reaction by GC/MS. Cool the reaction and extract with heptane (3 × 100 mL). Wash the combined organic portions with water (100 mL) and brine (100 mL). Concentrate under vacuum at 50 °C to remove most of the heptane. Obtain 23.29g of an oil and use directly in the next step.

Step 3: 7-(1-Propyl-butyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

Mix 5-propyl-octane-2,4-dione (15 g, 81.40 mmoles) in acetic acid (15 mL) and cool in an ice bath. Add 5-amino-3-methylpyrazole (7.91 g, 81.40 mmol) portionwise and stir at ambient temperature. Check for completion of the reaction by GC/MS after 3 h. GC indicates correct region isomer in comparison with an authentic sample. Distill off the excess acetic acid. Add water (50 mL) and extract with heptane (50 mL). Wash the heptane with brine (50 mL). Dry the organic layer over $MgSO_4$, filter, and concentrate under vacuum to give a crude oil (15.8 g, 79%). ^{1}H NMR ($CDCl_3$): 6.39 (s, 1H); 6.31 (s, 1H); 3.75 (m, 1H); 2.55 (s, 3H); 2.45 (s, 3H); 1.71 (q, 4H); 1.23 (m, 4H); 0.85 (t, 6H).

Prepare the following compound essentially as described in Preparation 3, using either procedure. Use 3-bromopentane to prepare the Grignard reagent.

Prep. No.	Chemical name	Physical data
4	7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine	ES/MS m/z 218.1 $(M+1)^+$

Preparation 5

7-(1-Propyl-butyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-*a*] pyrimidine

Dissolve 7-(1-propyl-butyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (3.08 g, 12.5 mmol) in anhydrous acetonitrile (25 mL) and add 6 portions (0.70 g each) of N-iodosuccinimide (4.2 g, 18.7 mmol) at 10 minute intervals. Stir over the weekend at room temperature. Strip off the acetonitrile and dilute the oil with dichloromethane (100 mL). Wash with saturated ammonium chloride solution (2 × 50 mL). Collect the organic phase, dry over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure to yield a dark red oil. Purify the oil using flash chromatography, eluting with 100% hexane/0% (20% ethyl acetate/hexane) to 0% hexane/100% (20% ethyl acetate/hexane) in a step gradient of 50% increments to give an orange oil (10.97 g, 87%).

^{1}H NMR (400 MHz, $CDCl_3$): 6.42 (s, 1H), 3.74-3.70 (m, 1H), 2.58 (s, 3H), 2.46 (s, 3H), 1.74-1.68 (m, 4H), 1.28-1.14 (m, 4H), 0.84 (t, J = 7.0 Hz, 6H).

Prepare the compound below essentially as described in Preparation 5.

Prep. No.	Chemical name	Physical data
6	7-(1-Ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-*a*] pyrimidine	1H NMR (400 MHz, $CDCl_3$): 6.44 (s, 1H), 3.59 (m, 1H), 2.61 (s, 3H), 2.49 (s, 3H), 1.86-1.76 (m, 4H), 0.85 (t, J = 7.5 Hz, 6H).

Alternate procedure for Preparation 6:

Add acetic acid (1 mL) and N-iodosuccinimide (6.7 g, 29.9 mmol) in one portion to a solution of 7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (6 g, 27.5 mmol) in acetonitrile (60 mL). Stir the mixture at 22 °C for 2 h. Evaporate the solvent and take the residue up in water (50 mL) and methyl-*t*-butyl ether (100 mL). Separate the organic portions, dry over sodium sulfate, filter, and evaporate the solvent to afford the title compound (9.2 g, 96%). ES/MS m/z 344 $(M+1)^+$.

Preparation 7

2-Trimethylsilanyl-thiazole

Mix *n*-butyl lithium (20.4 mL, 51.0 mmol, 2.5 M in hexane) with diethyl ether (50 mL) in a three-necked flask, equipped with a dropping funnel and thermometer. Cool to –78 °C and add dropwise a solution of thiazole (4.25 g, 50.0 mmol) in diethyl ether (50 mL). After the addition is complete, stir the reaction mixture at –78 °C for 30 min, followed by addition of chlorotrimethylsilane (5.4 g, 50 .0 mmol). Stir at –78 °C for an hour and then warm to room temperature. Quench the reaction by adding saturated sodium bicarbonate. Extract the aqueous layer with diethyl ether. Wash the combined organic portions with brine and dry over sodium sulfate. Filter and concentrate under reduced pressure to give a residue. Purify by distillation to give 8.33 g (52-56 °C/ 15 mm Hg) of title compound. ^{1}H NMR (400 MHz, $CDCl_3$) 8.13 (d, 1H, J=2.6 Hz), 7.54 (d, 1H, J = 2.6 Hz), 0.43 (s, 9H).

Preparation 8

5-Bromo-4-methylthiazole

Add bromine (9.27 mL, 182 mmol) to a solution of 4-methylthiazole (15.0 g, 152 mmol) in acetic acid (30 mL) at 0 °C. Slowly warm the reaction mixture to room temperature and stir overnight. Dilute with dichloromethane and wash with 1 N NaOH and brine. Dry the organic layer over sodium sulfate, filter, and concentrate under vacuum. Purify the crude product by silica gel column chromatography, elueting with hexanes/ethyl acetate (5/1) to obtain the title compound (9.94 g, 37%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.69 (s, 1H), 2.43 (s, 3H).

Preparation 9

Thiazole-2-carboxylic acid ethyl ester

To a mixture of 2-trimethylsilylthiazole (135 g, 858.1 mmol) in toluene (1350 mL) add a solution of ethyl chloroformate (98.4 mL, 1.03 mol) in toluene (1350 mL) over 15 min. Stir the reaction at 22 °C for 2 h. Add the solution over aqueous sodium carbonate 25% (wt/wt) (5 L) and stir for 30 min. Separate the organic layer and re-extract the aqueous layer with methylene chloride (2 × 1 L). Combine the organic layers and evaporate the solvent to provide the title compound (134 g, 99%). ES/MS m/z 158 $(M+1)^+$.

Preparation 10

Thiazole-2-carboxylic acid amide

Add thiazole-2-carboxylic acid ethyl ester (150 g, 0.9 mol) to a mixture of methanol (75 mL) and 30% aqueous ammonium hydroxide (750 mL) and heat the mixture at reflux for 1 h. Then cool to 22 °C and evaporate the methanol under vacuum. Stir the mixture for 30 min at room temperature and filter the solid. Dry the isolated solid under vacuum to afford the title compound (98 g, 85%). ES/MS m/z 129 $(M+1)^+$.

Preparation 11

1-Methyl-5-thiazol-2-yl-1H-[1,2,4]triazole

Cool 1,1-dimethoxy-*N*,*N*-dimethyl-methanamine (240 mL) to 10 °C and add thiazole-2-carboxylic acid amide (60 g, 421 mmol) in three portions. Stir the mixture at

10 °C for 30 min. Then heat the mixture gradually to reflux in 45 min. Distill off the methanol formed and then heat the reaction to 100 °C for 1.5 h. Cool the mixture to 60 °C and remove the excess 1,1-dimethoxy-N,N-dimethyl-methanamine by vacuum distillation. Cool the residue to 22 °C and add hexanes (200 mL). Triturate the mixture for 15 min, filter, and dry the solid to constant weight before using in the next step.

Add the solid isolated above (68 g) to acetic acid (680 mL) and cool the mixture to 10 °C. Add *N*-methyl-hydrazine (27 mL, 509 mmol) at such a rate as to keep the temperature below 15 °C. Warm the mixture to 20 °C in 30 min and then heat gradually to 90 °C. Stir at 90 °C for 30 min and then cool to 22 °C. Remove the acetic acid by vacuum distillation. Add the residue over water and adjust to pH = 8 by adding 25% aqueous sodium hydroxide solution. Extract the aqueous layer with methyl-*t*-butyl ether (3 × 600 mL). Combine the organic layers and evaporate the solvent. Purify the resulting residue by silica gel chromatography using an eluent of hexanes/isopropanol (9/1) to provide the title compound (49 g, 70%). ES/MS m/z 167 $(M+1)^+$.

Preparation 12

5-(5-Bromo-thiazol-2-yl)-1-methyl-1*H*-[1,2,4]triazole

To a mixture of methyl-5-thiazol-2-yl-1H-[1,2,4]triazole (6.55 g; 39.4 mmol) and dimethylformamide (32 mL), add N-bromosuccinimide (14 g, 78.8 mmol) in three portions over 1 h. Stir the mixture at 22 °C for 18 h and then add to water (300 mL) chilled to 0–5 °C. Separate the aqueous layer and extract with methyl-*t*-butyl ether (2 × 200 mL). Combine the organic layers and wash with 7% aqueous sodium hydrogen carbonate (100 mL) and then dry over sodium sulfate. Filter and evaporate the solvent to afford the title compound (9.5 g, 93%). ES/MS m/z ($^{79}Br/^{81}Br$) 245/247 $(M+1)^+$.

Preparation 13

2,5-Dimethyl-3-[2-(2-methyl-2*H*-[1,2,4]triazol-3-yl)-thiazol-5-yl]-7-(1-propyl-butyl)-pyrazolo[1,5-*a*]pyrimidine

Combine 5-(5-bromo-thiazol-2-yl)-1-methyl-1H-[1,2,4]triazole (6.5 g, 23.8 mmol) and 2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*]pyrimidine (6 g, 24.5 mmol) in N-methylpyrrolidinone (58 mL) and stir to complete solution under nitrogen. Then add tetra-N-butylammonium bromide (5.47 g, 16.7 mmol) and potassium acetate (11.8 g, 119

mmol) and heat the mixture to 100 °C under a nitrogen atmosphere. Degas the hot mixture by three cycles of vacuum/nitrogen purge. Then add palladium acetate (216 mg, 0.94 mmol) and tris(2,4-di-*tert*-butyl-phenyl)-phosphane (787 mg, 1.2 mmol). Heat the mixture 4 h at 125 °C under nitrogen. Cool the mixture to 22 °C and add to water (750 mL). Extract the aqueous layer with methyl-*t*-butyl ether (3 × 200 mL), combine the organic portions, and evaporate. Purify the residue by filtration through a silica gel pad eluting with hexanes/ethyl acetate (4/1). Combine the product containing fractions and evaporate the solvent to afford the title compound (7 g, 72%). ES/MS m/z 410 $(M+1)^+$.

Preparation 14

2,5-Dimethyl-7-(1-propyl-butyl)-3-thiazol-5-yl-pyrazolo[1,5-*a*] pyrimidine

Charge an oven dried flask with 2-trimethylsilanylthiazole (1.765 g, 11.24 mmol) dissolved in anhydrous THF (30 mL) and chill under an inert atmosphere to -78 °C. Slowly add *n*-butyl lithium (2.5 M hexane solution, 4.5 mL, 11.24 mmol) and stir 30 min at -78 °C. Add anhydrous zinc chloride (2.26 g, 16.58 mmol) in one aliquot and stir 30 min at -78 °C. Allow the reaction to rise to room temperature, stir 30 min, and add 7-(1-propyl-butyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-*a*] pyrimidine (1.624 g, 5.18 mmol) and dichloro[1,1'-bis(diphenylphosphino) ferrocene] palladium (II) dichloromethane (0.423 g, 0.518 mmol). Reflux overnight in an oil bath (90 °C) under an inert atmosphere. Cool the reaction to room temperature, quench with saturated sodium bicarbonate, and dilute with ethyl acetate (150 mL). Separate and extract the aqueous with ethyl acetate (75 mL). Combine the organic phases, dry over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure. Purify the resulting residue using flash chromatography, eluting with 100% hexane/0% (30% THF/hexane) to 0% hexane/100% (30% THF/hexane) in a step gradient of 10% increments to give a white solid (0.720 g, 42%). ES/MS m/z 329.0 $(M+1)^+$.

Prepare the compound below essentially as described in Preparation 14.

Prep. No.	Chemical name	Physical data
15	7-(1-Ethyl-propyl)-2,5-dimethyl-3-thiazol-5-yl-pyrazolo[1,5-*a*]pyrimidine	1H NMR (400 MHz, $CDCl_3$): d 8.75 (s, 1H), 8.75 (s, 1H), 8.28 (s, 1H), 6.47 (s, 1H), 3.62-3.55 (m, 1H), 2.65 (s, 3H), 2.61 (s, 3H), 1.89-1.75 (m, 4H), 0.85 (t, 6H, J = 7.5 Hz), 0.85 (t, 6H, J = 7.5 Hz).

Alternate procedure for Preparation 15:

Add n-butyl lithium (76.5 mL, 191 mmol, 2.5 M in hexanes) to a solution of 2-trimethylsilylthiazole (30 g, 191 mmol) in THF (450 mL) at -78 ºC under nitrogen, keeping the temperature below -74 ºC during the addition. Stir the mixture at -78 ºC for 30 min and then add zinc chloride, dry powder, (39.9 g, 286 mmol) in one portion and warm the mixture to 22 °C over one hour. Add 7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine, (30 g, 87 mmol) and 1,1'-bis(diphenylphosphino)ferrocene palladium(II) chloride (6.5 g, 8 mmol) and heat the mixture to reflux under nitrogen for 8 h. Cool the mixture to 22 °C and add 10% aqueous ammonium chloride (450 mL). Separate the organic layer and wash the aqueous layer with methyl-*t*-butyl ether (2 × 100 mL). Combine the organic portions, dry over sodium sulfate, filter, and evaporate the solvent to afford the title compound (20.4 g, 78%). ES/MS m/z 301 $(M+1)^+$.

Preparation 16

7-(1-Ethyl-propyl)-3-(2-iodo-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

Add a freshly prepared solution of lithium di-*i*-propylamide (150 ml; 62.4 mmol, 0.6 M in THF) to a mixture of 7-(1-ethyl-propyl)-2,5-dimethyl-3-thiazol-5-yl-pyrazolo[1,5-*a*]pyrimidine, (17.8 g, 62.4 mmol) in THF (100 mL) at -78 °C under a nitrogen atmosphere, keeping the temperature below -74 °C during the addition. Stir the mixture at -78 °C for one hour and then add a solution of N-iodosuccinimide (15 g, 63 mmol) in THF (100 mL) keeping temperature below -74 °C. Warm the reaction gradually to 22 °C and then add a 10% aqueous solution of ammonium chloride (300 mL). Separate the organic layer and wash the aqueous layer with methyl-*t*-butyl ether (2 × 200 mL). Combine the organic layers, dry over sodium sulfate, filter, and evaporate the

solvent. Purify the resulting residue by silica gel chromatography, eluting with hexanes/acetone (5/1) to afford the title compound (15 g, 60%). ES/MS m/z 427 $(M+1)^+$.

Example 1

7-(1-Ethyl-propyl)-2,5-dimethyl-3-(4-methyl-thiazol-5-yl)-pyrazolo[1,5-*a*]pyrimidine

Add Rieke® zinc (10 g in 100 mL of THF, 13.2 mL, 18.48 mmol) to 5-bromo-4-methylthiazole (2.13 g, 18.48 mmol) and heat at reflux for 2 h. Cool the mixture to room temperature and settle the zinc down by centrifuge. Bubble nitrogen gas through 7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (900 mg, 2.62 mmol) in dry tetrahydrofuran (10 mL) and add the organozinc bromide solution followed by addition of [1,1'-bis(diphenylphosphino)ferrocene]-dichloropalladium(II) (106 mg, 0.13 mmol). Stir the reaction mixture under reflux overnight and cool to room temperature. Add ammonium chloride solution to the reaction mixture and extract with dichloromethane. Dry the organic portion over sodium sulfate, filter, and remove the solvent under vacuum. Purify the crude product by silica gel column chromatography, elueting with hexane/ethyl acetate (3/1) to obtain the title compound (652 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.80 (s, 1H), 6.48 (s, 1H), 3.63 (m, 1H), 2.57 (s, 3H), 2.43 (s, 3H), 2.40 (s, 3H), 1.85 (m, 4H), 0.90 (t, 6H, J = 7.3Hz). ES/MS m/z 315 $(M+1)^+$.

Preparation 17

3-(2,4-dibromo-thiazol-5-yl)-2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*] pyrimidine

Dissolve 2,5-dimethyl-7-(1-propyl-butyl)-3-thiazol-5-yl-pyrazolo[1,5-*a*] pyrimidine (3.15 g, 9.59 mmol) in acetonitrile (100 mL) and add N-bromosuccinimide (4.27 g, 24.0 mmol) in one aliquot. Stir overnight under an inert atmosphere and confirm the reaction is complete using TLC. Concentrate under reduced pressure, dilute the oil with dichloromethane (150 mL), and wash with water (75 mL). Dry the organic phase over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure. Purify the resulting oil using flash chromatography, eluting with 100% hexane/0% (30% THF/hexane) to 0% hexane/100% (30% THF/hexane) in a step gradient (0-10-15-20-25-30-35-40-45-50-100% of 30% THF/hexane) to give yellow crystals (3.70 g, 79%). ES/MS m/z (^{79}Br^{81}Br) 486.7 $(M+1)^+$.

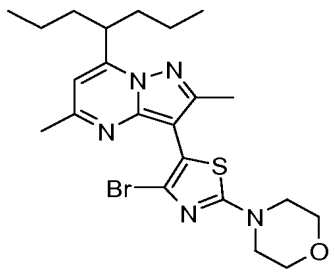
Prepare the compounds below essentially as described in Preparation 17, with the exception that dichloromethane is used as the solvent.

Ex. No.	Chemical name	Physical data
2	3-(2,4-Dibromo-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine	MS (APCI) m/z ($^{79}Br^{81}Br$) 459.1 $(M+1)^+$
3*	3-(2-Bromo-4-methyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine	ES/MS m/z (^{79}Br) 393 $(M+1)^+$

*Use 1.1 eq NBS and stir for 3 days.

Example 4

3-(4-Bromo-2-morpholin-4-yl-thiazol-5-yl)-2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*] pyrimidine



Charge an oven dried flask with 3-(2,4-dibromothiazol-5-yl)-2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*] pyrimidine (0.973 g, 2.00 mmol), anhydrous dioxane (20 mL), morpholine (0.872 g, 10.0 mmol), and cesium carbonate (1.95 g, 6.00 mmol). Reflux in an oil bath (105 °C) under an inert atmosphere overnight. Confirm the reaction is complete using LC/MS. Dilute with ethyl acetate (100 mL), wash with water (50 mL), and back extract the aqueous with ethyl acetate (50 mL). Combine the organic phases, dry over magnesium sulfate, filter, and concentrate under reduced pressure. Purify the resulting residue using flash chromatography, eluting with 100% hexane/0% (40% dichloromethane/20% ethyl acetate/2% 7 N ammonia in methanol/38% hexane) to 0% hexane/100% (40% dichloromethane/20% ethyl acetate/2% 7 N ammonia in methanol/38% hexane) in a step gradient of 10% increments to yield an off-white solid (0.878 g, 89%). ES/MS m/z (^{79}Br) 491.7 $(M+1)^+$.

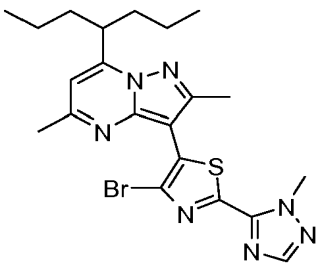
Prepare the following examples essentially as described in Example 4, using as the amine either 2.0 dimethylamine/THF or morpholine, and using THF or dioxane as the solvent. Run reactions in a sealed vessel or Schlenk tube.

Ex. No.	Chemical name	Physical data
5*	{4-Bromo-5-[2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*] pyrimidin-3-yl]-thiazol-2-yl}-dimethylamine	ES/MS (^{79}Br) 449.8 $(M+1)^+$.
6	3-(4-Bromo-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine	MS (APCI) m/z (^{81}Br) 466.5 $(M+1)^+$
7	{4-Bromo-5-[7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-thiazol-2-yl}-dimethyl-amine	MS (APCI) m/z (^{81}Br) 424.4 $(M+1)^+$
8	{5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-methyl-thiazol-2-yl}-dimethyl-amine	ES/MS m/z 358 $(M+1)^+$
9	7-(1-Ethyl-propyl)-2,5-dimethyl-3-(4-methyl-2-morpholin-4-yl-thiazol-5-yl)-pyrazolo[1,5-*a*]pyrimidine	ES/MS m/z 400 $(M+1)^+$

*Use 6 eq of cesium carbonate.

Example 10

3-[4-Bromo-2-(2-methyl-2*H*-[1,2,4]triazol-3-yl)-thiazol-5-yl]-2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*]pyrimidine



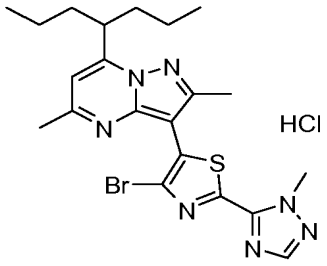
Charge an oven dried flask with 1-methyl-1,2,4-triazole (0.498, 6.00 mmol) and anhydrous THF (20 mL) and chill under an inert atmosphere to -78 °C. Slowly add *n*-butyl lithium (2.5 M hexane solution, 2.4 mL, 6.0 mmol) and stir 30 min. Add anhydrous zinc chloride (1.36 g, 10.0 mmol) in one aliquot and stir 30 min at -78 °C. Allow the reaction to warm to room temperature, stir 30 min, and add 3-(2,4-dibromothiazol-5-yl)-2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*] pyrimidine (0.973 g, 2.00 mmol) and dichloro[1,1'-bis(diphenylphosphino) ferrocene] palladium (II) dichloromethane (0.163 g, 0.200 mmol). Reflux the reaction overnight in an oil bath (90 °C) under an inert atmosphere. Cool the reaction to room temperature, quench with water, and dilute with ethyl acetate (100 mL). Separate and extract the aqueous portion with dichloromethane (50 mL). Combine the organic phases, dry over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure. Purify the resulting residue using flash chromatography, eluting with 100% hexane/0% (10% acetonitrile/40% THF/50% hexane) to 0% hexane/100% (10% acetonitrile/40% THF/50% hexane) in a step gradient of 10% increments to give a white solid (0.090 g, 9%). ES/MS m/z (^{79}Br) 487.7 $(M+1)^+$.

Alternate preparation:

To a solution of 2,5-dimethyl-3-[2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl]-7-(1-propyl-butyl)-pyrazolo[1,5-*a*]pyrimidine (6 g, 14.65 mmol) in acetonitrile (60 mL) add N-bromosuccinimide (2.74 g, 15.4 mmol) in one portion and stir at 22 °C for 10 h. Evaporate the solvent and dissolve the residue in a mixture of water (50 mL) and methyl-*t*-butyl ether (100 mL). Separate the organic layer and extract the aqueous layer with additional methyl-*t*-butyl ether (2 × 50 mL). Combine the organic portions and evaporate the solvent. Purify the resulting material by filtration through a silica gel pad, eluting with hexanes/ethyl acetate (3/1). Combine the product containing fractions and evaporate the solvent. Add heptanes (25 mL) and triturate the solid. Filter the solid and dry under vacuum to afford the title compound (5.5 g, 77%). ES/MS m/z (^{79}Br/^{81}Br) 488/490 $(M+1)^+$.

Example 10a

3-[4-Bromo-2-(2-methyl-2*H*-[1,2,4]triazol-3-yl)-thiazol-5-yl]-2,5-dimethyl-7-(1-propyl-butyl)- pyrazolo[1,5-*a*]pyrimidine, hydrochloride



Dissolve 3-[4-bromo-2-(2-methyl-2*H*-[1,2,4]triazol-3-yl)-thiazol-5-yl]-2,5-dimethyl-7-(1-propyl-butyl)- pyrazolo[1,5-*a*]pyrimidine (750 mg, 1.54 mmol) in acetone (5 mL) and add 1 M HCl in diethyl ether (1.84 mL, 1.84 mmol). Stir the mixture at room temperature for 3 h and concentrate *in vacuo*. Dissolve the residue in diethyl ether/hexane = 1/1 (5 mL) and crystallize the desired HCl salt (526 mg, 65%). ES/MS m/z (^{81}Br) 490 $(M+1)^+$; ^{1}H-NMR($CDCl_3$): 8.20 (s, 1H), 6.82 (s, 1H), 4.21 (s, 3H), 3.64 (m, 1H), 2.49 (m, 3H), 2.44 (s, 3H), 1.75 (m, 4H), 1.96(m, 4H), 0.81 (m, 6H).

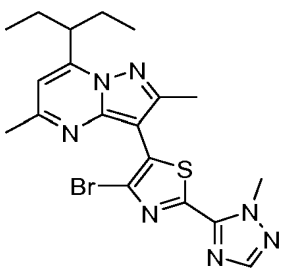
Prepare the following compound essentially as described in Example 10.

Ex. No.	Chemical name	Physical data
11*	7-(1-Ethyl-propyl)-2,5-dimethyl-3-[4-methyl-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl]-pyrazolo[1,5-*a*]pyrimidine	ES/MS m/z 396 $(M+1)^+$

* Use 0.5 M zinc chloride in THF instead of anhydrous zinc chloride. Heat at 80 °C for 3 days.

Example 12

3-(4-Bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine



Under nitrogen atmosphere, add *n*-butyl lithium (2.5 M in hexane, 0.6 mL, 1.5 mmol) to a solution of 1-methyl-1,2,4-triazole (124.5 mg, 1.5 mmol) in THF (3 mL) at –78 °C and stir for 30 min. Add anhydrous zinc chloride (409 mg, 3.0 mmol), continue stirring for 30 min, warm up to room temperature and stir for 2 h. Add 3-(2,4-dibromo-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (229 mg, 0.5 mmol), followed by tetrakis(triphenylphosine)palladium (58 mg, 0.05 mmol) and reflux overnight. Cool to room temperature, dilute with ethyl acetate, and wash with saturated ammonia chloride. Dry the organic portion over sodium sulfate, filter, and concentrate to a residue. Purify the crude material by flash chromatography, eluting with hexanes:ethyl acetate (10:2.5) to give the title compound as a yellow foam (77 mg). MS (APCI) m/z (^{79}Br) 460.4 $(M+1)^+$.

Alternate preparation from Example 21:

Add acetic acid (1 mL) and N-bromosuccinimide (4.1 g, 22 mmol) to a solution of 7-(1-ethyl-propyl)-2,5-dimethyl-3-[2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl]-pyrazolo[1,5-*a*]pyrimidine (8 g, 21 mmol) in acetonitrile (80 mL). Stir the mixture 2 h at 22 °C. Then evaporate the solvent and add water (50 mL) and methyl-*t*-butyl ether (100 mL) to the resulting residue. Separate the organic portion, dry over sodium sulfate, filter, and evaporate the solvent. Recrystallize the resulting residue from isopropyl alcohol to afford the title compound (8.7 g, 90%). ES/MS (m/z): (^{79}Br/^{81}Br) 460/462 $(M+1)^+$.

Example 12a

3-(4-Bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine, *p*-toluene sulfonic acid

Dissolve 3-(4-bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (50 mg, 0.109 mmol) in acetone (3 mL). Add a 0.25 M aqueous solution of *p*-toluene sulfonic acid (434.4 µL, 0.109 mmol) and evaporate the resulting mixture to dryness. Add ethyl acetate (12 mL) to get partial dissolution of the solids. Add methanol (1 mL) to achieve a clear solution. Concentrate the solution by slow evaporation until crystals are observed. Isolate the crystals by filtration and dry under vacuum at 25 °C to obtain about 50 mg of the title compound.

Determine the stoichiometry of the salt by ion chromatography using the following HPLC conditions: column: Phenomenex Phenosphere SAX, 4.6 × 150 mm at

30 °C; mobile phase: 50% acetonitrile/50% 0.025 M sodium phosphate buffer at pH = 4.5; flow rate = 1.5 mL/min; detection: UV at 205 nm; injection volume = 5 μL; run time = 3 min. Theoretical amount calc: 27.2% tosylate; found: 28.4% tosylate (average of three HPLC runs).

Example 13

{4-Chloro-5-[2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*] pyrimidin-3-yl]-thiazol-2-yl}-dimethylamine

Charge an oven dried flask with {4-bromo-5-[2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*] pyrimidin-3-yl]-thiazol-2-yl-dimethylamine (0.20, 0.44 mmol) and anhydrous THF (3.0 mL) and chill under an inert atmosphere to -78°C. Slowly add n-butyl lithium (1.6 M hexane solution, 0.42 mL, 0.67 mmol) and stir 30 min. Add N-chlorosuccinimide (0.120 g, 0.889 mmol) in one aliquot and stir 30 min at -78 °C. Allow the reaction to warm to room temperature, and stir for 5 h, checking the progress using LC/MS. Dilute with ethyl acetate (100 mL), wash with saturated ammonium chloride (50 mL), and back-extract the aqueous with dichloromethane (50 mL). Combine the organic phases, dry over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure. Purify the resulting residue using flash chromatography, eluting with 100% hexane/0% (25% THF/hexane) to 0% hexane/100% (25% THF/hexane) in a step gradient of 10% increments to give a white solid (0.087 g, 48%). ES/MS m/z (^{35}Cl) 406.0 $(M+1)^+$.

Prepare the following examples essentially as described in Example 13 using the appropriate bromothiazole prepared above.

Ex. No.	Chemical name	Physical data
14	3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-*a*] pyrimidine	ES/MS m/z (^{35}Cl) 448.0 $(M+1)^+$
15	{4-Chloro-5-[7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-thiazol-2-yl}-dimethyl-amine	MS (APCI) m/z (^{35}Cl) 378.0 $(M+1)^+$

Preparation 18

2,4-dichlorothiazole

Cool to 5 °C a mixture of thiazolidine-2,4-dione (50 g, 0.43 mol) in phosphorus oxychloride (240 mL) and add pyridine (34 mL, 0.43 mol) over 15 min. Heat the mixture to 125 °C for 4 h and then cool to 22 °C. Remove the excess phosphorus oxychloride by vacuum distillation and add the residue to water (1 L) chilled to a temperature of 5 °C. Extract the mixture with methylene chloride (3 × 400 mL). Combine the organic portions and evaporate the solvent to afford the title compound (50 g, 76%). EI/MS m/z : ($^{35}Cl^{35}Cl/^{35}Cl^{37}Cl/^{37}Cl^{37}Cl$) 153/155/157 $(M+1)^+$.

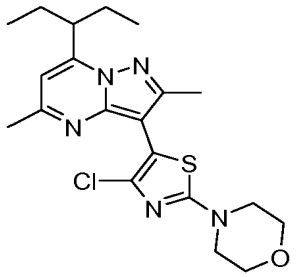
Preparation 19

4-chloro-2-morpholino-thiazole

To a mixture of 2,4-dichlorothiazole (34 g, 0.22 mol) in acetonitrile (425 mL) add potassium carbonate (60.9 g, 0.44 mol) and then morpholine (21.2 mL, 0.225 mol) dropwise over 30 min. Reflux the mixture at 40 °C and then cool to 22 °C. Filter the mixture and evaporate the filtrate. Triturate the residue with *i*-propyl alcohol (60 mL) at 22 °C for one hour. Filter the solids and dry under vacuum to a constant weight to afford the title compound (34.5 g, 76%). ES/MS m/z (^{35}Cl) 205 $(M+1)^+$.

Example 16

3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine



Under a nitrogen atmosphere dissolve 3-(4-bromo-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (116 mg, 0.25 mmol) in THF (1.5 mL) and chill to -78 °C. Add n-butyl lithium (0.1 mL. 2.5 M in hexane, 0.25 mmol) and stir at -78 °C for 30 min. Add N-chlorosuccinimide (33.4 mg, 0.25 mmol) and stir

for another 30 min, slowly warming to room temperature. After stirring overnight, quench the reaction by adding a solution of saturated ammonia chloride and extract with ethyl acetate. Wash the organic layer with brine, dry over sodium sulfate, filter, and concentrate to a residue. Purify the crude material by flash chromatography, eluting with hexanes:dichloromethane:ethyl acetate (5:5:2) to provide the title compound (54 mg). MS (APCI) m/z (^{35}Cl) 420.6 $(M+1)^+$; ^{1}H NMR (400 MHz, $CDCl_3$): 6.44 (s, 1H), 3.79 (t, 4H, *J*=4.8 Hz), 3.63-3.56 (m, 1H), 3.47 (t, 4H, *J*=4.8 Hz), 2.55 (s, 3H), 2.45 (s, 3H), 1.88-1.75 (m, 4H), 0.87 (t, 6H, *J*=7.5 Hz).

Alternate Preparation from Preparation 6:

Combine 7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine, (9 g, 26.2 mmol) and 4-chloro-2-morpholino-thiazole (7.5 g, 36.7 mmol) in dimethylformamide (90 mL) previously degassed with nitrogen. Add cesium carbonate (17.8 g, 55 mmol), copper iodide (250 mg, 1.31 mmol), triphenylphosphine (550 mg, 2.09 mmol) and palladium acetate (117 mg, 0.52 mmol). Heat the mixture to 125 °C for 16 h and then cool to 22 °C. Add water (900 mL) and extract with methyl-*t*-butyl ether (3 × 200 mL). Combine the organic portions and evaporate the solvent. Purify by silica gel chromatography eluting with hexanes/ethyl acetate (4/1) to afford the title compound (6.4 g, 62%). ES/MS m/z (^{35}Cl) 420 $(M+1)^+$.

Example 16a

3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine, hydrochloride

Dissolve 3-(4-chloro-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (1.40 g, 3.33 mmol) in acetone (10 mL) at 50 °C and cool to room temperature. Add hydrogen chloride (2 M in diethyl ether, 2.0 mL, 4.0 mmol) and stir well in a sonicator. Concentrate the solution a little and add a minimal amount of diethyl ether to crystallize the HCl salt. Cool the mixture in a refrigerator overnight. Add additional hydrogen chloride (2 M in diethyl ether, 2.0 mL, 4.0 mmol) and cool in a refrigerator. Filter the crystalline material and dry to obtain the title compound (1.15 g, 75%). ES/MS m/z (^{35}Cl) 420 $(M+1)^+$; ^{1}H NMR(CDCl3): 9.18 (br,

1H), 6.86 (s, 1H), 3.72 (m, 4H), 3.49(m, 1H), 3.39 (m, 4H), 2.48 (s, 3H), 2.38(s, 3H), 1.79 (m, 4H), 0.79 (m, 6H).

Example 17

3-(4-Bromo-2-butyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

Under a nitrogen atmosphere, add *n*-butyl lithium (2.5 M in hexane, 0.2 mL, 0.5 mmol) to a solution of 3-(2,4-dibromo-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (230 mg, 0.5 mmol) in THF (3 mL) at –78 °C. After 30 min, add 1-iodobutane (138 mg, 0.75 mmol) and continue stirring for 1 hour. Warm up to room temperature and stir for one hour. Quench the reaction by adding saturated ammonia chloride solution and extract with ethyl acetate. Dry the organic portion over sodium sulfate, filter, and concentrate to a residue. Purify the crude material by flash chromatography, eluting with hexanes/ethyl acetate (10/1.5) to give the title compound as orange foam (78 mg). ES/MS m/z (^{81}Br) 437.4 $(M+1)^+$.

Prepare the following example essentially as described in Example 17.

Ex. No.	Chemical name	Physical data
18	3-(4-Bromo-2-methoxymethyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine	MS (APCI) m/z (^{81}Br) 424.6 $(M+1)^+$

Example 19

7-(1-Ethyl-propyl)-3-(2-methoxymethyl-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

Under a nitrogen atmosphere, add *n*-butyl lithium (2.5 M in hexane, 0.4 mL, 1.0 mmol) to a solution of 7-(1-ethyl-propyl)-2,5-dimethyl-3-thiazol-5-yl-pyrazolo[1,5-a]pyrimidine (300 mg, 1.0 mmol) in THF (3 mL) at –78 °C. Stir 30 min and add 1-iodomethyl methyl ether (205 mg, 1.2 mmol). Continue stirring for one hour and then slowly warm up to room temperature and stir overnight. Quench the reaction by adding saturated ammonium chloride solution and extract with ethyl acetate. Wash the organic layer with brine, dry over sodium sulfate, filter, and concentrate under vacuum to a

residue. Purify the crude material by flash chromatography, eluting with hexanes/ethyl acetate (10/2) to give the title compound as a yellow foam (184 mg). MS (APCI) m/z 345.3 $(M+1)^+$.

Example 20

3-(4-Chloro-2-methoxymethyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

Add *n*-butyl lithium (2.5 M in hexanes, 174 mL, 0.43 mmol) to a stirred solution of 7-(1-ethyl-propyl)-3-(2-methoxymethyl-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine (150 mg, 0.43 mmol) in THF (3 mL) at –78 °C. Stir for 30 min and add N-chlorosuccinimide (87 mg, 0.653 mmol). Stir 30 min and then warm the reaction slowly to room temperature allowing the reaction to continue overnight. Quench the reaction by adding saturated ammonium chloride solution, extract with ethyl acetate, dry over sodium sulfate, filter, and concentrate to a residue. Purify by flash chromatography, eluting with hexanes/ethyl acetate (10/2) to give the title compound (7 mg). MS (APCI) m/z (^{35}Cl) 379.3 $(M+1)^+$.

Example 21

7-(1-Ethyl-propyl)-2,5-dimethyl-3-[2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-pyrazolo[1,5-*a*]pyrimidine

Mix 3-(4-bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (175 mg, 0.38 mmol) and copper(I) chloride (132 mg, 1.33 mmol) in DMF (5 mL) and heat to 120 °C for 24 h. Cool to room temperature, dilute with ethyl acetate, and wash with brine. Dry the organic layer over sodium sulfate, filter, and concentrate under vacuum to a residue. Purify by flash chromatography, eluting with hexanes and then hexanes/EtOAc (10/1.8) to give a yellow-orange solid (45 mg). ES/MS m/z 382.0 $(M+1)^+$.

Alternate Preparation:

Add *n*-butyl lithium (2.5 M in hexanes, 57.6 mL, 144 mmol) to a solution of *N*-methyltriazole (11.95 g, 144 mmol) in THF (600 mL) at -78 °C under a nitrogen atmosphere, keeping the temperature below -74 °C during the addition. Then add zinc chloride, dry powder, (26 g, 192 mmol) in one portion and warm the mixture to 22 °C in

one hour. Add 7-(1-ethyl-propyl)-3-(2-iodo-thiazol-5-yl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (12.5 g, 29 mmol) and tetrakis(triphenyl)phosphine palladium catalyst (1.15 g, 0.01 mol) in one portion and heat the mixture to reflux under nitrogen for 8 h. Cool the mixture to 22 °C and add water (300 mL). Separate the organic layer and extract the aqueous layer with methyl-*t*-butyl ether (2 × 200 mL). Combine the organic portions, dry over sodium sulfate, filter, and evaporate the solvent. Purify by passing over a silica gel pad eluting with hexanes/ethyl acetate (4/1) to afford the title compound (8 g, 72%). ES/MS m/z 382 $(M+1)^+$.

Example 22

3-(4-Chloro-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

Stir a mixture of 7-(1-ethyl-propyl)-2,5-dimethyl-3-[2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-pyrazolo[1,5-*a*]pyrimidine (20 mg, 0.052 mmol) and N-chlorosuccinimide (7.6 mg, 0.0569 mmol) in dichloromethane (0.5 mL) and acetonitrile (0.5 mL) at room temperature overnight in a vial. Concentrate to a residue. Purify by flash chromatography, eluting with hexanes and then with hexanes/ethyl acetate (10/1.5) to give the title compound (16 mg). ES/MS m/z (^{35}Cl) 416.0 $(M+1)^+$.

Example 23

7-(1-Ethyl-propyl)-3-(4-methoxy-2-morpholin-4-yl-thiazol-5-yl)- 2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

Stir a mixture of 3-(4-bromo-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (162 mg, 0.35 mmol), sodium methoxide (57 mg, 1.05 mmol) and copper(I) iodide (67 mg, 0.35 mmol) in methanol (3 mL) in a sealed 4-mL vial for 15 h at 120 °C. Cool to room temperature, remove the solid by filtration, and concentrate the filtrate under vacuum. Purify the residue by flash chromatography, eluting with hexanes/THF (10/2). Recrystallize the material from methanol to give the title compound (20 mg). ES/MS m/z 416.0 $(M+1)^+$.

Preparation 20

4-Bromo-5-[7-(1-ethyl-propyl)- 2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-thiazole-2-carbaldehyde

Under a nitrogen atmosphere, add *n*-butyl lithium (1.6 M in hexanes, 0.312 mL, 0.50 mmol) to a THF solution (2.5 mL) of 3-(2,4-dibromo-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (230 mg, 0.50 mmol) at -78 °C and stir for 30 min. Add a THF (0.5 mL) solution of N-formyl morpholine (58 mg, 0.50 mmol). Stir for one hour, then store the reaction at –20 °C overnight. Warm the reaction to room temperature, dilute with ether, and quench by adding 4 N HCl (4 mL). Separate and extract the organic phase with 4 N HCl (2 × 4 mL). Combine the aqueous portions, treat with solid sodium bicarbonate to pH = 8 to 9 and then extract with diethyl ether. Combine all the organic layers, wash with brine, dry over sodium sulfate, filter, and concentrate to a residue. Purify the crude material by flash chromatography, eluting with hexanes/dichloromethane/ethyl acetate (5/5/1) to give the title compound (154 mg). MS (APCI) m/z (^{81}Br) 409.0 $(M+1)^+$.

Example 24

3-(4-Bromo-2-morpholin-4-yl-methyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

Combine 4-bromo-5-[7-(1-ethyl-propyl)- 2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-thiazole-2-carbaldehyde (150 mg, 0.368 mmol), morpholine (35 mg, 0.405 mmol) and sodium triacetoxyborohydride (97 mg, 0.46 mmol), in dichloromethane (3 mL) and methanol (0.5 mL). Stir overnight, add additional morpholine (35 mg, 0.405 mmol), and sodium triacetoxyborohydride (97 mg, 0.46 mmol), and stir 4 h more. Remove the solvent under vacuum, dilute with dichloromethane, and wash with brine. Dry the organic phase over sodium sulfate, filter, and concentrate under vacuum. Purify the resulting material using flash chromatography, eluting with dichloromethane:2 M ammonia in methanol (10:1) to give a mixture. Purify the mixture using a reverse phase column, eluting with water/acetonitrile (80/20) to water/acetonitrile (10/90) to give the title compound (20 mg). ES/MS m/z (^{81}Br) 480.0 $(M+1)^+$.

Example 25

3-(4-Bromo-2-pyridin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine

Under a nitrogen atmosphere, add *n*-butyl lithium (1.6 M in hexane, 0.312 mL, 0.5 mmol) to a solution of 3-(2,4-dibromo-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (229 mg, 0.5 mmol) in THF (4 mL) at –78 °C. After stirring for 30 min, add anhydrous zinc chloride (264 mg, 1.5 mmol) and continue stirring for 30 min. Warm the reaction to room temperature and stir for one hour. Add 4-iodopyridine (103 mg, 0.5 mmol), followed by 1,1'-bis (diphenylphosphino)ferrocene palladium (II) chloride (dichloromethane adduct) (0.40.8 mg, 0.05 mmol). Heat the reaction to reflux overnight. Cool to room temperature, dilute with ethyl acetate, and wash with water and brine. Dry the organic layer over sodium sulfate, filter, and concentrate to a residue. Purify the crude material by flash chromatography, eluting with dichloromethane:2 N ammonia in methanol (10:0.75) to give a mixture. Purify the mixture using a reverse phase column, eluting with water:acetonitrile = 80:20 to water: acetonitrile = 10:90, to give the title compound (19 mg). ES/MS m/z (^{79}Br) 456.0 $(M+1)^+$.

Preparation 21

4-Trifluoromethyl-thiazole-2-ylamine

Add thiourea (4.0 g, 52.3 mmol) and 3-bromo-1,1,1-trifluoropropan-2-one (5.5 mL, 10 g, 52.3 mmol) to ethanol (100 mL) and heat at 50 °C for 2 h. Cool to room temperature and concentrate to dryness. Dissolve the residue in water and adjust the pH to >12 with 2 M NaOH. Extract with diethyl ether (4×). Dry the combined organic extracts with sodium sulfate, filter, and concentrate under vacuum. Purify the resulting material by silica gel chromatography (CH_2Cl_2) to obtain the title compound (6.9 g, 79%). ES/MS m/z 169 $(M+1)^+$.

Preparation 22

5-Bromo-4-trifluoromethyl-thiazol-2-ylamine, hydrobromide

Add bromine (2.0 mL, 6.28 g, 39.3 mmol) dropwise to an ice-bath cooled solution of 4-trifluoromethyl-thiazole-2-ylamine (6.0 g, 35.7 mmol) in diethyl ether (60 mL). Stir for one hour after the addition is complete and then warm to room temperature. Collect

the solids by filtration and wash with diethyl ether to obtain the title compound (10.5 g, 90%). ES/MS m/z ($^{79}Br/^{81}Br$) 247/249 $(M+1)^+$.

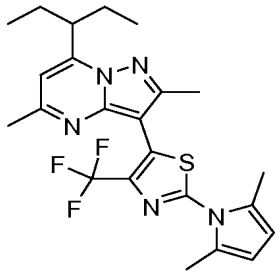
Preparation 23

5-Bromo-2-(2,5-dimethyl-pyrrol-1-yl)-4-trifluoromethyl-thiazole

Add hexane-2,5-dione (3.5 mL, 3.4 g, 30.2 mmol) to a solution of 5-bromo-4-trifluoromethyl-thiazol-2-ylamine hydrobromide (9.0 g, 27.4 mmol) in methanol (60 mL). Stir at room temperature overnight. Add phosphate buffer (50 mL, pH = 7). Collect the resulting precipitate by filtration, washing with water. Dissolve the filter cake in CH_2Cl_2 and dry over sodium sulfate. Filter and concentrate under vacuum to obtain the title compound (8.2 g, 92%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.91 (s, 2H), 2.27 (s, 6H).

Example 26

3-[2-(2,5-Dimethyl-pyrrol-1-yl)-4-trifluoromethyl-thiazol-5-yl]-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine



Cool a solution of 5-bromo-2-(2,5-dimethyl-pyrrol-1-yl)-4-trifluoromethyl-thiazole (2.2 g, 6.6 mmol) in THF (25 mL) in a dry ice bath. Add *t*-butyl lithium (1.7 M in pentane, 8.5 mL, 14.5 mmol) dropwise. Stir for 45 min and then add zinc chloride (0.5 M in THF, 14.6 mL, 7.3 mmol) dropwise. Stir 5 min and remove the cooling bath. Stir 30 min and then add 7-(1-ethyl-propyl)-3-iodo-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (1.5 g, 4.4 mmol) and bis(tri-*t*-butylphosphine)palladium (0) (450 mg, 0.9 mmol). Reflux for 24 h. Cool the reaction, pour the mixture into diethyl ether, and wash with water (2×). Extract the combined water layers with diethyl ether. Dry the combined organic portions over sodium sulfate, filter, and concentrate to dryness under vacuum. Purify the resulting residue by silica gel chromatography (75-100% CH_2Cl_2 in hexanes) to give the title

compound (1.47 g, 72%). HR-ToF-MS m/z calcd for $C_{23}H_{26}F_3N_5S+H^+$: 462.1939, found: 462.1915.

Example 27

5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-ylamine

Add hydroxylamine (2 mL, 50% in water) to a solution of 3-[2-(2,5-dimethyl-pyrrol-1-yl)-4-trifluoromethyl-thiazol-5-yl]-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (1.1 g, 2.3 mmol) in acetic acid (10 mL). Heat the reaction to 80 °C for 6 h. Cool to room temperature. Pour into diethyl ether and wash with 2 M NaOH (2×) and then once with water. Dry the organic portion over sodium sulfate, filter, and concentrate to dryness under vacuum. Purify the resulting residue by silica gel chromatography (40% ethyl acetate in hexanes) to give the title compound (0.76 g, 87%). ES/MS m/z 384 $(M+1)^+$.

Preparation 24

3-(2-Bromo-4-trifluoromethyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine

Heat a mixture of copper(II) bromide (540 mg, 2.4 mmol) and *t*-butylnitrite (0.36 mL, 310 mg, 3.0 mmol) in acetonitrile (20 mL) to 60 °C. Add 5-[7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-ylamine (755 mg, 2.0 mmol) as a solid. Heat the reaction to 80 °C for 2 h. Cool the reaction, pour into diethyl ether and extract with water (3×). Dry the organic layer over sodium sulfate, filter, and concentrate to dryness under vacuum. Purify the resulting residue by silica gel chromatography in CH_2Cl_2 to give the title compound (0.77 g, 87%). ES/MS m/z ($^{79}Br/^{81}Br$) 447, 449 $(M+1)^+$.

Example 28

{5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}-dimethyl-amine

Heat a sealed tube containing 3-(2-bromo-4-trifluoromethyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (313 mg, 0.7 mmol), and dimethyl amine (2 M in THF, 4 mL, 8 mmol) in methanol (4 mL) to 80 °C for 2 h. Cool the reaction and concentrate under vacuum. Purify the resulting residue by silica gel chromatography, eluting with 0-30% ethyl acetate in CH_2Cl_2, to give the title compound (0.28 g, 97%). HR-ToF-MS m/z calcd for $C_{19}H_{24}F_3N_5S+H^+$ 412.1770, found: 412.1783.

Prepare the following example essentially as described for Example 28 with the exception that the reaction is refluxed for 8 h.

Ex. No.	Chemical name	Physical data
29	7-(1-Ethyl-propyl)-2,5-dimethyl-3-(2-morpholin-4-yl-4-trifluoromethyl-thiazol-5-yl)-pyrazolo[1,5-*a*]pyrimidine	HR-ToF-MS m/z calcd for $C_{21}H_{26}F_3N_5OS+H^+$: 454.1873, found: 454.1888

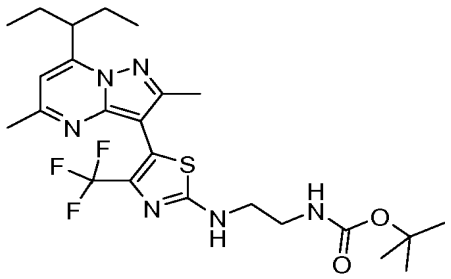
Example 30

N-{5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}-acetamide

Add hydroxylamine (50% in water, 5 mL,) to a solution of 3-[2-(2,5-dimethyl-pyrrol-1-yl)-4-trifluoromethyl-thiazol-5-yl]-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (2.4 g, 5.2 mmol) in acetic acid (25 mL). Heat the reaction to 80 °C for 72 h. Cool the reaction to room temperature, pour into diethyl ether and wash with 2 M NaOH (2×) and then once with water. Dry the organic portion over sodium sulfate, filter, and concentrate to dryness. Purify the resulting residue by column chromatography, eluting with 40% ethyl acetate in hexanes to give the title compound (0.28 g, 13%). HR-ToF-MS m/z calcd for $C_{19}H_{22}F_3N_5OS+H^+$ 426.1575, found: 426.1565.

Example 31

(2-{5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-ylamino}-ethyl)-carbamic acid *tert*-butyl ester



Add (2-amino-ethyl)-carbamic acid *tert*-butyl ester (0.5 mL, 506 mg, 3.2 mmol) to a solution of 3-(2-bromo-4-trifluoromethyl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine (100 mg, 0.22 mmol) and triethylamine (0.3 mL, 222 mg, 2.2 mmol) in methanol (1 mL). Evaporate off the methanol and heat to 80 °C overnight. Cool the reaction to room temperature and concentrate under vacuum. Purify the resulting residue by column chromatography, eluting with 10-50% ethyl acetate in CH_2Cl_2, to give the title compound (0.11 g, 97%). ES/MS m/z 527.2 $(M+1)^+$.

Prepare the compounds below as essentially described in Example 31 using the appropriate amine.

Prep. or Ex. No.	Chemical name	Physical data
Prep. 25*	[2-({5-[7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}-propyl-amino)-ethyl]-carbamic acid tert-butyl ester	ES/MS m/z 569.3 $(M+1)^+$
Prep. 26**	(S)-(1-{5-[7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}-pyrrolidin-3-yl)-carbamic acid tert-butyl ester	ES/MS m/z 553.3 $(M+1)^+$

Ex. 32[***]	*N*-{5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}-*N*,*N*'-dipropyl-ethane-1,2-diamine, hydrochloride	ES/MS m/z 511.2 $(M+1)^+$

[*] Heat at reflux in EtOH overnight. Evaporate EtOH and heat at 110 °C for 24 h.

[**] Heat at 80 °C in EtOH overnight.

[***] Make the HCl salt and recrystallize from EtOAc/hexanes.

Example 33

N^1-{5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}-ethane-1,2-diamine, hydrochloride

Add (2-{5-[7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-ylamino}-ethyl)-carbamic acid *tert*-butyl ester (101 mg, 0.192 mmol) to 1 M HCl in methanol (1 mL). Heat the reaction at 70 °C overnight. Cool the reaction and concentrate under vacuum from methanol/ethyl acetate. Triturate the resulting residue with ethyl acetate to obtain the title compound (64 mg, 78%). ES/MS m/z 427.0 $(M+1)^+$.

Prepare the following examples essentially as described for Example 33.

Ex. No.	Chemical name	Physical data
34	N^1-{5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}-N^1-propyl-ethane-1,2-diamine, hydrochloride	ES/MS m/z 469.2 $(M+1)^+$
35	(*S*)-1-{5-[7-(1-Ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-3-yl]-4-trifluoromethyl-thiazol-2-yl}-pyrrolidin-3-ylamine, hydrochloride	ES/MS m/z 453.0 $(M+1)^+$

Example A

In Vivo Potency Assessment Using Ex Vivo Binding

To assess in vivo potency, a compound of the present invention is evaluated using ex vivo binding. Using the procedures as provided in D. R. Gehlert *et al.*, *EJP* 509: 145-153 (2005), a compound is administered to a rat via the oral route. The binding of ^{125}I-

sauvagine to the cerebellum is then assessed ex vivo as described in Gehlert *et al.* For example, Example 15 provides 65% inhibition at 10 mg/kg.

Example B

CRF1 Filter Binding Assay

The limitations of plasmid-based human CRF1 expression, in terms of generating a recombinant cell line with sufficient receptor density to develop a binding assay, are overcome by using a Phoenix retroviral expression system licensed from Stanford. The stable HEK-hCRF1 cell line is used to prepare membranes and binding reactions (200 µL) are set up as follows: 50 µL of ^{125}I-sauvagine (0.2 nM final), 50 µL compound and 100 µL CRF1 membrane (25 µg/reaction). The reactions are incubated at room temperature for 2 h and then terminated by filtration through pre-treated FB Millipore glass fiber filter plates (96 well). The plates are washed twice with ice-cold assay buffer (50 mM tris, 12.5 mM NaCl, 1 mM EDTA, 10 mM $MgCl_2$, 0.05% BSA, pH 7.2), air dried over night and counted with 100 µL Microscint 40 in a MicroBeta counter. Non-specific binding (NSB) is determined in the presence of 0.5 µM non-labeled sauvagine. Triplicate determinations are typically run and the median data points plotted by Graph Pad Prism.

Using this assay, the exemplified compounds of the present invention inhibit the binding of ^{125}I-Sauvagine (4 nM) in roller/adherent cells with a Ki (inhibition constant) below 1 µM. For example, Example 15 exhibits a Ki of 6.2 nM.

Example C

CRF2 Filter Binding Assay

The limitations of plasmid-based human CRF2 expression, in terms of generating a recombinant cell line with sufficient receptor density to develop a binding assay, are overcome by using a Phoenix retroviral expression system licensed from Stanford. The stable HEK-hCRF2 cell line is used to prepare membranes and binding reactions (200 µL) are set up as follows: 50 µL of ^{125}I-sauvagine (0.2 nM final concentration), 50 µL compound and 100 µL CRF2 membrane (25 µg/reaction). The reactions are incubated at room temperature for 2 hours and then terminated by filtration through pre-treated FB Millipore glass fiber filter plates (96 well). The plates are washed twice with ice-cold assay buffer (50 mM tris, 12.5 mM NaCl, 1 mM EDTA, 10 mM $MgCl_2$, 0.05% BSA, pH

7.2), air dried over night and counted with 100 μL Microscint 40 in a MicroBeta counter. Non-specific binding (NSB) is determined in the presence of 0.5 μM non-labeled sauvagine. Alternatively, compounds are evaluated using a Scintillation Proximity assay. This assay is set up as follows: 50 μL of ^{125}I-Sauvagine (0.2 nM final concentration), 50 μL compound or non-labelled sauvagine (NSB) and 100 μL containing 250 μg wheat germ agglutinin (WGA) SPA beads and CRF2 membrane (1.5 μg/reaction). Plates are incubated for 4-5 h at room temperature and then centrifuged at 200 X g for 10 min. Bound radioactivity is assessed using a Wallac Trilux scintillation counter. Binding is assessed typically using triplicate determinations and the median data points plotted by Graph Pad Prism. Compounds are initially screened at a fixed concentration and, if sufficient activity is noted, subsequent concentration-response curves are generated.

Particular exemplified compounds of the present invention are tested in the CRF2 binding assay and exhibit weak affinity for the CRF2 receptor. For example, Example 15 exhibits 11% inhibition at a concentration of 50 μM. This result suggests that the compounds of the present invention are selective for the CRF1 receptor, (relative to CRF2).

Example D

Bioavailability and Pharmacokinetic Properties

The volume of distribution (Vdist) relates the amount of the drug in the body to the concentration of the drug in the blood or plasma. The volume of distribution refers to the fluid volume that would be required to contain the total amount of the drug in the body at the same concentration as in the blood or plasma: Vdist = amount of drug in the body/ concentration of drug in blood or plasma (Goodman and Gillman's). For a 10 mg dose and a plasma concentration of 10 mg/L, the volume of distribution would be 1 liter. The volume of distribution reflects the extent to which the drug is present in the extravascular tissue. A large volume of distribution reflects the tendency of a compound to bind to the tissue components compared with plasma protein binding. In a clinical setting, Vdist can be used to determine a loading dose to achieve a steady state concentration.

To test for volume of distribution, Male Sprague Dawley rats (N=3) are administered a single 1 mg/kg intravenous dose of compound. Multiple plasma samples

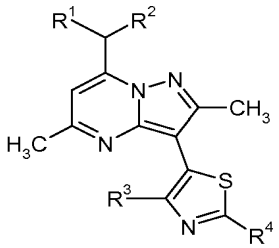
are collected at time points from 0.08 to 24 hours post-dose. The plasma samples are analyzed by LC/MS/MS to determine the plasma concentrations. Plasma pharmacokinetic calculations are performed to determine the pharmacokinetic parameters including Vdist and plasma clearance (Clp).

Compounds of the present invention preferably have favorable bioavailability profiles. For example, a majority of commercial CNS and cardiovascular drugs exhibit a human Vdist of <10 L/Kg. In comparison with CRF antagonists, CP154526 (Schulz et al., *Proc. Natl. Acad. Sci. (USA)*, 93:10477 (1996)) and NBI30775 (Chen et al., *Drug Development Research*, 65:216 (2005)), which exhibit a rat Vdist of 114 L/Kg and 76 L/Kg, respectively, when analyzed separately. Example 15 of the present invention, when analyzed separately, exhibits a rat Vdist of only 7.2 L/Kg following a single intravenous dose of 1 mg/kg.

Further, the plasma clearance (CLp) is a measure of the rate of removal of the drug from the body. Following an intravenous dose and first-order kinetics, the plasma clearance may be determined using the following equation: CLp = Dose/ AUC, where AUC is the total area under the curve that describes the concentration of the drug in the plasma as a function of time from zero to infinity. Reference CRF antagonists CP154526 and NBI37582 exhibit rat clearance (CLp) of 83 and 306 mL/min/kg, respectively, when analyzed separately, following a single intravenous dose, while Example 15 of the present invention, when analyzed separately, exhibits a rat CLp of only 23.6 mL/min/kg.

WE CLAIM:

1. A compound of Formula I

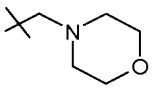
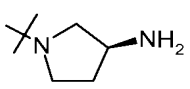
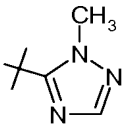
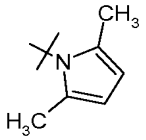


Formula I

wherein:

R^1 and R^2 are independently ethyl or *n*-propyl;

R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl or methoxy;

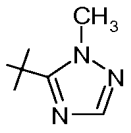
R^4 is hydrogen, Br, R^aR^bN-, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl,  ,  ,  or  ;

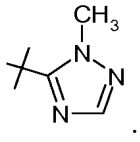
R^a and R^b are independently hydrogen, C_1-C_3alkyl, $H_2NCH_2CH_2$-, $(CH_3)_3COC(O)NHCH_2CH_2$-, or $CH_3CH_2CH_2NHCH_2CH_2$-;

or a pharmaceutically acceptable salt thereof.

2. The compound according to claim 1, or a pharmaceutically acceptable salt thereof, wherein R^3 is Cl, Br, methyl or trifluoromethyl.

3. The compound according to claim 1 or 2, or a pharmaceutically acceptable salt thereof, wherein R^3 is Cl or Br.

4. The compound according to any one of claims 1 to 3, or a pharmaceutically acceptable salt thereof, wherein R^4 is R^aR^bN-, pyridin-4-yl, morpholin-4-yl, or  .

5. The compound according to any one of claims 1 to 4, or a pharmaceutically acceptable salt thereof, wherein R^4 is morpholin-4-yl or  .

6. The compound according to any one of claims 1 to 4, or a pharmaceuticaly acceptable salt thereof, wherein R^4 is R^aR^bN- and R^a and R^b are independently C_1-C_3alkyl.

7. The compound according to claim 1, or a pharmaceutically acceptable salt thereof, which is 3-[4-bromo-2-(2-methyl-2*H*-[1,2,4]triazol-3-yl)-thiazol-5-yl]-2,5-dimethyl-7-(1-propyl-butyl)- pyrazolo[1,5-*a*]pyrimidine.

8. The compound according to claim 1, or a pharmaceutically acceptable salt thereof, which is 3-(4-bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine.

9. The compound according to claim 1, or a pharmaceutically acceptable salt thereof, which is 3-(4-chloro-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine.

10. A pharmaceutical composition comprising: a compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

11. A method of treating depression, anxiety, alcohol or substance abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, stroke, sleep disorders, allergy, migraine, premenstrual syndrome, infertility, sexual dysfunction, congenital adrenal hyperplasia, Cushing's disease, premature labor, stress-induced gastric ulceration, inflammatory disorders, pituitary or ectopic pituitary-derived tumors, chronic fatigue syndrome, fibromyalgia, visceral pain or multiple sclerosis in a patient, comprising: administering to a patient in need thereof a therapeutically effective amount

of a compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof.

12. A method of treating anxiety or depression in a patient, comprising: administering to a patient in need thereof a therapeutically effective amount of a compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof.

13. A method of treating alcohol or substance abuse in a patient, comprising: administering to a patient in need thereof a therapeutically effective amount of a compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof.

14. A compound according to any one of Claims 1 to 9, or a pharmaceutically acceptable salt thereof, for use in therapy.

15. A compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, for use in the treatment of depression, anxiety, alcohol or substance abuse, obesity, hypertension, metabolic syndrome, irritable bowel syndrome, epilepsy, stroke, sleep disorders, allergy, migraine, premenstrual syndrome, infertility, sexual dysfunction, congenital adrenal hyperplasia, Cushing's disease, premature labor, stress-induced gastric ulceration, inflammatory disorders, pituitary or ectopic pituitary-derived tumors, chronic fatigue syndrome, fibromyalgia, visceral pain or multiple sclerosis.

16. A compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, for use in the treatment of anxiety or depression.

17. A compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, for use in the treatment of alcohol or substance abuse.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/078605

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D487/04 A61K31/505 A61P29/00 A61P25/22 A61P25/24
A61P25/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	GEHLERT ET AL: "3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-b]pyridazine: a novel brain-penetrant, orally available corticotropin-releasing factor receptor 1 antagonist with efficacy in animal models of alcoholism." THE JOURNAL OF NEUROSCIENCE, vol. 27, no. 10, 7 March 2007 (2007-03-07), pages 2718-2726, XP002465426 figure 1; table 1	1-17
Y	WO 01/23388 A (NEUROGEN CORP [US]; PFIZER [US]; DARROW JAMES W [US]; LOMBAERT STEPHAN) 5 April 2001 (2001-04-05) page 69; example 95 page 13, line 3	1-17

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 January 2008	31/01/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gettins, Marc

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/072202 A (BRISTOL MYERS SQUIBB PHARMA CO [US]) 19 September 2002 (2002-09-19) claims 1,16	1-17
A	HE LIQI ET AL: "4-(1,3-Dimethoxyprop-2-ylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)pyrazolo[1,5-a]-1,3,5-triazine: A Potent, Orally Bioavailable CRF1 Receptor Antagonist" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 43, 2000, pages 449-456, XP002196777 ISSN: 0022-2623 table 1	1-17
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Y	CHEN C ET AL: "DESIGN OF 2,5-DIMETHYL-3-(6-DIMETHYL-4-METHYLPYRIDIN-3-YL)-7-DIPROPYL AMINOPYRAZOLO[1,5-ALPHA]PYRIMIDINE (NBI 30775/R121919) AND STRUCTURE-ACTIVITY RELATIONSHIPS OF A SERIES OF POTENT AND ORALLY ACTIVE CORTICOTROPIN-RELEASING FACTOR RECEPTOR ANTAGONISTS" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 47, no. 19, 9 September 2004 (2004-09-09), pages 4787-4798, XP001206057 ISSN: 0022-2623 figure 1	1-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/078605

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 11-13 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2007/078605

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2007/078605

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 February 2019 (21.02.2019)

WIPO | PCT

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C07D 471/04 (2006.01)

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PCT/US2018/046707

(22) International Filing Date:
14 August 2018 (14.08.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/545,393 14 August 2017 (14.08.2017) US

(71) Applicant: **SPRUCE BIOSCIENCES, INC.** [US/US]; 548 Market Street, Suite 74589, San Francisco, CA 94105 (US).

(72) Inventors: **HOWERTON, Alexis**; 548 Market Street, Suite 74589, San Francisco, CA 94105 (US). **GERBER, Hal**; 548 Market Street, Suite 74589, San Francisco, CA 94105 (US). **KARABORNI, Sami**; 548 Market Street, Suite 74589, San Francisco, CA 94105 (US).

(74) Agent: **DELANEY, Simren**; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, CA 94304 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: CORTICOTROPIN RELEASING FACTOR RECEPTOR ANTAGONISTS

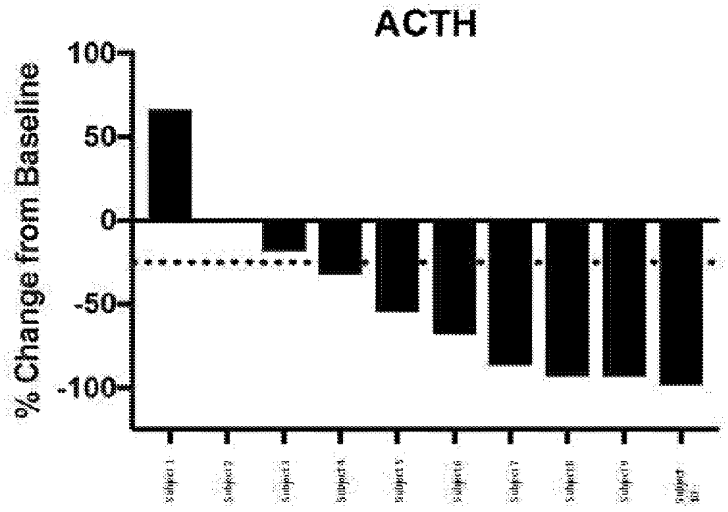

FIG. 2

(57) **Abstract:** The present invention provides novel pharmaceutical compositions comprising 3-(4-Chloro-2-(morpholin-4-yl)thiazol-5-yl)-7-(1-ethylpropyl)-2,5-dimethylpyrazolo(1,5- a)pyriinidine and methods of using the same for the treatment of Congenital adrenal hyperplasia (CAH).

[Continued on next page]

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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

CORTICOTROPIN RELEASING FACTOR RECEPTOR ANTAGONISTS

CROSS-REFERENCE

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 62/545,393, filed August 14, 2017, which is incorporated herein by reference in its entirety.

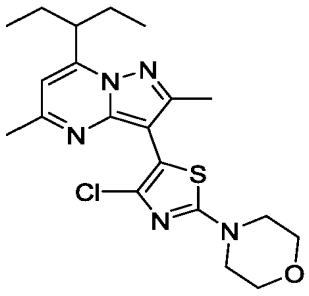
BACKGROUND OF THE INVENTION

[0002] Corticotropin releasing factor (CRF) is a 41 amino acid peptide that is the primary physiological regulator of proopiomelanocortin (POMC) derived peptide secretion from the anterior pituitary gland. In addition to its endocrine role at the pituitary gland, immunohistochemical localization of CRF has demonstrated that the hormone has a broad extrahypothalamic distribution in the central nervous system and produces a wide spectrum of autonomic, electrophysiological and behavioral effects consistent with a neurotransmitter or neuromodulator role in the brain. There is also evidence that CRF plays a significant role in integrating the response in the immune system to physiological, psychological, and immunological stressors.

SUMMARY OF THE INVENTION

[0003] The present invention provides novel pharmaceutical compositions comprising 3-(4-Chloro-2-(morpholin-4-yl)thiazol-5-yl)-7-(1-ethylpropyl)-2,5-dimethylpyrazolo(1,5-a)pyrimidine and methods using such pharmaceutical compositions for treating congenital adrenal hyperplasia (CAH).

[0004] In one aspect, the present disclosure provides a pharmaceutical composition in the form of a capsule comprising Compound 1:



or a pharmaceutically acceptable salt or solvate thereof.

[0005] In one embodiment, the pharmaceutical composition comprises between about 1 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises between about 5 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment the pharmaceutical composition comprises between about 10 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical

composition comprises between about 10 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises between about 10 mg and about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0006] In one embodiment, the pharmaceutical composition comprises between about 50 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises between about 100 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises between about 100 mg and about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises between about 100 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises between about 150 mg and about 250 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0007] In one embodiment, the pharmaceutical composition comprises about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 250 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 200 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 150 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 80 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 60 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 50 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 30 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

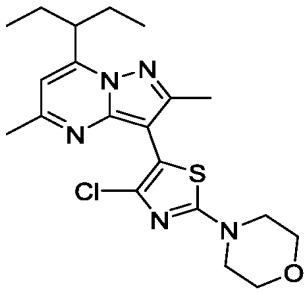
[0008] In one embodiment, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is in the form of microparticles. In one embodiment, the average size of the microparticles is between about 1 μm and about 20 μm. In one embodiment, the average size of

the microparticles is between about 5 μm and about 15 μm. In one embodiment, the average size of the microparticles is less than about 10 μm.

[0009] In one embodiment, the capsule is a hard gelatin capsule. In one embodiment, the capsule is a soft gelatin capsule. In one embodiment, the capsule is formed using materials selected from the group consisting of natural gelatin, synthetic gelatin, pectin, casein, collagen, protein, modified starch, polyvinylpyrrolidone, acrylic polymers, cellulose derivatives, and any combinations thereof.

[0010] In one embodiment, the pharmaceutical composition is free of additional excipients. In one embodiment, the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

[0011] In one aspect, the present disclosure provides a pharmaceutical composition in the form of a tablet comprising Compound 1:



or a pharmaceutically acceptable salt or solvate thereof.

[0012] In one embodiment, the pharmaceutical composition comprises between about 1 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises between about 5 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises between about 10 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises between about 10 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises between about 10 mg and about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0013] In one embodiment, the pharmaceutical composition comprises between about 50 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises between about 100 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises between about 100 mg and about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises between about 100 mg and about 300

mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises between about 150 mg and about 250 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0014] In one embodiment, the pharmaceutical composition comprises about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 250 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 200 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 150 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 80 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 60 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 50 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the pharmaceutical composition comprises about 30 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0015] In one embodiment, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is in the form of microparticles. In one embodiment, the average size of the microparticles is between about 1 μm and about 20 μm. In one embodiment, the average size of the microparticles is between about 5 μm and about 15 μm. In one embodiment, the average size of the microparticles is less than about 10 μm.

[0016] In one embodiment, the tablet is made by compression, molding, or extrusion. In one embodiment, he tablet is made by hot-melt extrusion. In one embodiment, the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

[0017] In one embodiment, the pharmaceutical composition is stable for at least 1 month at 25 °C. In one embodiment, the pharmaceutical composition is stable for at least 3 months at 25 °C. In one embodiment, the pharmaceutical composition is stable for at least 6 months at 25 °C. In one embodiment, the pharmaceutical composition is stable for at least 9 months at 25 °C. In one embodiment, the pharmaceutical composition is stable for at least 12 months at 25 °C.

INCORPORATION BY REFERENCE

[0018] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0020] FIG. 1 shows Compound 1 in patients with CAH following 14-days of once daily dosing at each level;

[0021] FIG. 2 demonstrates the attenuation of ACTH across different subjects due to the administration of Compound 1;

[0022] FIG. 3 demonstrates the reduction in 17-OHP due to the administration of Compound 1;

[0023] FIG. 4 demonstrates the reduction of Androstenedione due to the administration of Compound 1;

[0024] FIG. 5 demonstrates the percentage release of Manufacturing Formulae A-1, A-2 and A-3 in 0.1N HCL + 1.0% SDS, media at 50 rpm and 900 mL using USP-II (Paddle);

[0025] FIG. 6 demonstrates the percentage release of Manufacturing Formulae B-1, B-2 and B-3 in 0.1N HCL + 1.0% SDS, media at 50 rpm and 900 mL using USP-II (Paddle); and

[0026] FIG. 7 demonstrates the percentage release of Manufacturing Formulae C-1, C-2 and C-3 in 0.1N HCL + 1.0% SDS, media at 50 rpm and 900 mL using USP-II (Paddle).

DETAILED DESCRIPTION OF THE INVENTION

[0027] CRF has been implicated in psychiatric disorders and neurological diseases including depression and anxiety, as well as the following: Alzheimer's disease, Huntington's disease, progressive supranuclear palsy, amyotrophic lateral sclerosis, Parkinson's disease, epilepsy, migraine, alcohol and substance abuse and associated withdrawal symptoms, obesity, metabolic syndrome, congenital adrenal hyperplasia (CAH), Cushing's disease, hypertension, stroke, irritable bowel syndrome, stress-induced gastric ulceration, premenstrual syndrome, sexual dysfunction, premature labor, inflammatory disorders, allergies, multiple sclerosis, visceral pain, sleep disorders, pituitary tumors or ectopic pituitary derived tumors, chronic fatigue syndrome, and fibromyalgia.

[0028] CRF receptor subtypes, CRF1 and CRF2, have been identified and are distributed heterogeneously within the brain thereby suggesting potential functional diversity. For example, widely distributed brain CRF1 receptors are strongly implicated in emotionality accompanying exposure to environmental stressors. Significantly, CRF1, not CRF2, receptors appear to mediate select anxiogenic like behaviors. A more discrete septallhypothalmic distribution and the availability of alternative endogenous ligands suggest a different functional role for the CRF2 receptor. For example, a novel CRF-family neuropeptide with preferential affinity for CRF2 relative to CRF 1 receptors is reported to suppress appetite without producing the profile of behavioral activation observed with selective CRF1 agonism. In other cases, CRF2 agonism produces similar effects to those reported for CRF 1 antagonists or CRF 1 gene deletion. For example, while CRF2 agonists have been proposed as antiobesity agents, CRF1 antagonists may be an important treatment for obesity as well.

[0029] Treatment of CAH is based on normalization of hormone and steroid levels using a variety of medications from diagnosis in infancy through adulthood. Glucocorticoids are the current standard treatment in CAH and are used both to correct the endogenous Cortisol deficiency and for reducing the elevated ACTH levels from the pituitary, which drives increased androgen production. Unlike the treatment of Addison's disease (adrenal insufficiency), in which Cortisol replacement is sufficient, the treatment of CAH must also reduce ACTH production, to control the subsequent androgen excess as well. Thus, the goals of glucocorticoid treatment include Cortisol replacement and suppression of ACTH to prevent virilization and menstrual disturbances in women. Mineralocorticoid replacement is needed to achieve normal plasma renin activity for maintenance of regular blood pressure, electrolyte balance, and volume status in those patients with the salt-wasting form of CAH.

[0030] The regimen of glucocorticoid treatment must support normal physiology and also ensure that sufficient Cortisol is available during events that may elicit a strong stress response (e.g., intercurrent illness, exercise, hypotension). Careful monitoring is also necessary to avoid the development of Addisonian syndrome due to under-treatment. Overtreatment with mineralocorticoids may cause hypertension while under-treatment may lead to low blood pressure, salt loss, fatigue and increased requirements for glucocorticoids. Typical laboratory tests for monitoring treatment efficacy include measurement of plasma concentrations of 17-OHP, androstenedione, testosterone, renin activity, and electrolytes.

[0031] Adult patients with CAH have an increased prevalence of risk factors for cardiovascular disease including obesity, hypertension, and insulin resistance. A study of a large cohort of pediatric and adult CAH patients (n=244) demonstrated that patients are prescribed a variety of glucocorticoid treatment regimens yet frequently suffer from poor hormonal control and the

aforementioned adverse outcomes. Treatment of CAH includes efforts to normalize the Cortisol deficiency with glucocorticoids (usually hydrocortisone in children but often more potent agents with narrow therapeutic indices, such as dexamethasone, in adults) and, if necessary for salt-wasting, mineralocorticoids (usually fludrocortisone). The glucocorticoid doses required to achieve sufficient suppression of excess androgens, however, are usually well above the normal physiologic dose used for Cortisol replacement alone as in patients with Addison's disease. This increased exposure to glucocorticoids can lead to increased cardiovascular risk factors, glucose intolerance, and decreased bone mineral density in CAH patients.

[0032] CRF is believed to be the major physiological regulator of the basal and stress-induced release of adrenocorticotropic hormone ("ACTH"), β-endorphin, and other proopiomelanocortin ("POMC")-derived peptides from the anterior pituitary. Secretion of CRF causes release of ACTH from corticotrophs in the anterior pituitary via binding to the CRF_1 receptor, a member of the class B family of G-protein coupled receptors.

[0033] Due to the physiological significance of CRF_1, the development of biologically-active small molecules having significant CRF receptor binding activity and which are capable of antagonizing the CRF_1 receptor remains a desirable goal and has been the subject of ongoing research and development for the treatment of anxiety, depression, irritable bowel syndrome, post-traumatic stress disorder, and substance abuse.

[0034] The pituitary hormone ACTH, under the control of hypothalamic corticotropin-releasing factor (CRF), stimulates uptake of cholesterol and drives the synthesis of pregnenolone initiating steroidogenesis in the adrenal gland. The adrenal cortex is comprised of three zones, which produce distinct classes of hormones many of which are driven by ACTH mobilizing cholesterol through this pathway. Deficiencies in these enzymes as a result of mutation or deletion cause the substrate concentrations to increase. In the most common form of CAH resulting from mutations or deletions in the 21 -hydroxylase gene (CYP21A2), potent androgens are produced by the adrenal because of the accumulation of the steroid precursors, progesterone and 17-hydroxyprogesterone (17-OHP). Plasma levels of 17-OHP can reach 10-1000 times the normal concentration in these cases. These increases result in the overproduction of androgens, specifically androstenedione, testosterone, and dihydroxytestosterone causing virilization in females. In addition, 21 -hydroxylase deficiency in CAH causes insufficient biosynthesis of glucocorticoids and mineralocorticoids, specifically Cortisol and aldosterone. Cortisol is a critical negative feedback regulator of hypothalamic CRF secretion and pituitary ACTH release. The lack of glucocorticoid synthesis and release eliminates the restraint on the hypothalamus and pituitary, which causes ACTH levels to increase. The excessive ACTH stimulation causes hypertrophy of the zona fasciculata and zona reticularis resulting in adrenal hyperplasia.

[0035] In one embodiment, the CRF receptor antagonist useful for the treatment of CAH is 3-(4-Chloro-2-(morpholin-4-yl)thiazol-5-yl)-7-(1-ethylpropyl)-2,5-dimethylpyrazolo(1,5-a)pyrimidine.

Certain Definitions

[0036] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments described herein, certain preferred methods, devices, and materials are now described.

[0037] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "an excipient" is a reference to one or more excipients and equivalents thereof known to those skilled in the art, and so forth.

[0038] The term "about" is used to indicate that a value includes the standard level of error for the device or method being employed to determine the value.

[0039] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and to "and/or."

[0040] The terms "comprise," "have" and "include" are open-ended linking verbs. Any forms or tenses of one or more of these verbs, such as "comprises," "comprising," "has," "having," "includes" and "including," are also open-ended. For example, any method that "comprises," "has" or "includes" one or more steps is not limited to possessing only those one or more steps and also covers other unlisted steps.

[0041] "Administering" when used in conjunction with a therapeutic means to administer a therapeutic systemically or locally, as directly into or onto a target tissue, or to administer a therapeutic to a patient whereby the therapeutic positively impacts the tissue to which it is targeted. "Administering" a pharmaceutical composition may be accomplished by injection, topical administration, and oral administration or by other methods alone or in combination with other known techniques.

[0042] By "pharmaceutically acceptable", it is meant the carrier, diluent or excipient must be compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

[0043] The term "pharmaceutical composition" means a composition comprising at least one active ingredient, such as Compound 1, whereby the composition is amenable to investigation for a specified, efficacious outcome in a mammal (for example, without limitation, a human). Those of ordinary skill in the art will understand and appreciate the techniques appropriate for

determining whether an active ingredient has a desired efficacious outcome based upon the needs of the artisan.

[0044] The term "supraphysiologic" amount" describes hormones levels that are elevated compared to average levels found in healthy individuals.

[0045] The term "physiologic amount" describes average hormone levels found in healthy individuals.

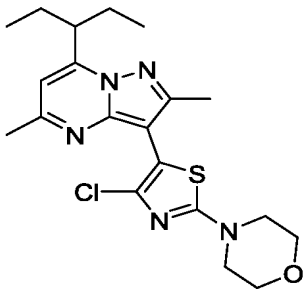
[0046] A "therapeutically effective amount" or "effective amount" as used herein refers to the amount of active compound or pharmaceutical agent that elicits a biological or medicinal response in a tissue, system, animal, individual or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes one or more of the following: (1) preventing the disease; for example, preventing a disease, condition or disorder in an individual that may be predisposed to the disease, condition or disorder but does not yet experience or display the pathology or symptomatology of the disease, (2) inhibiting the disease; for example, inhibiting a disease, condition or disorder in an individual that is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., arresting further development of the pathology and/or symptomatology), and (3) ameliorating the disease; for example, ameliorating a disease, condition or disorder in an individual that is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., reversing the pathology and/or symptomatology).

[0047] The terms "treat," "treated," "treatment," or "treating" as used herein refers to both therapeutic treatment in some embodiments and prophylactic or preventative measures in other embodiments, wherein the object is to prevent or slow (lessen) an undesired physiological condition, disorder or disease, or to obtain beneficial or desired clinical results. For the purposes described herein, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of the extent of the condition, disorder or disease; stabilization (i.e., not worsening) of the state of the condition, disorder or disease; delay in onset or slowing of the progression of the condition, disorder or disease; amelioration of the condition, disorder or disease state; and remission (whether partial or total), whether detectable or undetectable, or enhancement or improvement of the condition, disorder or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment. A prophylactic benefit of treatment includes prevention of a condition, retarding the progress of a condition, stabilization of a condition, or decreasing the likelihood of occurrence of a condition. As used herein, "treat," "treated," "treatment," or "treating" includes prophylaxis in some embodiments.

[0048] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

Compound

[0049] Disclosed herein is 3-(4-Chloro-2-(morpholin-4-yl)thiazol-5-yl)-7-(1-ethylpropyl)-2,5-dimethylpyrazolo(1,5-a)pyrimidine (or alternatively 4-(4-chloro-5-(2,5-dimethyl-7-(pentan-3-yl)pyrazolo[1,5-a]pyrimidin-3-yl)thiazol-2-yl)morpholine), a pharmaceutically acceptable salt, and/or a solvate thereof:



. In some embodiments, 4-(4-chloro-5-(2,5-dimethyl-7-(pentan-3-yl)pyrazolo[1,5-a]pyrimidin-3-yl)thiazol-2-yl)morpholine is referred to as Compound 1. In some embodiments, 3-(4-Chloro-2-(morpholin-4-yl)thiazol-5-yl)-7-(1-ethylpropyl)-2,5-dimethylpyrazolo(1,5-a)pyrimidine is referred to as Compound 1.

Pharmaceutical Compositions

[0050] Poorly soluble drugs may be difficult to formulate using technologies such as high shear wet granulation. Optimum delivery of poorly soluble drugs may require complex technologies such as solid solutions or amorphous dispersions (for example hot melt extrusion or spray drying), nano-formulations or lipid-based formulations. Hydrophobic drug substances which may be considered poorly soluble according to USP criteria may also be difficult to granulate with water and other excipients as most excipients for immediate-release formulations may be water soluble or water-swellable.

[0051] Making a small tablet of a high dose drug substance that is poorly soluble may require a high concentration of the drug substance. However, as the drug concentration is increased above a certain level, formation of granules may become more difficult, and at a certain drug load, it may become impossible.

[0052] In one embodiment, the pharmaceutical compositions described herein may be for a pediatric population. Hence, it may be necessary to maintain the pharmaceutical composition as small as possible to facilitate swallowing of pills and therefore increase patient compliance. In some embodiments, tablet weights are less than 400 mg. In some embodiments, tablet weights are less than 300 mg. In some embodiments, where dose strength is 200 mg, the drug load in the tablet is higher than 50%. In some embodiments, where dose strength is 200 mg, the drug load in the tablet is higher than 66%. In some embodiments, where dose strength is 200 mg, the drug load is as high as possible.

[0053] Disclosed herein is a pharmaceutical composition comprising Compound 1, a pharmaceutically acceptable salt, and/or a solvate thereof.

Dosage Form

[0054] In some embodiments, the pharmaceutical compositions described herein are provided in unit dosage form. As used herein, a "unit dosage form" is a composition containing an amount of Compound 1 that is suitable for administration to an animal, preferably mammal, subject in a single dose, according to good medical practice. The preparation of a single or unit dosage form however, does not imply that the dosage form is administered once per day or once per course of therapy. Such dosage forms are contemplated to be administered once, twice, thrice or more per day and may be administered as infusion over a period of time (e.g., from about 30 minutes to about 2-6 hours), or administered as a continuous infusion, and may be given more than once during a course of therapy, though a single administration is not specifically excluded.

[0055] Pharmaceutical compositions are administered in a manner appropriate to the disease to be treated (or prevented). An appropriate dose and a suitable duration and frequency of administration will be determined by such factors as the condition of the patient, the type and severity of the patient's disease, the particular form of the active ingredient, and the method of administration. In general, an appropriate dose and treatment regimen provides the composition(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit (e.g., an improved clinical outcome, such as more frequent complete or partial remissions, or longer disease-free and/or overall survival, or a lessening of symptom severity. Optimal doses are generally determined using experimental models and/or clinical trials. The optimal dose depends upon the body mass, weight, or blood volume of the patient.

[0056] In some embodiments, the pharmaceutical compositions described herein are formulated as oral dosage forms. Suitable oral dosage forms include, for example, tablets, pills, sachets, or capsules. In some embodiments, the pharmaceutical composition comprises one or more additional pharmaceutically acceptable excipients. *See*, *e.g.*, *Remington: The Science and*

Practice of Pharmacy (Gennaro, 21st Ed. Mack Pub. Co., Easton, PA (2005) for a list of pharmaceutically acceptable excipients.

Capsule

[0057] In some embodiments, the pharmaceutical composition is formulated as a capsule. In some embodiments, the pharmaceutical composition is formulated as a hard gel capsule. In some embodiments, the pharmaceutical composition is formulated as a soft gel capsule.

[0058] In some embodiments, the capsule is formed using materials which include, but are not limited to, natural or synthetic gelatin, pectin, casein, collagen, protein, modified starch, polyvinylpyrrolidone, acrylic polymers, cellulose derivatives, or any combinations thereof. In some embodiments, the capsule is formed using preservatives, coloring and opacifying agents, flavorings and sweeteners, sugars, gastroresistant substances, or any combinations thereof. In some embodiments, the capsule is coated. In some embodiments, the coating covering the capsule includes, but is not limited to, immediate release coatings, protective coatings, enteric or delayed release coatings, sustained release coatings, barrier coatings, seal coatings, or combinations thereof. In some embodiments, a capsule herein is hard or soft. In some embodiments, the capsule is seamless. In some embodiments, the capsule is broken such that the particulates are sprinkled on soft foods and swallowed without chewing. In some embodiments, the shape and size of the capsule also vary. Examples of capsule shapes include, but are not limited to, round, oval, tubular, oblong, twist off, or a non-standard shape. The size of the capsule may vary according to the volume of the particulates. In some embodiments, the size of the capsule is adjusted based on the volume of the particulates and powders. Hard or soft gelatin capsules may be manufactured in accordance with conventional methods as a single body unit comprising the standard capsule shape. A single-body soft gelatin capsule typically may be provided, for example, in sizes from 3 to 22 minims (1 minims being equal to 0.0616 ml) and in shapes of oval, oblong or others. The gelatin capsule may also be manufactured in accordance with conventional methods, for example, as a two-piece hard gelatin capsule, sealed or unsealed, typically in standard shape and various standard sizes, conventionally designated as (000), (00), (0), (1), (2), (3), (4), and (5). The largest number corresponds to the smallest size. In some embodiments, the pharmaceutical composition described herein (e.g., capsule) is swallowed as a whole.

[0059] In some embodiments, the capsule comprises one or more pharmaceutically acceptable excipients. In some embodiments, the capsule is free of additional excipients.

[0060] In some embodiments, a capsule is developed, manufactured and commercialized for a drug substance that is insoluble. In some embodiments, a drug substance is insoluble if solubility is less than 0.002 mg/mL in water. In some embodiments, the capsule has a dose

strength of up to 200 mg. In some embodiments, drug substance in the capsule is immediately released in a dissolution medium using USP apparatus I. In some embodiments, drug substance in the capsule is immediately released in a dissolution medium using USP apparatus II.

Tablet

[0061] Insoluble drugs may be difficult to formulate using standard technologies such as high shear wet granulation. Optimum delivery of insoluble drugs may require complex technologies such as solid solutions amorphous dispersions (hot melt extrusion or spray drying), nano-formulations or lipid-based formulations. Hydrophobic drug substances may be considered insoluble according to USP criteria and may be known to be difficult to granulate with water and other excipients. This is likely due to most known excipients for immediate release formulations being water soluble or water-swellable. Making a tablet of a high dose drug substance that is insoluble may require a high concentration of the drug substance. However, as the drug concentration is increased above a certain level, formation of granules may become more and more difficult. Furthermore, at a certain drug load, it may become impossible.

[0062] In some embodiments, the pharmaceutical composition is formulated as a tablet.

[0063] In some embodiments, the tablet is made by compression, molding, or extrusion, optionally with one or more pharmaceutically acceptable excipient. In some embodiments, compressed tablets are prepared by compressing Compound 1 in a free-flowing form, optionally mixed with pharmaceutically acceptable excipients. In some embodiments, molded tablets are made by molding a mixture of the powdered Compound 1 moistened with an inert liquid diluent. In some embodiments, the tablet is prepared by hot-melt extrusion. In some embodiments, extruded tablets are made by forcing a mixture comprising Compound 1 through an orifice or die under controlled conditions. In some embodiments, the tablet is coated or scored. In some embodiments, the tablet is formulated so as to provide slow or controlled release of Compound 1. In some embodiments, a tablet is developed, manufactured and commercialized for a drug substance that is insoluble. In some embodiments, a drug substance is insoluble if solubility is less than 0.002 mg/mL in water. In some embodiments, the tablet has a dose strength of up to 200 mg. In some embodiments, drug substance in the tablet is immediately released in a dissolution medium using USP apparatus I. In some embodiments, drug substance in the tablet is immediately released in a dissolution medium using USP apparatus II.

[0064] In some embodiments, the tablet size is less than about 1000 mg, less than about 800 mg, less than about 600 mg, less than about 400 mg or less than about 200 mg. In some embodiments, the tablet has a dose strength of more than about 50 mg, more than about 100 mg, more than about 150 mg, more than about 200 mg, or more than about 250 mg. In some embodiments, the tablet size is less than about 1000 mg for a dose strength of more than about

50 mg. In some embodiments, the tablet size is less than 800 mg for a dose strength of more than about 100 mg. In some embodiments, the tablet size is less than 600 mg for a dose strength of more than about 150 mg. In some embodiment, the tablet size is less than 400 mg for a dose strength of more than about 200 mg. In some embodiments, the tablet size is less than 400 mg for a dose strength of 200 mg.

In some embodiments, more than about 20% of the tablet is dissolved in conventional dissolution media. In some embodiments, more than about 40% of the tablet is dissolved in conventional dissolution media. In some embodiments, more than about 50% of the tablet is dissolved in conventional dissolution media. In some embodiments, more than about 60% of the tablet is dissolved in conventional dissolution media. In some embodiments, more than about 70% of the tablet is dissolved in conventional dissolution media. In some embodiments, more than about 80% of the tablet is dissolved in conventional dissolution media. In some embodiments, more than about 20% of the tablet is dissolved in less than 24 hours in conventional dissolution media. In some embodiments, more than about 20% of the tablet is dissolved in less than 12 hours in conventional dissolution media. In some embodiments, more than about 20% of the tablet is dissolved in less than 6 hours in conventional dissolution media. In some embodiments, more than about 20% of the tablet is dissolved in less than 3 hours in conventional dissolution media. In some embodiments, more than about 20% of the tablet is dissolved in less than 2 hours in conventional dissolution media. In some embodiments, more than about 20% of the tablet is dissolved in less than 60 minutes in conventional dissolution media. In some embodiments, more than about 40% of the tablet is dissolved in less than 60 minutes in conventional dissolution media. In some embodiments, more than about 50% of the tablet is dissolved in less than 60 minutes in conventional dissolution media. In some embodiments, more than about 60% of the tablet is dissolved in less than 60 minutes in conventional dissolution media. In some embodiments, more than about 70% of the tablet is dissolved in less than 60 minutes in conventional dissolution media. In some embodiments, more than about 80% of the tablet is dissolved in less than 60 minutes in conventional dissolution media. In some embodiments, more than 70% of the tablet is dissolved in 60 minutes in conventional dissolution media.

[0065] In some embodiments, the tablet is produced at a commercial scale.

[0066] In some embodiments, the tablet comprises one or more pharmaceutically acceptable excipients.

[0067] In some embodiments, the tablet is coated with a coating material, e.g., a sealant. In some embodiments, the coating material is water soluble. In some embodiments, the coating material comprises a polymer, plasticizer, a pigment, or any combination thereof. In some

embodiments, the coating material is in the form of a film coating, e.g., a glossy film, a pH independent film coating, an aqueous film coating, a dry powder film coating (e.g., complete dry powder film coating), or any combination thereof. In some embodiments, the coating material is highly adhesive. In some embodiments, the coating material provides low level of water permeation. In some embodiments, the coating material provides oxygen barrier protection. In some embodiments, the coating material allows immediate disintegration for fast release of Compound 1. In some embodiments, the coating material is pigmented, clear, or white. In some embodiments, the coating is an enteric coating. Exemplary coating materials include, without limitation, polyvinylpyrrolidone, polyvinyl alcohol, an acrylate-methacrylic acid copolymer, a methacrylate-methacrylic acid copolymer, cellulose acetate phthalate, cellulose acetate succinate, hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, polyvinyl acetate phthalate, shellac, cellulose acetate trimellitate, sodium alginate, zein, and any combinations thereof.

Pharmaceutically Acceptable Excipients

[0068] In some embodiments, the pharmaceutical composition comprises a pharmaceutically acceptable excipient. In some embodiments, the composition is free of pharmaceutically acceptable excipients. The term "pharmaceutically acceptable excipient", as used herein, means one or more compatible solid or encapsulating substances, which are suitable for administration to a mammal. The term "compatible", as used herein, means that the components of the composition are capable of being commingled with the subject compound, and with each other, in a manner such that there is no interaction, which would substantially reduce the pharmaceutical efficacy of the composition under ordinary use situations. In some embodiments, the pharmaceutically acceptable excipient is of sufficiently high purity and sufficiently low toxicity to render them suitable for administration preferably to an animal, preferably mammal, being treated.

[0069] Some examples of substances, which can serve as pharmaceutically acceptable excipients include:

- Amino acids such as alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. In some embodiments, the amino acid is arginine. In some embodiments, the amino acid is L-arginine.
- Monosaccharides such as glucose (dextrose), arabinose, mannitol, fructose (levulose), and galactose.
- Cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose, and methyl cellulose.

- Solid lubricants such as talc, stearic acid, magnesium stearate and sodium stearyl fumarate.
- Polyols such as propylene glycol, glycerin, sorbitol, mannitol, and polyethylene glycol.
- Emulsifiers such as the polysorbates.
- Wetting agents such as sodium lauryl sulfate, Tween®, Span , alkyl sulphates, and alkyl ethoxylate sulphates.
- Cationic surfactants such as cetrimide, benzalkonium chloride, and cetylpyridinium chloride.
- Diluents such as calcium carbonate, microcrystalline cellulose, calcium phosphate, starch, pregelatinized starch, sodium carbonate, mannitol, and lactose.
- Binders such as starches (corn starch and potato starch), gelatin, sucrose hydroxypropyl cellulose (HPC), polyvinylpyrrolidone (PVP), and hydroxypropyl methyl cellulose (HPMC).
- Disintegrants such as starch, and alginic acid.
- Super-disintegrants such as ac-di-sol, croscarmellose sodium, sodium starch glycolate and crospovidone.
- Glidants such as silicon dioxide.
- Coloring agents such as the FD&C dyes.
- Sweeteners and flavoring agents, such as aspartame, saccharin, menthol, peppermint, and fruit flavors.
- Preservatives such as benzalkonium chloride, PHMB, chlorobutanol, thimerosal, phenylmercuric, acetate, phenylmercuric nitrate, parabens, and sodium benzoate.
- Tonicity adjustors such as sodium chloride, potassium chloride, mannitol, and glycerin.
- Antioxidants such as sodium bisulfite, acetone sodium bisulfite, sodium formaldehyde, sulfoxylate, thiourea, and EDTA.
- pH adjuster such as NaOH, sodium carbonate, sodium acetate, HCl, and citric acid.
- Cryoprotectants such as sodium or potassium phosphates, citric acid, tartaric acid, gelatin, and carbohydrates such as dextrose, mannitol, and dextran.
- Surfactants such as sodium lauryl sulfate. For example, cationic surfactants such as cetrimide (including tetradecyl trimethyl ammonium bromide with dodecyl and hexadecyl compounds), benzalkonium chloride, and cetylpyridinium chloride. Some examples of anionic surfactants are alkylsulphates, alkylethoxylate sulphates, soaps, carxylate ions, sulfate ions, and sulfonate ions. Some examples of non-ionic surfactants are polyoxyethylene derivatives, polyoxypropylene derivatives, polyol derivatives,

polyol esters, polyoxyethylene esters, poloxamers, glocol, glycerol esters, sorbitan derivatives, polyethylene glycol (such as PEG-40, PEG-50, or PEG-55) and esters of fatty alcohols.

- Organic materials such as carbohydrates, modified carbohydrates, lactose (including a-lactose, monohydrate spray dried lactose or anhydrous lactose), starch, pregelatinized starch, sucrose, mannitol, sorbital, cellulose (including powdered cellulose and microcrystalline cellulose).
- Inorganic materials such as calcium phosphates (including anhydrous dibasic calcium hosphate, dibasic calcium phosphate or tribasic calcium phosphate).
- Co-processed diluents.
- Compression aids .
- Anti-tacking agents such as silicon dioxide and talc.

Amounts

[0070] In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 200 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 90 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 80 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 70 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 60 mg

of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 50 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 40 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 30 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 20 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 10 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 5 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule comprises about 1 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule comprises about 5 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0071] In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 5 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 200 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 100 mg of Compound 1, or a pharmaceutically

acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 90 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 80 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 70 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 60 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0072] In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 20 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 20 mg and about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 20 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 20 mg and about 200 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 20 mg and about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 20 mg and about 90 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 20 mg and about 80 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 20 mg and about 70 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 20 mg and about 60 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0073] In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 30 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 30 mg and about 400

mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 30 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 30 mg and about 200 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 30 mg and about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 30 mg and about 90 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 30 mg and about 80 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 30 mg and about 70 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 30 mg and about 60 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0074] In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 40 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 40 mg and about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 40 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 40 mg and about 200 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 40 mg and about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 40 mg and about 90 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 40 mg and about 80 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 40 mg and about 70 mg

of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 40 mg and about 60 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. . In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises about 50 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0075] In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 50 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 50 mg and about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 50 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 50 mg and about 200 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 50 mg and about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 50 mg and about 90 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 50 mg and about 80 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 50 mg and about 70 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0076] In some embodiments, the pharmaceutical composition comprises between about 100 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises between about 100 mg and about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises between about 100 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises between about 150 mg and about 250 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises between about 100 mg and about 200 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0077] In some embodiments, the pharmaceutical composition comprises about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 250 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 200 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 150 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 90 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 80 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 70 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 60 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 50 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 40 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 30 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 20 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 10 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

Particle size

[0078] In some embodiments, the pharmaceutical composition, in the form of a tablet or a capsule, comprises Compound 1, or a pharmaceutically acceptable salt or solvate thereof, in the form of microparticles. In some embodiments, microparticles of Compound 1 have an average size from about 1 μm to about 100 μm. In some embodiments, microparticles of Compound 1 have an average size from about 1 μm to about 50 μm. In some embodiments, microparticles of Compound 1 have an average size from about 1 μm to about 30 μm. In some embodiments, microparticles of Compound 1 have an average size from about 1 μm to about 20 μm. In some

embodiments, microparticles of Compound 1 have an average size from about 5 μm to about 15 μm. In some embodiments, microparticles of Compound 1 have an average size from about 1 μm to about 10 μm. In some embodiments, microparticles of Compound 1 have an average size from about 3 μm to about 10 μm. In some embodiments, microparticles of Compound 1 have an average size from about 4 μm to about 9 μm.

[0079] In some embodiments, microparticles of Compound 1 have an average size less than about 100 μm. In some embodiments, microparticles of Compound 1 have an average size less than about 80 μm. In some embodiments, microparticles of Compound 1 have an average size less than about 60 μm. In some embodiments, microparticles of Compound 1 have an average size less than about 50 μm. In some embodiments, microparticles of Compound 1 have an average size less than about 40 μm. In some embodiments, microparticles of Compound 1 have an average size less than about 30 μm. In some embodiments, microparticles of Compound 1 have an average size less than about 20 μm. In some embodiments, microparticles of Compound 1 have an average size less than about 10 μm.

Pharmacokinetics

[0080] In some embodiments, Compound 1 is formulated as a capsule or a tablet as to provide a Tmax of about 1 to about 8 hours in a subject. In some embodiments, Compound 1 is formulated as a capsule or a tablet as to provide a Tmax of about 2 to about 7 hours in a subject. In some embodiments, Compound 1 is formulated as a capsule or a tablet as to provide a Tmax of about 2 to about 6 hours in a subject. In some embodiments, Compound 1 is formulated as a capsule or a tablet as to provide a Tmax of about 3 to about 5 hours in a subject.

[0081] In some embodiments, Compound 1 is formulated as a capsule or a tablet as to provide a Tmax of about 8 hours in a subject. In some embodiments, Compound 1 is formulated as a capsule or a tablet as to provide a Tmax of about 7 hours in a subject. In some embodiments, Compound 1 is formulated as a capsule or a tablet as to provide a Tmax of about 6 hours in a subject. In some embodiments, Compound 1 is formulated as a capsule or a tablet as to provide a Tmax of about 5 hours in a subject. In some embodiments, Compound 1 is formulated as a capsule or a tablet as to provide a Tmax of about 4 hours in a subject. In some embodiments, Compound 1 is formulated as a capsule or a tablet as to provide a Tmax of about 3 hours in a subject. In some embodiments, Compound 1 is formulated as a capsule or a tablet as to provide a Tmax of about 2 hours in a subject. In some embodiments, Compound 1 is formulated as a capsule or a tablet as to provide a Tmax of about 1 hour in a subject.

Stability

[0082] The pharmaceutical compositions described herein are stable in various storage conditions including refrigerated, ambient and accelerated conditions. Stable as used herein

refers to pharmaceutical compositions having about 95% or greater of the initial Compound 1 amount and about 5% w/w or less total impurities or related substances at the end of a given storage period. The percentage of impurities is calculated from the amount of impurities relative to the amount of Compound 1. Stability is assessed by HPLC or any other known testing method. In some embodiments, the stable pharmaceutical compositions have about 5% w/w, about 4% w/w, about 3% w/w, about 2.5% w/w, about 2% w/w, about 1.5% w/w, about 1% w/w, or about 0.5% w/w total impurities or related substances. In other embodiments, the stable pharmaceutical compositions have about 5% w/w total impurities or related substances. In yet other embodiments, the stable pharmaceutical compositions have about 4% w/w total impurities or related substances. In yet other embodiments, the stable pharmaceutical compositions have about 3% w/w total impurities or related substances. In yet other embodiments, the stable pharmaceutical compositions have about 2% w/w total impurities or related substances. In yet other embodiments, the stable pharmaceutical compositions have about 1% w/w total impurities or related substances.

[0083] At refrigerated condition, the pharmaceutical compositions described herein are stable for at least 1 month, at least 2 months, at least 3 months, at least 6 months, at least 9 months, at least 12 months, at least 15 months, at least 18 months, at least 24 months, at least 30 months and at least 36 months. In some embodiments, refrigerated condition is 5±5 °C. In some embodiments, refrigerated condition is about 0 °C, about 0.1 °C, about 0.2 °C, about 0.3 °C, about 0.4 °C, about 0.5 °C, about 0.6 °C, about 0.7 °C, about 0.8 °C, about 0.9 °C, about 1 °C, about 1.1 °C, about 1.2 °C, about 1.3 °C, about 1.4 °C, about 1.5 °C, about 1.6 °C, about 1.7 °C, about 1.8 °C, about 1.9 °C, about 2 °C, about 2.1 °C, about 2.2 °C, about 2.3 °C, about 2.4 °C, about 2.5 °C, about 2.6 °C, about 2.7 °C, about 2.8 °C, about 2.9 °C, about 3 °C, about 3.1 °C, about 3.2 °C, about 3.3 °C, about 3.4 °C, about 3.5 °C, about 3.6 °C, about 3.7 °C, about 3.8 °C, about 3.9 °C, about 4 °C, about 4.1 °C, about 4.2 °C, about 4.3 °C, about 4.4 °C, about 4.5 °C, about 4.6 °C, about 4.7 °C, about 4.8 °C, about 4.9 °C, about 5 °C, about 5.1 °C, about 5.2 °C, about 5.3 °C, about 5.4 °C, about 5.5 °C, about 5.6 °C, about 5.7 °C, about 5.8 °C, about 5.9 °C, about 6 °C, about 6.1 °C, about 6.2 °C, about 6.3 °C, about 6.4 °C, about 6.5 °C, about 6.6 °C, about 6.7 °C, about 6.8 °C, about 6.9 °C, about 7 °C, about 7.1 °C, about 7.2 °C, about 7.3 °C, about 7.4 °C, about 7.5 °C, about 7.6 °C, about 7.7 °C, about 7.8 °C, about 7.9 °C, about 8 °C, about 8.1 °C, about 8.2 °C, about 8.3 °C, about 8.4 °C, about 8.5 °C, about 8.6 °C, about 8.7 °C, about 8.8 °C, about 8.9 °C, about 9 °C, about 9.1 °C, about 9.2 °C, about 9.3 °C, about 9.4 °C, about 9.5 °C, about 9.6 °C, about 9.7 °C, about 9.8 °C, about 9.9 °C, or about 10 °C. At accelerated conditions, the pharmaceutical compositions described herein are stable for at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6

months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, at least 18 months, or at least 24 month. Accelerated conditions for the pharmaceutical compositions described herein include temperatures that are at or above ambient levels (e.g. 25±5 °C). In some instances, an accelerated condition is at about 40±2 °C. In some instances, an accelerated condition is at about 35 °C, about 40 °C, about 45 °C, about 50 °C, about 55 °C, or about 60 °C. Accelerated conditions for the pharmaceutical compositions described herein also include relative humidity (RH) that are at or above ambient levels (55±10% RH). In other instances, an accelerated condition is above about 65% RH, about 70% RH, about 75% RH, or about 80% RH. In further instances, an accelerated condition is about 40 °C or 60 °C at ambient humidity. In yet further instances, an accelerated condition is about 40±2 °C at 75±5% RH humidity.

[0084] In some embodiments, the pharmaceutical compositions are stable at about 5±5 °C to about 25±5 °C for at least 12 months. In one embodiment, the pharmaceutical compositions are stable at about 5±5 °C for at least 12 months. In one embodiment, the pharmaceutical compositions are stable at about 25±5 °C for at least 12 months. In one embodiment, the pharmaceutical compositions are stable at about 5±5 °C for at least 24 months. In one embodiment, the pharmaceutical compositions are stable at about 25±5 °C for at least 24 months.

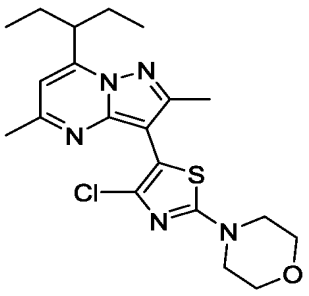
Methods of Use

[0085] Disclosed herein is a method of treating congenital adrenal hyperplasia (CAH) in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, CAH is classic CAH. In some embodiments, CAH is non-classic CAH. In some embodiments, the methods described herein result in the reduction of a hormone level. Such hormones include deoxycorticosterone, 11-deoxycortisol, cortisol, corticosterone, aldosterone, pregnenolone, 17α-hydroxy pregnenolone, progesterone, 17α-hydroxy progesterone (17-OHP), dehydroepiandrosterone, androstenediol, androstenedione, testosterone, dihydrotestosterone, estrone, estradiol, estriol, and adrenocorticotropic hormone (ACTH). In some embodiments, the methods described herein result in the reduction of 17α-hydroxy progesterone (17-OHP). In some embodiments, the methods described herein result in the reduction of adrenocorticotropic hormone (ACTH), also known as corticotropin.

[0086] Also disclosed herein is a method of treating congenital adrenal hyperplasia (CAH) in a subject in need thereof, the method comprising:

(i) measuring a hormone level in the subject in need thereof;

(ii) administering Compound 1:



or a pharmaceutically acceptable salt or solvate thereof;

(iii) repeating steps (i) and (ii) until the hormone level reaches a pre-determined range followed by a maintenance therapy of a daily dosing of compound 1.

[0087] In some embodiment, the hormone is 17α-Hydroxyprogesterone (17-OHP), adrenocorticotropic hormone (ACTH), testosterone, or androstenedione.

[0088] In some embodiment, the hormone is 17-OHP, and the pre-determined range is from about 200 ng/dL to about 400 ng/dL. In some embodiment, the hormone is 17-OHP, and the pre-determined range is less than about 400 ng/dL, less than about 350 ng/dL, less than about 300 ng/dL, less than about 250 ng/dL, or less than about 200 ng/dL.

[0089] In some embodiment, the hormone is ACTH, and the pre-determined range is below about 100 pg/mL. In some embodiment, the hormone is ACTH, and the pre-determined range is below about 100 pg/mL, below about 90 pg/mL, or below about 80 pg/mL.

[0090] In some embodiment, the hormone is testosterone and the pre-determined range is from about 14 ng/dL to about 76 ng/dL. In some embodiment, the hormone is testosterone and the pre-determined range is less than about 76 ng/dL, less than about 70 ng/dL, less than about 65 ng/dL, less than about 60 ng/dL, less than about 55 ng/dL, less than about 50 ng/dL, less than about 45 ng/dL, less than about 40 ng/dL, less than about 35 ng/dL, less than about 30 ng/dL, less than about 25 ng/dL, less than about 20 ng/dL, or less than about 15 ng/dL.

[0091] In some embodiment, the hormone is androstenedione and the pre-determined range is from about 30 ng/dL to about 200 ng/dL in males. In some embodiment, the hormone is androstenedione and the pre-determined range is less than about 200 ng/dL, less than about 150 ng/dL, less than about 100 ng/dL, less than about 50 ng/dL, or less than about 30 ng/dL in males

[0092] In some embodiment, the hormone is androstenedione and the pre-determined range is from about 40 ng/dL to about 150 ng/dL in females. In some embodiment, the hormone is androstenedione and the pre-determined range is less about 150 ng/dL, less about 100 ng/dL, less about 50 ng/dL, or less about 40 ng/dL in females.

[0093] In some embodiments, the methods described herein include administration of the pharmaceutical composition comprising Compound 1, or a pharmaceutically acceptable salt or solvate thereof once a month, twice a month, three times a month, once a week, twice a week, three times a week, once every two days, once a day, twice a day, three times a day, or four

times a day. In some embodiments, the methods described herein administer Compound 1, or a pharmaceutically acceptable salt or solvate thereof once a day. In some embodiments, the methods described herein administer Compound 1, or a pharmaceutically acceptable salt or solvate thereof twice a day.

[0094] In some embodiments, the methods described herein include administration of about 1 mg to about 2000 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof, per day. In some embodiments, about 100 mg to about 1600 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 1600 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 1200 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 1000 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 800 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 100 mg to about 800 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 800 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 100 mg to about 600 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 600 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 300 mg to about 600 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 100 mg to about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 300 mg to about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered each day.

[0095] In some embodiments, less than about 2000 mg Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 1800 mg Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 1600 mg Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 1400 mg Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 1200 mg Compound 1, or a pharmaceutically

acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 1000 mg Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 800 mg Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 600 mg Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 500 mg Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 400 mg Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 300 mg Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 200 mg Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered per day.

[0096] In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein wherein the subject is in the fed state. In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein wherein the subject is in the fasted state.

[0097] In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein at bedtime.

[0098] In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein less than about 4 hours before sleep. In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein less than about 3 hours before sleep. In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein less than about 2 hours before sleep. In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein less than about 1 hour before sleep. In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein less than about 30 mins before sleep.

[0099] In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein in the evening.

[0100] In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein at about 11 pm at night. In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein at about 10 pm at night. In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein at about 9 pm at

night. In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein at about 8 pm at night.

[0101] In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein at or before the expected circadian release of adrenocorticotropic hormone (ACTH). In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein about 3-4 hours before the expected circadian release of adrenocorticotropic hormone (ACTH).

Combination Therapy

[0102] Disclosed herein is a method of treating congenital adrenal hyperplasia (CAH) in a subject in need thereof, comprising administering a combination of Compound 1, or a pharmaceutically acceptable salt or solvate thereof; and a glucocorticoid. In some embodiments, the amount of glucocorticoid administered is reduced as compared to a method not comprising administering Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0103] In some embodiments, the methods described herein reduce the amount of a glucocorticoid administered from a supraphysiologic amount to a physiologic amount.

[0104] In some embodiments, the methods described herein reduce the symptoms associated with high-dose glucocorticoid therapy. In some embodiments, the symptoms associated with high-dose glucocorticoid therapy are obesity, insulin resistance, metabolic abnormalities, hypertension, cardiovascular diseases, or osteoporosis.

[0105] In some embodiments, the amount of glucocorticoid administered is reduced by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 80, or about 90% as compared to a method not comprising administering Compound 1, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the amount of glucocorticoid administered is reduced by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, or about 60% as compared to a method not comprising administering Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0106] In some embodiments, the amount of glucocorticoid administered is reduced by about 1% to about 90%, about 1% to about 60%, about 1% to about 30%, about 1% to about 10%, about 10% to about 50%, about 10% to about 40%, about 10% to about 30%, about 15% to about 25%, about 20% to about 30%, about 5% to about 25%, about 20% to about 50%, about 30% to about 60%, or about 40% to about 70% as compared to a method not comprising administering Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0107] In some embodiments, the glucocorticoid is administered at a dose between about 0.1 mg/day and about 25 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 1 mg/day and about 20 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 1 mg/day and about 15 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 1 mg/day and about 12 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 1 mg/day and about 11 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 1 mg/day and about 10 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 1 mg/day and about 9 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 1 mg/day and about 8 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 1 mg/day and about 7 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 1 mg/day and about 6 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 1 mg/day and about 5 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 1 mg/day and about 4 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 1 mg/day and about 3 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 1 mg/day and about 2 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 3 mg/day and about 13 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 5 mg/day and about 11 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 8 mg/day and about 11 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 9 mg/day and about 12 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 9 mg/day and about 10 mg/day. In some embodiments, the glucocorticoid is administered at a dose between about 5 mg/day and about 10 mg/day.

[0108] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered concurrently. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered in one pharmaceutical composition. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered concurrently in separate pharmaceutical compositions.

[0109] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered within 24 hours. In some embodiments, Compound 1, or a pharmaceutically

acceptable salt or solvate thereof, and the glucocorticoid are administered within 12 hours. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered within 8 hours. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered within 6 hours. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered within 4 hours. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered within 2 hours. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered within 1 hour. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered within 30 minutes. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered within 10 minutes.

[0110] In some embodiments, the glucocorticoid is beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone. In some embodiments, the glucocorticoid is hydrocortisone.

[0111] In some embodiments, the glucocorticoid is hydrocortisone and the dose administered is less than the recommended dose of 15-25 mg/day.

[0112] In some embodiments, the glucocorticoid is prednisone and the dose administered is less than the recommended dose of 5-7.5 mg/day.

[0113] In some embodiments, the glucocorticoid is prednisolone and the dose administered is less than the recommended dose of 4-6 mg/day.

[0114] In some embodiments, the glucocorticoid is dexamethasone and the dose administered is less than the recommended dose of 0.25-0.5 mg/day.

[0115] Disclosed herein is a method of treating congenital adrenal hyperplasia (CAH) in a subject in need thereof, comprising administering a combination of Compound 1, or a pharmaceutically acceptable salt or solvate thereof; a glucocorticoid; and optionally a mineralcorticoid. In some embodiments, the mineralocorticoid is fludrocortisone and the dose is less than the recommended dose of 0.05-0.2 mg/day.

EXAMPLES

[0116] The following examples further illustrate the invention but should not be construed as in any way limiting its scope. In particular, the processing conditions are merely exemplary and can be readily varied by one of ordinary skill in the art.

[0117] All methods described herein can be performed in a suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples,

or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. Unless defined otherwise, technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs.

Example 1: Pharmaceutical Composition

[0118] The pharmaceutical composition is manufactured as size 1 white hard gelatin capsules containing 200 mg of Compound 1 micronized to and average size of 10 microns or less. The pharmaceutical composition contains no additional excipients.

Example 2: Stability of the Pharmaceutical Composition

Stability Data Summary

[0119] A summary of the pharmaceutical composition stability studies is provided in Table 1. The pharmaceutical composition is a Compound 1 neat-filled into size 0 capsules with no added excipients, in 3 strength configurations: 1-mg, 5-mg, and 50-mg. The capsules were blister packaged in a polyvinyl chloride (PVC)-based film.

[0120] Under long term and accelerated conditions, no significant trend was observed in the three lots for any of the attributes evaluated throughout the course of the stability study.

Table 1. Summary of Stability

Lot Number	Strength	CCS	Stability Conditions	Available Data
#1	1-mg	PVC-based blister pack	25 °C/60% RH	6 months
			40 °C/75% RH	6 months
#2	5-mg	PVC-based blister pack	25 °C/60% RH	6 months
			40 °C/75% RH	6 months
#3	50-mg	PVC-based blister pack	25 °C/60% RH	6 months
			40 °C/75% RH	6 months
#4	200-mg	75 mL HDPE bottle	25 °C/60% RH	1 month
			40 °C/75% RH	9 months
#5	200-mg	30 mL HDPE bottle	25 °C/60% RH	6 months
			40 °C/75% RH	6 months
#6	200-mg	30 mL HDPE bottle	25 °C/60% RH	6 months
			40 °C/75% RH	6 months

CCS = container closure system; CRC = child-resistant closure; DoM = date of manufacture; HDPE = high density polyethylene; PVC = polyvinyl chloride

Stability Protocols

[0121] The stability protocol for various pharmaceutical compositions is provided in Table 2, 3, and 4.

Table 2. Stability Protocol

Test	Acceptance Criteria	Time (months)			
		T_0	1	3	6
Appearance	Size 0 blue capsule containing yellow powder, blister pack and foil backing intact	X	A,B	A,B	A,B
Assay	Report (% Label Claim)	X	A,B	A,B	A,B
Related Substances	Report RRT and % each individual species and total	X	A,B	A,B	A,B
Disintegration	Report	X	A,B	A,B	A,B

RRT = relative retention time
X-testing performed at the time study was initiated
A-samples stored under long term conditions of 25 ± 2 °C/60 ± 5 % RH
B-samples stored under accelerated conditions of 40 ± 2 °C/75 ± 5 % RH

Table 3. Stability Protocol (200-mg capsules in 30 mL HDPE bottles)

Test	Acceptance Criteria	Time (months)			
		T_0	1	3	6
Appearance	Size 1 white capsule containing an off-white to yellow powder	X	A,B	A,B	A,B
Assay (%LC)	90.0 – 110.0	X	A,B	A,B	A,B
Related Substances Any Unspecified Impurity (%) Total Impurities (%)	 ≤1.0 ≤2.0	X	A,B	A,B	A,B
Disintegration (min)	NMT 15	X	A,B	A,B	A,B
Water Activity	Report result	X	A,B	A,B	A,B

LC = label claim; NMT = not more than
X-testing performed at the time study was initiated
A-samples stored under long term conditions of 25 ± 2 °C/60 ± 5 % RH
B-samples stored under accelerated conditions of 40 ± 2 °C/75 ± 5 % RH

Table 4. Stability Protocol (200-mg capsules in 30 mL HDPE bottles) for Lots to be Used in Phase 2 Clinical Study

Test	Acceptance Criteria	Time (months)					
		T_0	1	3	6	9	12
Appearance	Size 1 white capsule containing an off-white to yellow powder	X	B	A,B	A,B	A	A
Assay (%LC)	90.0 – 110.0	X	B	A,B	A,B	A	A
Related Substances Any Unspecified Impurity (%) Total Impurities (%)	 ≤1.0 ≤2.0	X	B	A,B	A,B	A	A
Disintegration (min)	NMT 15	X	B	A,B	A,B	A	A
Water Activity	Report result	X	n/a	n/a	n/a	A	A
Microbial Enumeration Total Aerobic Microbial Count (CFU/g) Total Combined Yeast and Mold (CFU/g) Count	NMT 2000 CFU/g NMT 200 CFU/g	X	n/a	n/a	n/a	A	A
Test for Specified Microorganisms *E. coli* (/g)	Absent	X	n/a	n/a	n/a	A	A

CFU = colony forming units; LC = label claim; n/a = not applicable; NMT = not more than
X-testing performed at the time study was initiated
A-samples stored under long term conditions of 25 ± 2 °C/60 ± 5 % RH
B-samples stored under accelerated conditions of 40 ± 2 °C/75 ± 5 % RH

[0122] The supportive data demonstrate that the pharmaceutical composition is stable for a minimum of 6 months (end of study). No adverse trends were observed under long term and accelerated conditions. The assay results were consistent through the entire study and no new related substances species were observed during the stability study. The reported stability results for lots stored in a blister packaging configuration are considered supportive for the updated packaging configuration of a 30-mL HDPE bottle, induction seal, and a child resistant cap. There are no excipients in either configuration and both configurations provide protection from light.

Example 3: Phase 1 Clinical Studies

[0123] Compound 1 has been investigated in 2 Phase 1 studies in healthy adult volunteers.

[0124] Study 1 was the first-in-human study that investigated the safety, tolerability, and PK of single-escalating doses of Compound 1, given orally, to healthy adult subjects. Safety and tolerability assessments were made over a wide range of single oral doses, and dose escalation did not proceed until safety data from the preceding doses had been reviewed. The data from this study were used for the selection of doses for Study 2.

[0125] The 2-part multiple-dose study, Study 2, determined the safety and tolerability of repeated daily doses of Compound 1 and investigated the effects on biomarkers of relevance for the treatment of alcohol dependence. Part B investigated the interaction of Compound 1 with midazolam (a cytochrome P450 3A4 [CYP3A4] substrate), determining whether Compound 1 significantly inhibited the metabolism of drugs that are metabolized by CYP3A4.

[0126] In Study 1, Compound 1 was administered to healthy adult subjects as a single PO dose of 2, 10, 50, 150, 400, or 800 mg in the fed state and 150 mg in the fasted state. Absorption occurred moderately late, achieving peak C_{max} between 4 to 6 hours following dosing in the fed state.

[0127] Table 5 provides a summary of the PK parameters at each dose level. When Compound 1 was given in the fed state, median time to reach maximum plasma concentration (T_{max}) occurred between 4 and 6 hours. The median T_{max} was 10.05 hours when Compound 1 was given in the fasted state at 150 mg and ranged between 6 and 12 hours, suggesting possible delayed absorption in the fasted state. Mean half-life ($t_{1/2}$) after a single PO dose (fed and fasted state) was between 31 and 44 hours, ranging from 11 to 101 hours. Apparent volume of distribution (V_z/F) was large and appeared highly variable with greatest variability observed at the 2 highest dose levels of 400 mg and 800 mg.

Table 5. Summary of Pharmacokinetic Parameters of Compound 1 Following Single Oral Dose Administration in Healthy Volunteers (all Fed)

PK Parameters	Geometric Mean (CV%) Analyte = Plasma Compound 1					
	2 mg	**10 mg**	**50 mg**	**150 mg**	**400 mg**	**800 mg**
N	6	6	6	6	6	6
C_{max} (ng/mL)	0.867 (53)	3.01 (87)	19.5 (39)	93.4 (22)	207 (92)	382 (110)
T_{max}[a] (h)	6.00 (4.00-6.00)	4.00 (2.00 -6.00)	4.00 (3.00 - 6.00)	4.00 (3.00 - 6.00)	4.00 (3.00 - 6.00)	6.00 (4.00 - 6.00)
$t_{1/2}$[b] (h)	NC (NC)	NC (NC)	31 (10.8-53.4)	29.2 (20.0 - 41.5)	44.2 (20.2 - 101)	41.9 (24.1 - 67.8)
$AUC_{0\text{-tlast}}$ (ng•h/mL)	NC (NC)	NC (NC)	152 (60)	891 (26)	2300 (103)	4390 (100)

$AUC_{0-\infty}$ (ng•h/mL)	NC (NC)	NC (NC)	165 (64)	956 (25)	2580 (93)	4850 (95)
CL/F (L/h)	NC (NC)	NC (NC)	302 (64)	157 (25)	155 (93)	165 (95)
V_z/F (L)	NC (NC)	NC (NC)	13500 (45)	6620 (38)	9890 (212)	9970 (144)
V_{ss}/F (L)	NC (NC)	NC (NC)	8080 (36)	4600 (33)	6580 (152)	6820 (120)

AUC = area under the plasma concentration-time curve; CL/F = oral clearance; C_{max} = maximum plasma concentration; CV = coefficient of variation; NC = not calculable; $t_{1/2}$ = elimination half-life; T_{max} = time to reach maximum plasma concentration; V_{ss}/F = volume of distribution at steady state; V_z/F = volume of distribution at terminal phase

a Median (range)

b Geometric mean (range)

[0128] As part of this single-dose escalation study, a food effect PK investigation was performed to examine Compound 1 exposures in both the fed and fasted states at the 150-mg dose level. A total of 6 subjects were administered 150 mg Compound 1 in each of these 2 dosing groups. Of these 6 subjects, 4 subjects received Compound 1 at the same dose in both fed and fasted states. The administration of Compound 1 in the fasted state resulted in a much flatter mean concentration-time (i.e., with significantly lower absorption) profile when compared against the mean profile at the same dose, given within 5 minutes after a standardized breakfast meal. The mean $AUC_{0-\infty}$, and C_{max} values for a 150-mg dose in the fed state was approximately 3- and 11-fold greater than that of the fasted state, respectively. Table 6 provides a summary of the PK parameters at the 150-mg dose level under both fed and fasted state conditions.

Table 6. Summary of Pharmacokinetic Parameters of Compound 1 Following Single Oral Dose Administration in Healthy Volunteers – Fed vs. Fasted State

Pharmacokinetic Parameters	**Geometric Mean (CV %)**	
	Analyte = Plasma Compound 1	
	150 mg Fed	**150 mg Fasted**
N	6	6
C_{max} (ng/mL)	93.4 (22)	8.22 (83)
T_{max}[a] (h)	4.00 (3.00 – 6.00)	10.05 (6.00 – 12.00)
$t_{1/2}$[b] (h)	29.2 (20.0 – 41.5)	38.4 (24.2 – 88.5)
$AUC_{0-tlast}$ (ng•h/mL)	891 (26)	288 (48)
$AUC_{0-\infty}$ (ng•h/mL)	956	331

	(25)	(44)
CL/F (L/h)	157 (25)	454 (44)
V_Z/F (L)	6620 (38)	25100 (81)
V_{SS}/F (L)	4600 (33)	25200 (68)

AUC = area under the plasma concentration-time curve; CL/F = apparent total body clearance; C_{max} = maximum plasma concentration; CV = coefficient of variation; N = number of subjects; NC = not calculable; PK = pharmacokinetic; T_{max} = time to reach maximum plasma concentration; t1/2 = elimination half-life; $V_{ss}F$ = apparent volume of distribution at steady state during the terminal phase after extravascular administration; V_z/F = apparent volume of distribution during the terminal phase after extravascular administration.

a Median (range)

b Geometric mean (range)

[0129] PK parameters $AUC_{0-\infty}$ and C_{max} were analyzed separately for dose proportionality for Compound 1 from 50 to 800 mg when administered in the fed state. The analysis results suggested that for every doubling of dose, $AUC_{0-\infty}$ can be expected to increase 1.74 times more than what would be expected under dose proportionality. C_{max} appeared more than dose proportional but the formal test was inconclusive as the 90% confidence interval were partially within the 0.8 – 1.25 interval. Dose proportionality across administered doses in the fed state could not be concluded on the basis of $AUC_{0-\infty}$ or C_{max}.

[0130] PK were also evaluated in the multiple-dose, dose-escalation study, Study 2. In Part A of the study, subjects were divided into 3 cohorts and received 50, 150, or 200 mg Compound 1 or placebo for 14 consecutive days (at least 6 subjects received Compound 1 and 2 subjects received placebo in each cohort). Blood concentrations of Compound 1 were close to steady-state levels after 2 weeks of dosing and the accumulation ratio was between 2.51 to 3.65. Part B investigated the interaction of Compound 1 with midazolam (a CYP3A4 substrate), thereby determining whether this compound significantly inhibited the metabolism of drugs that are metabolized by CYP3A4 serial blood samples were collected to determine plasma concentrations of study drug after a single dose of Compound 1 had been administered and at steady state. All dosing occurred in the fed state. An assessment of diurnal cortisol levels, plus when under conditions of glucose clamp, were also carried out both prior to and during the dosing period.

[0131] Overall concentration time profiles of Compound 1 showed that absorption was moderately delayed with C_{max} achieved at a median of 5 hours following oral dosing. Consistent with the single-dose study (Study 1), concentrations appeared to decline in a bi-exponential

manner, characterized by a rapid decrease within the first 24 hours. Following multiple daily dosing for 2 weeks, the $t_{1/2}$ of Compound 1 exceeded 100 hours; therefore, an accumulation ratio of Compound 1 was between 2.51 to 3.65 (see Table 7). The T_{max} appeared to be consistent across doses. Overall half-lives, weight normalized CL/F and V/F were consistent for 150 and 200 mg. However, the values for these latter 2 parameters were almost doubled at the 50-mg dose level. Variability (CV%) for apparent clearance and volume of distribution were large and not reduced with weight-normalization.

Table 7. Summary of Noncompartmental Pharmacokinetic Parameters of Compound 1 After Single (Day 1) and Multiple (Day 14) Oral Doses of 50 mg, 150 mg, and 200 mg of Compound 1 in Part A of the Study

	Geometric Mean (CV%)					
	50 mg Day 1	**150 mg Day 1**	**200 mg Day 1**	**50 mg Day 14**	**150 mg Day 14**	**200 mg Day 14**
N	**8**	**9**	**7**	**8**	**9**	**6**
C_{max} (ng/mL)	22.7 (59)	127 (52)	143 (62)	50.3 (56)	222 (58)	314 (93)
T_{max} [a] (hr)	4.52 (3.00 – 10.00)	5.00 (5.00 - 6.00)	5.00 (2.00 - 5.00)	5.00 (3.00 - 5.00)	5.00 (3.00 - 5.03)	5.00 (3.00 - 5.00)
Effective $t_{1/2}$ [b] (hr)	NC NC	NC NC	NC NC	37.7 (27.4)	32.7 (29.7)	52.0
$AUC_{0-\infty}$ (ng•hr/mL)	NC NC	NC NC	NC NC	980 (53.8)	4680 (104)	5660 (122)
AUC_{0-24h} (ng•hr/mL)	97.6 (58)	590 (56)	559 (55[c])	273 (56)	1480 (66)	2040 (98)
C_{avg} (ng/mL)	NC NC	NC NC	NC NC	11.4 (56)	61.7 (66)	85.0 (98)
CL_{aa}/F (L/hr)	NC NC	NC NC	NC NC	183 (56)	101 (66)	98 (98)
WT-norm						
Cl_{aa}/F (L/hr/kg)	NC NC	NC NC	NC NC	2.88 (46.9)	1.42 (72.9)	1.50 (89.9)
Vz/F (L)	NC NC	NC NC	NC NC	33500 (90)	17600 (59)	16300 (76)
Vss/F (L)	NC NC	NC NC	NC NC	12900 (91)	6170 (40)	5080 (78)
WT-norm						
Vss/F (L/kg)	NC NC NC	NC NC NC	NC NC NC	204 (71.3) 2.80	86.5 (41.0) 2.51	77.4 (68.6) 3.65
R_A	NC	NC	NC	(27.4)	(29.7)	(34.4)

AUC = area under the plasma concentration-time curve; CL/F = apparent clearance; C_{max} = maximum plasma concentration; RA = accumulation ratio calculated as Day 14 AUC_{0-24}/Day 1 AUC_{0-24}; t1/2 = terminal half-life; effective t1/2 = half-life calculated by accumulation ratio; Tmax = time to maximum plasma concentration; Vss/F = volume of distribution at steady state; Vz/F = volume of distribution at terminal phase; WT-norm = weight normalized

[a] Median (range).

[b] Geometric mean (range).

[c] n = 6, Dropout Subject 306 not included in the calculation of summary statistics.

[0132] Table 8 presents the results of the dose proportionality assessment for the AUC_{0-24} and C_{max} over the tested dose range. For AUC_{0-24} and C_{max}, the adjusted mean slope at Day 1 and Day 14 were all above the value of 1, suggesting a slightly more than proportional increase of AUC_{0-24} and C_{max} values with increasing doses.

Table 8. Summary of Assessment of Dose Proportionality as Assessed by the Power Model for Plasma Compound 1

Parameter	Day	Mean Slope	Standard Error	90% CI for Slope	CV%
AUC_{0-24} (ng hr/mL)	1	1.40	0.194	(1.069, 1.737)	59.3
	14	1.48	0.221	(1.104, 1.864)	69.1
C_{max} (ng/mL)	1	1.41	0.185	(1.092, 1.728)	57.5
	14	1.33	0.210	(0.972, 1.693)	64.8

AUC = area under the plasma concentration-time curve; C_{max} = maximum plasma concentration

Safety

[0133] The safety of Compound 1 was evaluated in 2 Phase 1 studies in healthy adult volunteers (Study 1 and Study 2). In both studies, adverse events (AEs), clinical laboratory tests, vital signs (supine blood pressure and pulse rate), and electrocardiograms (ECGs) were evaluated. Overall, in Study 1, Compound 1 was well-tolerated. Compound 1, when administered as multiple doses up to 200 mg, was generally well tolerated in the healthy subject population studied.

[0134] In Study 1, the effects upon biomarkers of relevance for the treatment of alcohol dependence were investigated. Five clusters of an Addiction Research Center Inventory Questionnaire (ARCI-49) were used to compare the effect of Compound 1 vs. placebo: Morphine-Benzedrine Group Scale measuring euphoria; Lysergic-Acid-Diethylamide Group Scale estimating dysphoric and somatic changes; Pentobarbital-Chlorpromazine-Alcohol Group Scale measuring sedation; Benzedrine Group (BG) Scale measuring intellectual efficiency and energy; Amphetamine Group Scale measuring effects of d-amphetamine, respectively. No

systematic pattern or dose-response for the change from baseline or for the difference over placebo in each cluster was observed.

[0135] In summary, single, oral doses up to 800 mg and multiple doses up to 200 mg of Compound 1 were well-tolerated by healthy male and female subjects.

Example 4: Phase 2 Multiple-Dose, Dose-Escalation Clinical Studies

[0136] Cohort A of the Phase 2 Study includes a 6-week, multiple-dose, dose escalation study of Compound 1 for the treatment of adults with classic CAH. After screening, eligible patients will be enrolled into a 6-week treatment period followed by a 4-week washout/safety follow-up period.

[0137] This cohort will be conducted in approximately 9 patients, who will receive Compound 1 daily for up to 6 weeks. Compound 1 will be administered as an oral daily dose. Patients will undergo titration of Compound 1 through three escalating dosage strengths at 2-week intervals. Patients will have overnight PK/PD assessments performed at baseline, which include an pre-dose overnight assessment and a post-dose overnight assessment for PK/PD following administration of the first dose. At the end of each 2-week dosing period, patients will return for single overnight visits for steady-state PK/PD assessments. A follow-up outpatient visit will occur 30 days after their last dose. Upon completion of the initial cohort (Cohort A), the study will proceed to a multiple ascending dose (MAD) design with up to 3 sequential cohorts (Cohorts B, C, and D) to further evaluate the safety, PK, and PD of various SPR001 dosing regimens and to identify an optimal dose regimen. Each cohort will undergo a 2-week run-in period, a 2-week treatment period, and a 30-day washout and safety follow-up period. During the run-in period, which will occur during screening, subjects will document in a paper diary each dose of glucocorticoid medication taken, the time of each meal, and the time they went to bed and woke up each day, to ensure compliance with background glucocorticoid regimens and the stability of their daily routine.

[0138] Patients in Cohort B will receive study drug at 200 mg BID, with a dose in the morning and a dose in the evening, either with a meal or consumption of a standardized snack. For Cohorts C and D, the dose level and the frequency and timing of dosing will be determined based on interim data from the previous cohorts. However, the dose level of each successive cohort will be capped at twice the daily dose level of the previous cohort.

Study Design

- Study Type: Interventional
- Primary Purpose: Treatment
- Study Phase: Phase 2
- Interventional Study Model: Sequential Assignment

- Number of Cohorts: Up to 4
- Masking: No masking
- Allocation: Non-Randomized
- Enrollment: Up to approximately 27 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Cohort A The first cohort of 9 patients will be administered Compound 1 at dose strength of 200 mg daily for 2 weeks, and escalating through 600 mg per day for 2 weeks and 1,000 mg per day for 2 weeks.	Drug: Compound 1 200-mg capsules
Experimental: Cohort B Cohort B patients will be administered Compound 1 at a dose strength of 200 mg twice daily (BID) for 2 weeks.	Drug: Compound 1 200-mg capsules
Experimental: Cohort C The dose/dose regimen for Cohort C will be determined based on an interim review of safety and PK/PD data from the previous Cohorts	Drug: Compound 1 50-mg or 200 mg capsules
Experimental: Cohort D The dose/dose regimen for Cohort C will be determined based on an interim review of safety and PK/PD data from the previous Cohorts	Drug: Compound 1 50-mg or 200 mg capsules

Outcome Measures

Primary Outcome Measure:

1. To evaluate the safety of Compound 1 in subjects with CAH.

2. To assess the efficacy of Compound 1 in subjects with classic CAH as measured by percent and absolute change in 17-OHP compared to baseline.

Secondary Outcome Measure:

3. To explore the dose(s) of Compound 1 that cause pharmacodynamics changes in plasma concentrations of ACTH, androstenedione, and testosterone, as measured by the absolute and percent change from baseline by dose.

4. To determine pharmacokinetics of Compound 1 in subjects with CAH.

5. To explore potential relationships between pharmacodynamics and pharmacokinetics.

Exploratory

7. To explore the dose(s) of Compound 1 that cause changes in pharmacodynamics biomarkers in urine, as measured by the absolute and percent change from baseline by dose.

Eligibility

- Minimum Age: 18 Years
- Maximum Age:
- Sex: All

- Gender Based: No
- Accepts Healthy Volunteers: No
- Criteria: Inclusion

Criteria:

Inclusion Criteria:

- Male and female patients age 18 or older.
- Documented diagnosis of classic CAH due to 21-hydroxylase deficiency
- Elevated 17-OHP at screening
- On a stable glucocorticoid replacement regimen for a minimum of 30 days

Exclusion Criteria:

- Clinically significant unstable medical condition, illness, or chronic disease
- Clinically significant psychiatric disorder.
- Clinically significant abnormal laboratory finding or assessment
- History of bilateral adrenalectomy or hypopituitarism
- Pregnant or nursing females
- Use of any other investigational drug within 30 days
- Unable to understand and comply with the study procedures, understand the risks, and/or unwilling to provide written informed consent.

Results:

The phase 2 study showed that Compound 1 was generally well-tolerated. The study established a range of safe doses after exploring a wide range of doses (5-fold range) (see FIG. 1).

[0139] With respect to patient-level response to Compound 1, 80% showed reduced ACTH (see FIG. 2). Generally, attenuation of ACTH demonstrates target engagement and functional CRF_1 receptor antagonism. 80% of patient subjects demonstrated reduction in ACTH. 70% of subject demonstrated more than 25% reduction in ACTH. 40% of subjects were in the normal range after treatment.

[0140] A reduction of 17-OHP demonstrates "control" of the disease based on Standard guidelines. This allows for steroid taper. 80% of subjects demonstrated reduction in 17-OHP (see FIG. 3). 50% of subjects demonstrated more than 25% reduction in 17-OHP. 50% of subjects were within the guideline range (1200 ng/dL) after treatment.

Compound 1 attenuates morning rise in A4 which indicates an ability to control excess androgen production and associated symptoms (see FIG. 4). 100% of subjects demonstrated reduction in Androstenedione (at various doses). 60% of subjects demonstrated more than 25% reduction in Androstenedione. 50% of subjects were within normal reference range after treatment.

Example 5: Formulation Development

[0141] The objectives of this experiment was to (1) evaluate different formulations to obtain a Compound 1 200 mg immediate release core tablet; (2) evaluate dissolution profiles of

Compound 1 tablets in wet granulation process and in granulation with Gelucire 48/16 and/or Vitamin E TPGS; and (3) evaluate dissolution of the tablets/capsules in various bio-relevant media and sink conditions to compare dissolution of API in capsule.

Experimental Outcomes

[0142] The formulation of Compound 1 tablets were performed in two phases.

[0143] In the first phase of manufacturing, two immediate release trial formulations were manufactured. The first trial involved granulation of Compound 1 using Gelucire (10%) and included fillers and disintegrants. No surfactant was used in his formulation. The granules were final belnded and compressed at a tablet weight of 500 mg. No issues were observed in granulation and compression. The second trial involved wet granulation with HPC as the binder with fillers and disintegrants. Sodium lauryl sulphate was used as a surfactant at a concentration of 1%. The granules were softer when compared to the first trial and the final blend had poor flow. The filler in the extra granular portion was increased to improve flow and weight variation during compression. The tablets were compressed at 600 mg tablet weight. The first and second trial batches were tested for dissolution in different bio-relevant media – SGF (simulated gastric fluid), SIF (simulated intestinal fluid), FaSSIF (fasted state simulated intestinal fluid) and FeSSIF (fed state simulated intestinal fluid).

[0144] In the second phase of manufacturing, two additional formulations with Gelucire and Vitamin E TPGS at a percentage of 8% and with high concentration of sodium lauryl sulphate were manufactured. The percentage of the surfactant was increased to 7%. The tablets were compressed at 570 mg tablet weight. No issues were observed in the manufacturing process. Further development included manufacturing with high concentration of Gelucire (25%). Due to the high concentration of the Gelucire in the formulation, the granules could not be compressed and were manually filed into size 0 capsules.

[0145] Further development of wet granulation process was recommended as the flow was poor and particle size of granules was not optimum. The granules from the wet granulation formulation also showed weight variation during compression.

[0146] The release in SIF and FaSSIF was very low, ranging from between 0.5% to 3% at 60 minutes for the Gelucire and HPC formulations. The release in SGF and FeSSIF was higher and ranged from 11-16% in 60 minutes. The higher release in SGF and FeSSIF may have been due to the presence of surfactants in the media. However, the dissolution in the bio-relevant media did not show improvement with the lipid excipients or high concentration of surfactants. The release in SIF and FeSSIF was below 2%, in SGF approximately 15% and 10% in FeSSIR at 60 minutes. Dissolution showed no improvement with high concentration of Gelucire in the capsule formulation. A comparison of the dissolution of API in capsule and the different

formulations did not show any improvement in dissolution. An animal pK study was also prformed with the wet granulation formulation and the Gelucire formulation. The dissolution and pK studies indicate that the current formulation approach may not be suitable to improve bioavailability of Compound 1 or minimize the food effect. A different formulation approach was recommended for Compound 1 in Phase 2/3 studies.

Example 6: Preliminary Tablet formulation

[0147] The objective of the general experiments were to develop, manufacture and commercialize a small tablet or capsule for a drug substance that is insoluble (solubility less than 0.002 mg/ml in water and at all physiological pHs ranging from 1.2 to 7.5) and a dose strength up to 200 mg. Tablet should be immediately released in a dissolution medium using USP apparatus I or II.

Success criteria

1. A tablet size less than 400 mg for a dose strength of 200 mg
2. More than 70% dissolved in 60 minutes in conventional dissolution media.

General Manufacturing Procedure

[0148] The relevant excipients were manually sieved and then dry mixed. A binder solution was added and the mixture subjected to wet granulation. The granules were then wet milled and dried. The resulting granules were dry milled and added to other relevant common granules and excipients. The mixture was then compressed into tablets to a target tablet weight.

[0149] A granulation was developed with a minimum amount and number of excipients. The granulation consisted of at least 90% Compound 1 with a surfactant and a binder and in some cases, a super-disintegrant. After granulation was successful, other excipients were added to enable compression. These excipients included a compression aid, a lubricant, an anti-tacking agent and a super-disintegrant.

[0150] Two blends were granulated. One blend had 95% Compound 1, 1% sodium lauryl sulfate, and 4% HPC-EXF. The second blend had 91% Compound 1, 1% sodium lauryl sulfate (surfactant and wetting agent), 6% PVP (water soluble binder) and 2% Ac-di-sol (water-swellable super-disintegrant).

[0151] The granulation consisting of HPC-EXF (binder) was further blended with SiO_2 (flow aid and anti-tacking agent), ac-di-sol (water swellable disintegrant) and magnesium stearate (lubricant). Different levels of MCC were added to the tablets (0, 10 and 20% levels). All three formulations (90%, 81% and 71% DL) dissolved quickly, and there were no significant changes during accelerated stability in open dish conditions. The granulation with PVP as a binder was repeated three times. The first granulation consisted of 93% Compound 1, 6% PVP and 1%

SLS. This formulation demonstrated inferior manufacturing features (granular flow) however the tablets had acceptable dissolution. No further stability was conducted.

[0152] The second granulation (identical to first) had acceptable manufacturing features (good flow and compression) however dissolution was somewhat slower and there were significant changes during on stability in very stressful conditions.

[0153] The third granulation consisted of 91% Compound 1, 6% PVP, 1% SLS and 2% Ac-di-sol as intra-granular super-disintegrant. The granulation was further blended with SiO_2 (flow aid and anti-tacking agent), ac-di-sol (water swellable disintegrant) and magnesium stearate (lubricant). Different levels of MCC were added to the tablets (0, 10 and 20% levels). All three formulations (86%, 77% AND 68% DL) all dissolved quickly.

[0154] This demonstrated a successful tablet of Compound 1 with water solubility less than 0.002 mg/ml, while maintaining a drug load above 50% and resulting in acceptable dissolution in a conventional dissolution media (1% SLS and USP apparatus II). The tablet was manufactured using a conventional high shear wet granulation method.

Table 10. Summary of Compound 1 solubility in different aqueous media (n=2, 24 hours at room temperature)

Aqueous media	Equilibrium pH	Solubility mg/mL	Standard Deviation (n=2)	Description
Water	7.78	0.0003	0.0000	insoluble
0.1N HCl	1.06	0.0018	0.0000	insoluble
0.01N HCl	2.06	0.0004	0.0000	Insoluble
0.001N HCl	3.36	0.0003	0.0000	Insoluble
pH 4.5 Acetate (USP)	4.65	0.0005	0.0000	Insoluble
pH 6.0 Phosphate (USP)	6.08	0.0002	0.0000	Insoluble
pH 7.5 Phosphate (USP)	7.57	0.0006	0.0000	Insoluble
0.1N NaOH	11.65	0.0002	0.0000	Insoluble
SGF	2.25	0.0204	0.0001	Insoluble
FaSSIF	6.73	0.0036	0.0000	Insoluble
FaSSIF	5.16	0.0104	0.0000	insoluble

Example 7: Tablet Formulation A

[0155] Three formulations were prepared using wet granulations with common granules and PVP K30 as a binder (see Table 11). The granules were diluent with MCC PH102 with 95%, 85% and 75% drug loads respectively.

Table 11. Manufacturing Formulae A

Material	A-1		A-2		A-3	
	%w/w	mg/tablet	%w/w	mg/tablet	%w/w	mg/tablet
Common Granular						
Compound 1	93	200.00	93	200.00	93	200.00
Povidone Compendial [plasdoneK-29/32]	6	12.90	6	12.90	6	12.90
Kolliphor® SLS fine	1	2.15	1	2.15	1	2.15
Extra Granular						
Common granules	95	215.05	85	215.05	75	215.05
Microcrystalline Cellulose PH102	-	0.00	10	25.30	20	57.35
Croscarmellose Sodium, Compendial	3	6.79	3	7.59	3	8.60
Silica Dioxide (Aerosil200Pharma)	1	2.26	1	2.53	1	2.87
LIGAMEDMF-2-V Magnesium Stearate	1	2.26	1	2.53	1	2.87
Target Tablet Weight (mg)		226.37		253.00		286.73

[0156] Compound 1, Povidone and sodium lauryl sulfate were mixed to produce a dry mixture. Water was added to the dry mixture and subjected to a wet granulation at an impeller speed of about 550 – 560 rpm. The wet granules were then sifted and dried. The dry granules were dry-sieved after which the granules were compressed into tablets.

[0157] In process control tests, each tablet was satisfactory. Dissolution results of A-1 and A-3 showed similar curves whereas A-2 was slightly slower than the other two in the first few points (see table 12 and FIG. 5).

Table 12. Dissolution for Manufacturing Formulae A

Dissolution in 0.1N HCl + 1.0% SDS, media at 50 rpm and 900 ml using USP-II (Paddle)			
Time points (Minutes)	A-1	A-2	A-3
5 minutes	48.4	35.0	46.7
10 minutes	58.3	48.2	56.4
15 minutes	65.2	55.9	62.2
20 minutes	70.4	61.3	65.9
30 minutes	78.1	68.3	71.9
45 minutes	85.6	77.4	79.3
60 minutes	91.5	84.1	85.1

90 minutes	98.5	92.6	91.9
120 minutes	100.5	97.2	95.6

Example 8: Tablet Formulation B

[0158] Three formulations were prepared using wet granulation with common granules and PVP K30 as a binder (see Table 13). The granules were diluent with MCC PH102 with 95%, 85% and 75% drug loads respectively.

Table 13. Manufacturing Formulae B

Material	B-1		B-2		B-3	
	%w/w	mg/tablet	%w/w	mg/tablet	%w/w	mg/tablet
Common Granular						
Compound 1	93	200.00	93	200.00	93	200.00
Povidone Compendial [plasdoneK-29/32]	6	12.90	6	12.90	6	12.90
Kolliphor® SLS fine	1	2.15	1	2.15	1	2.15
Extra Granular						
Common granules	95	215.05	85	215.05	75	215.05
Microcrystalline Cellulose PH102	-	0.00	10	25.30	20	57.35
Croscarmellose Sodium, Compendial	3	6.79	3	7.59	3	8.60
Silica Dioxide (Aerosil200Pharma)	1	2.26	1	2.53	1	2.87
LIGAMEDMF-2-V Magnesium Stearate	1	2.26	1	2.53	1	2.87
Target Tablet Weight (mg)		226.37		253.00		286.73

[0159] Compound 1, Povidone and sodium lauryl sulfate were mixed to produce a dry mixture. Water was added to the dry mixture and subjected to a wet granulation at an impeller speed of about 600 – 610 rpm. The wet granules were then sifted and dried. The dry granules were dry-sieved after which the granules were compressed into tablets.

[0160] Dissolution results showed slower dissolution than previous tablets which may be caused by more granules and more hardness (see table 14 and FIG. 6).

Table 14. Dissolution for Manufacturing Formulae B

Dissolution in 0.1N HCl + 1.0% SDS, media at 50 rpm and 900 ml using USP-II (Paddle)			
Time points (Minutes)	B-1	B-2	B-3
5 minutes	9.4	13.7	20.4
10 minutes	21.2	33.3	47.0
15 minutes	28.6	47.8	56.4
20 minutes	34.7	54.3	61.9
30 minutes	41.8	62.0	68.2
45 minutes	49.5	70.1	74.4
60 minutes	56.3	75.8	78.8

90 minutes	66.9	84.0	84.3
120 minutes	74.8	89.5	87.4

Example 9: Tablet Formulation C

[0161] Three formulations were prepared using wet granulation with common granules, PVP K30 as a binder, and 2% Croscarmellose Sodium Intragranular (see Table 15).

Table 15. Manufacturing Formulae C

Material Name	C-1		C-2		C-3	
	%w/w	Mg/tablet	%w/w	Mg/tablet	%w/w	Mg/tablet
Common Granular						
C07102401-F	91	200	91	200	91	200
Povidone Compendial [plasdoneK-29/32]	6	13.2	6	13.2	6	13.2
Kolliphor® SLS Fine	1	2.2	1	2.2	1	2.2
Croscarmellose Sodium, Compendial	2	4.4	2	4.4	2	4.4
Extra Granular						
Common granules	95	219.78	85	219.78	75	219.78
Microcrystalline Cellulose PH102	-	-	10	25.86	20	58.61
Croscarmellose Sodium, Compendial	3	6.94	3	7.76	3	8.79
Silica Dioxide(Aerosil200Pharma)	1	2.31	1	2.59	1	2.93
LIGAMEDMF-2-V Magnesium Stearate	1	2.31	1	2.59	1	2.93
Target Tablet Weight (mg)		231.36		258.60		293.06

[0162] Compound 1, Povidone, croscarmellose sodium and sodium lauryl sulfate were mixed to produce a dry mixture. Water was added to the dry mixture and subjected to a wet granulation at an impeller speed of about 600 – 610 rpm. The wet granules were then sifted and dried. The dry granules were dry-sieved after which the granules were compressed into tablets.

[0163] Dissolution results showed faster dissolution than other tablets which may be caused by the additional quantity of croscarmellose sodium (see Table 16 and FIG. 7).

Table 16. Dissolution for Manufacturing Formulae C

Dissolution in 0.1N HCl + 1.0% SDS, media at 50 rpm and 900 ml using USP-II (Paddle)			
Time points (Minutes)	1806DFP2555-02A	1806DFP2555-02B	1806DFP2555-02C
5 minutes	26.3	25.1	26.2
10 minutes	66.3	62.7	65.8
15 minutes	79.9	81.7	77.8
20 minutes	85.4	89.3	83.6
30 minutes	90.6	94.9	88.5
45 minutes	94.2	98.3	92.4
60 minutes	95.4	99.6	94.1

90 minutes	95.3	100.2	95.0
120 minutes	95.5	100.4	95.7
250RPM/5min	95.6	100.5	98.0

Example 10: Tablet Formulation D

[0164] Two formulations were prepared using wet granulation (see Table 17).

Table 17. Manufacturing Formulae D

Material Name	**D-1**			**D-2**		
	%w/w	**mg/tablet**	**Batch formula**	**%w/w**	**Mg/tablet**	**Batch formula**
C07102401-F	71.25	200.000	570.00	77.35	200.000	546.00
Klucel(TM)EXF PHARM Hydroxypropyl cellulose	3.00	8.421	24.00	-	-	-
Povidone Compendial [plasdoneK-29/32]	-	-	-	5.10	13.187	36.00
Kolliphor® SLS Fine	0.75	2.105	6.00	0.85	2.198	6.00
Croscarmellose Sodium, Compendial	-	-	-	1.70	4.396	12.00
Extra granular material						
Microcrystalline Cellulose PH102	20.00	56.140	160.00	10.00	25.856	70.59
Croscarmellose Sodium, Compendial	3.00	8.421	24.00	3.00	7.757	21.18
Silica Dioxide (Aerosil200 Pharma)	1.00	2.807	8.00	1.00	2.586	7.06
LIGAMEDMF-2-V Magnesium Stearate	1.00	2.807	8.00	1.00	2.586	7.06
Target Tablet Weight (mg)		280.701	800.00		258.566	705.89

[0165] Compound 1, Povidone, croscarmellose sodium and sodium lauryl sulfate were mixed to produce a dry mixture. Water was added to the dry mixture and subject to a wet granulation at an impeller speed of about 600 – 610 rpm. The wet granules were then sifted and dried. The dry granules were dry-sieved after which the granules were compressed into tablets.

[0166] The release profile of Formulation D-1 and D2 were similar to previous formulations with no significant changes (see Table 18).

Table 18. Dissolution for Manufacturing Formulae C

Dissolution in 0.1N HCl + 1.0% SDS, media at 50 rpm and 900 ml using USP-II (Paddle)		
Time points (Minutes)	D-1	D-2
5 minutes	18.7	30.6
10 minutes	43.8	72.1
15 minutes	56.9	85.1
20 minutes	65.2	91.5
30 minutes	73.9	96.4
45 minutes	81.9	99.9
60 minutes	87.1	101.3

90 minutes	92.4	102.3
120 minutes	95.6	-
250RPM/5min	99.4	100.9

[0167] Both formulations had a faster relief profile in SGF media (see Table 19).

Table 19. Dissolution for Manufacturing Formulae C in SGF media

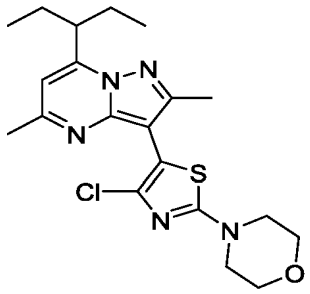
Dissolution in SGF, media at 60 rpm and 900 ml using USP-II (Paddle)			
Time points (Minutes)	D-1	D-2	Capsule with Compound 1
5 minutes	10.8	7.0	0.3
10 minutes	18.4	11.0	1.9
15 minutes	21.3	11.8	4.2
20 minutes	23.4	12.2	6.5
30 minutes	24.4	12.4	9.4
45 minutes	26.1	12.8	13.6
60 minutes	25.7	13.0	15.1
90 minutes	27.9	13.1	15.5
120 minutes	28.2	13.2	15.2
250RPM/5min	28.0	13.2	15.8

[0168] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A pharmaceutical composition in the form of a capsule comprising Compound 1:

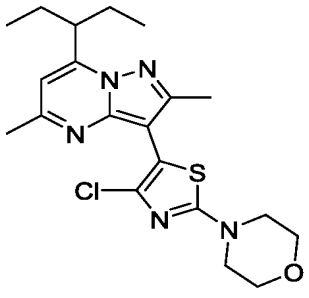


or a pharmaceutically acceptable salt or solvate thereof.

2. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition comprises between about 1 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
3. The pharmaceutical composition of claim 1 or 2, wherein the pharmaceutical composition comprises between about 5 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
4. The pharmaceutical composition of any one of claims 1 to 3, wherein the pharmaceutical composition comprises between about 10 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
5. The pharmaceutical composition of any one of claims 1 to 4, wherein the pharmaceutical composition comprises between about 10 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
6. The pharmaceutical composition of any one of claims 1 to 5, wherein the pharmaceutical composition comprises between about 10 mg and about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
7. The pharmaceutical composition of any one of claims 1 to 4, wherein the pharmaceutical composition comprises between about 50 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
8. The pharmaceutical composition of any one of claims 1 to 4, wherein the pharmaceutical composition comprises between about 100 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
9. The pharmaceutical composition of any one of claims 1 to 4, wherein the pharmaceutical composition comprises between about 100 mg and about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

10. The pharmaceutical composition of any one of claims 1 to 5, wherein the pharmaceutical composition comprises between about 100 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
11. The pharmaceutical composition of any one of claims 1 to 5, wherein the pharmaceutical composition comprises between about 150 mg and about 250 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
12. The pharmaceutical composition of any one of claims 1 to 4, wherein the pharmaceutical composition comprises about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
13. The pharmaceutical composition of any one of claims 1 to 5, wherein the pharmaceutical composition comprises about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
14. The pharmaceutical composition of any one of claims 1 to 5, wherein the pharmaceutical composition comprises about 250 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
15. The pharmaceutical composition of any one of claims 1 to 5, wherein the pharmaceutical composition comprises about 200 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
16. The pharmaceutical composition of any one of claims 1 to 5, wherein the pharmaceutical composition comprises about 150 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
17. The pharmaceutical composition of any one of claims 1 to 6, wherein the pharmaceutical composition comprises about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
18. The pharmaceutical composition of any one of claims 1 to 6, wherein the pharmaceutical composition comprises about 80 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
19. The pharmaceutical composition of any one of claims 1 to 6, wherein the pharmaceutical composition comprises about 60 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
20. The pharmaceutical composition of any one of claims 1 to 6, wherein the pharmaceutical composition comprises about 50 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

21. The pharmaceutical composition of any one of claims 1 to 6, wherein the pharmaceutical composition comprises about 30 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
22. The pharmaceutical composition of any one of claims 1-21, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is in the form of microparticles.
23. The pharmaceutical composition of claim 22, wherein the average size of the microparticles is between about 1 μm and about 20 μm.
24. The pharmaceutical composition of claim 22 or 23, wherein the average size of the microparticles is between about 5 μm and about 15 μm.
25. The pharmaceutical composition of any one of claims 22 to 24, wherein the average size of the microparticles is less than about 10 μm.
26. The pharmaceutical composition of any one of claims 1 to 25, wherein the capsule is a hard gelatin capsule.
27. The pharmaceutical composition of any one of claims 1 to 25, wherein the capsule is a soft gelatin capsule.
28. The pharmaceutical composition of any one of claims 1 to 27, wherein the capsule is formed using materials selected from the group consisting of natural gelatin, synthetic gelatin, pectin, casein, collagen, protein, modified starch, polyvinylpyrrolidone, acrylic polymers, cellulose derivatives, and any combinations thereof.
29. The pharmaceutical composition of any one of claims 1 to 28, wherein the pharmaceutical composition is free of additional excipients.
30. The pharmaceutical composition of any one of claims 1 to 28, wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.
31. A pharmaceutical composition in the form of a tablet comprising Compound 1:



or a pharmaceutically acceptable salt or solvate thereof.
32. The pharmaceutical composition of claim 31, wherein the pharmaceutical composition comprises between about 1 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

33. The pharmaceutical composition of claim 31 or 32, wherein the pharmaceutical composition comprises between about 5 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
34. The pharmaceutical composition of any one of claims 31 to 33, wherein the pharmaceutical composition comprises between about 10 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
35. The pharmaceutical composition of any one of claims 31 to 34, wherein the pharmaceutical composition comprises between about 10 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
36. The pharmaceutical composition of any one of claims 31 to 35, wherein the pharmaceutical composition comprises between about 10 mg and about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
37. The pharmaceutical composition of any one of claims 31 to 34, wherein the pharmaceutical composition comprises between about 50 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
38. The pharmaceutical composition of any one of claims 31 to 34, wherein the pharmaceutical composition comprises between about 100 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
39. The pharmaceutical composition of any one of claims 31 to 34, wherein the pharmaceutical composition comprises between about 100 mg and about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
40. The pharmaceutical composition of any one of claims 31 to 35, wherein the pharmaceutical composition comprises between about 100 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
41. The pharmaceutical composition of any one of claims 31 to 35, wherein the pharmaceutical composition comprises between about 150 mg and about 250 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
42. The pharmaceutical composition of any one of claims 31 to 34, wherein the pharmaceutical composition comprises about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
43. The pharmaceutical composition of any one of claims 31 to 35, wherein the pharmaceutical composition comprises about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

44. The pharmaceutical composition of any one of claims 31 to 35, wherein the pharmaceutical composition comprises about 250 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

45. The pharmaceutical composition of any one of claims 31 to 35, wherein the pharmaceutical composition comprises about 200 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

46. The pharmaceutical composition of any one of claims 31 to 35, wherein the pharmaceutical composition comprises about 150 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

47. The pharmaceutical composition of any one of claims 31 to 36, wherein the pharmaceutical composition comprises about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

48. The pharmaceutical composition of any one of claims 31 to 36, wherein the pharmaceutical composition comprises about 80 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

49. The pharmaceutical composition of any one of claims 31 to 36, wherein the pharmaceutical composition comprises about 60 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

50. The pharmaceutical composition of any one of claims 31 to 36, wherein the pharmaceutical composition comprises about 50 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

51. The pharmaceutical composition of any one of claims 31 to 36, wherein the pharmaceutical composition comprises about 30 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof. The pharmaceutical composition of any one of claims 31 to 50, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is in the form of microparticles.

52. The pharmaceutical composition of claim 51, wherein the average size of the microparticles is between about 1 μm and about 20 μm.

53. The pharmaceutical composition of claim 51 or 52, wherein the average size of the microparticles is between about 5 μm and about 15 μm.

54. The pharmaceutical composition of any one of claims 51 to 53, wherein the average size of the microparticles is less than about 10 μm.

55. The pharmaceutical composition of any one of claims 31 to 54, wherein the tablet is made by compression, molding, or extrusion.

56. The pharmaceutical composition of claim 55, wherein the tablet is made by hot-melt extrusion.

57. The pharmaceutical composition of any one of claims 31 to 56, wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

58. The pharmaceutical composition of any one of claims 1 to 57, wherein the pharmaceutical composition is stable for at least 1 month at 25 °C.

59. The pharmaceutical composition of any one of claims 1 to 58, wherein the pharmaceutical composition is stable for at least 3 months at 25 °C.

60. The pharmaceutical composition of any one of claims 1 to 59, wherein the pharmaceutical composition is stable for at least 6 months at 25 °C.

61. The pharmaceutical composition of any one of claims 1 to 60, wherein the pharmaceutical composition is stable for at least 9 months at 25 °C.

62. The pharmaceutical composition of any one of claims 1 to 61, wherein the pharmaceutical composition is stable for at least 12 months at 25 °C.

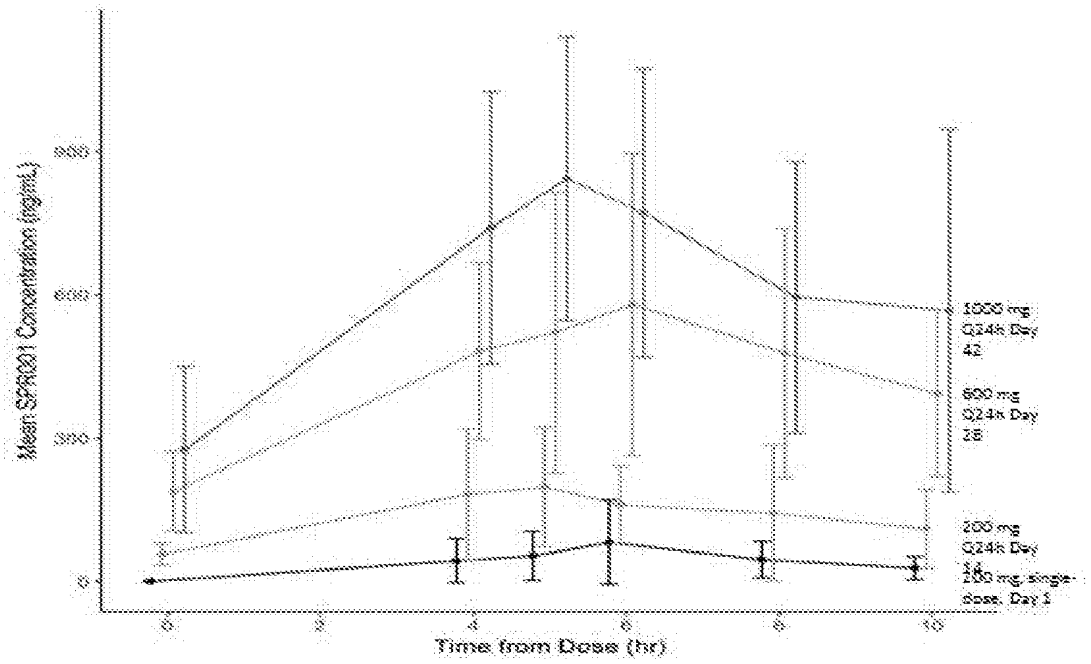

Mean SP4001 Concentration (ng/mL)
Time from Dose (hr)
1000 mg Q24h Day 42
800 mg Q24h Day 28
200 mg Q24h Day 14
100 mg, single dose, Day 1

FIG. 1

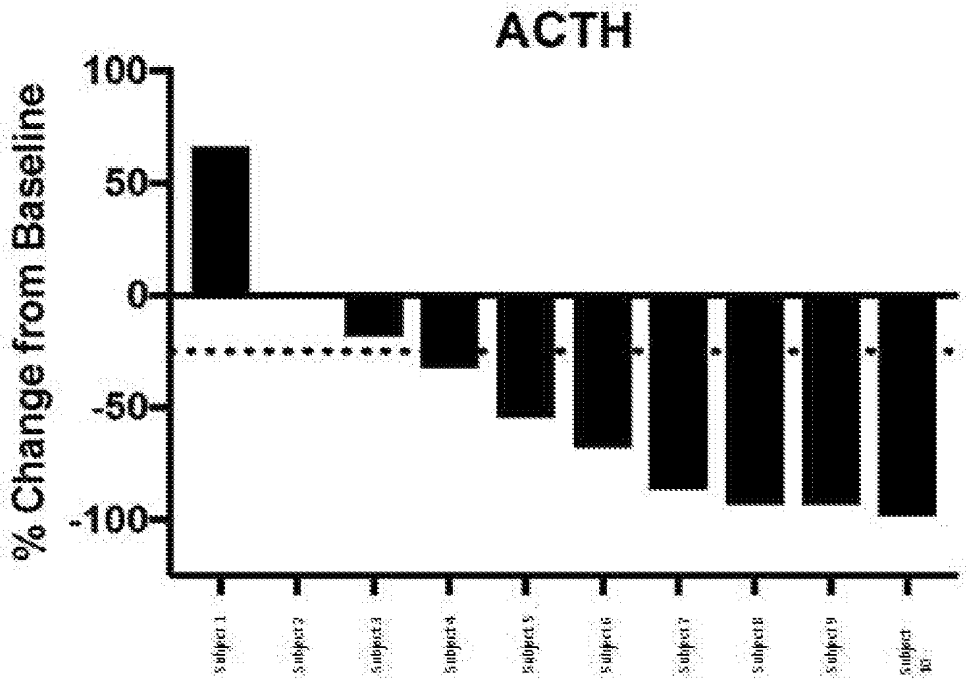

ACTH
% Change from Baseline
100
50
0
-50
-100
Subject 1
Subject 2
Subject 3
Subject 4
Subject 5
Subject 6
Subject 7
Subject 8
Subject 9
Subject 10

FIG. 2

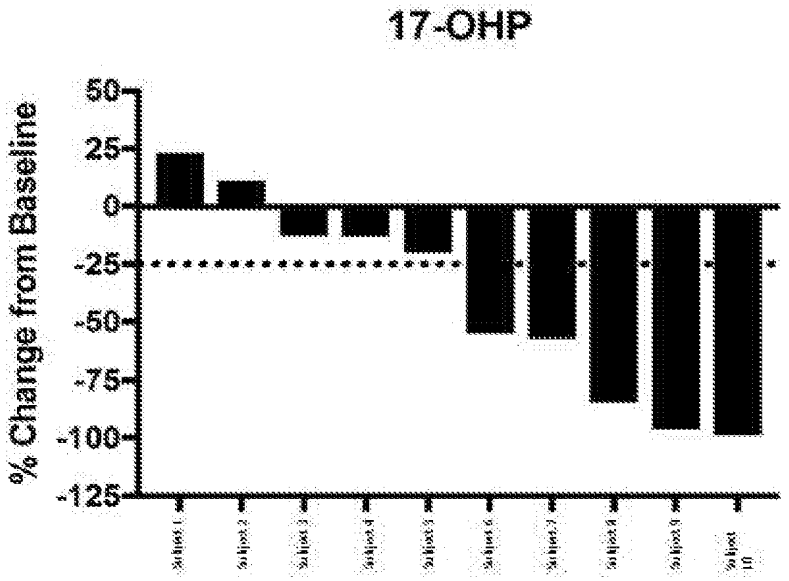

17-OHP
% Change from Baseline
50
25
0
-25
-50
-75
-100
-125

FIG. 3

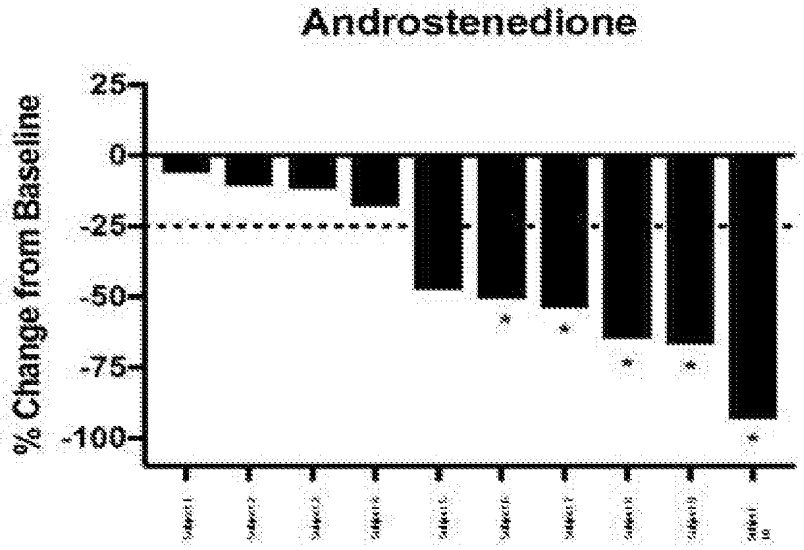

Androstenedione
% Change from Baseline
25
0
-25
-50
-75
-100
*

FIG. 4

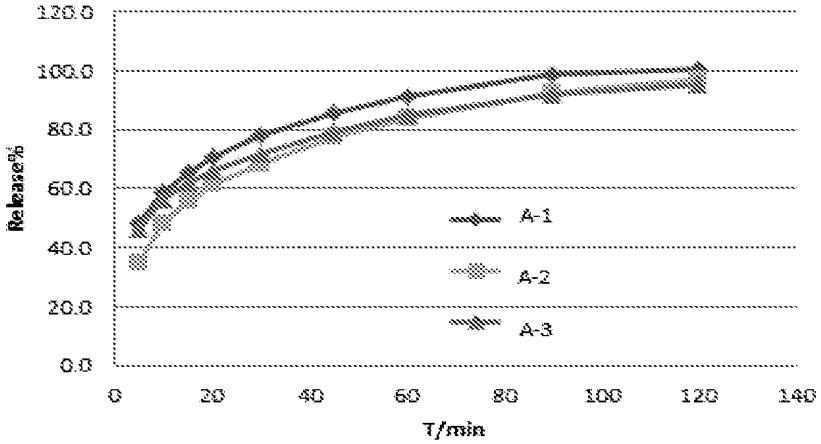

120.0
100.0
80.0
60.0
40.0
20.0
0.0
Release%
0
20
40
60
80
100
120
140
T/min
A-1
A-2
A-3

FIG. 5

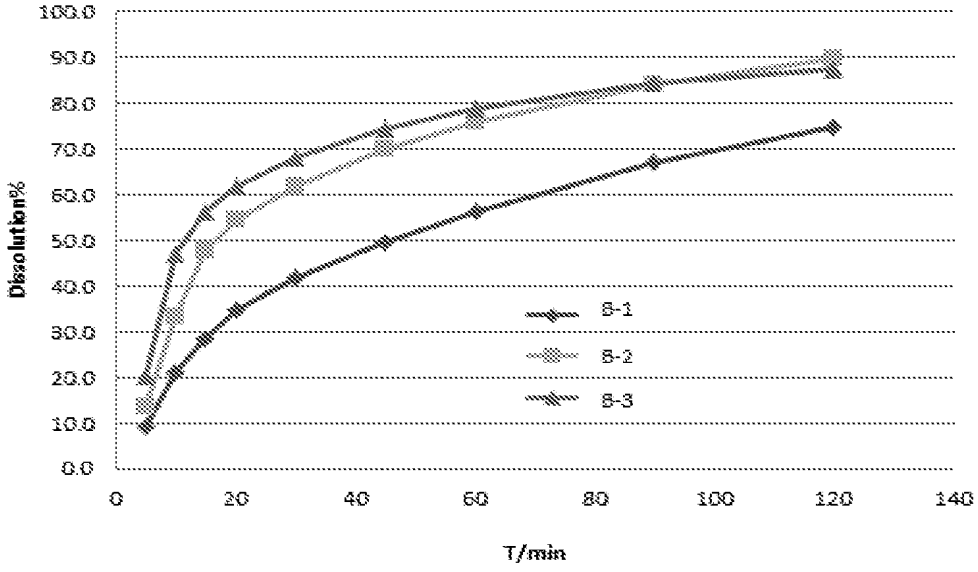

100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Dissolution%
0
20
40
60
80
100
120
140
T/min
S-1
S-2
S-3

FIG. 6

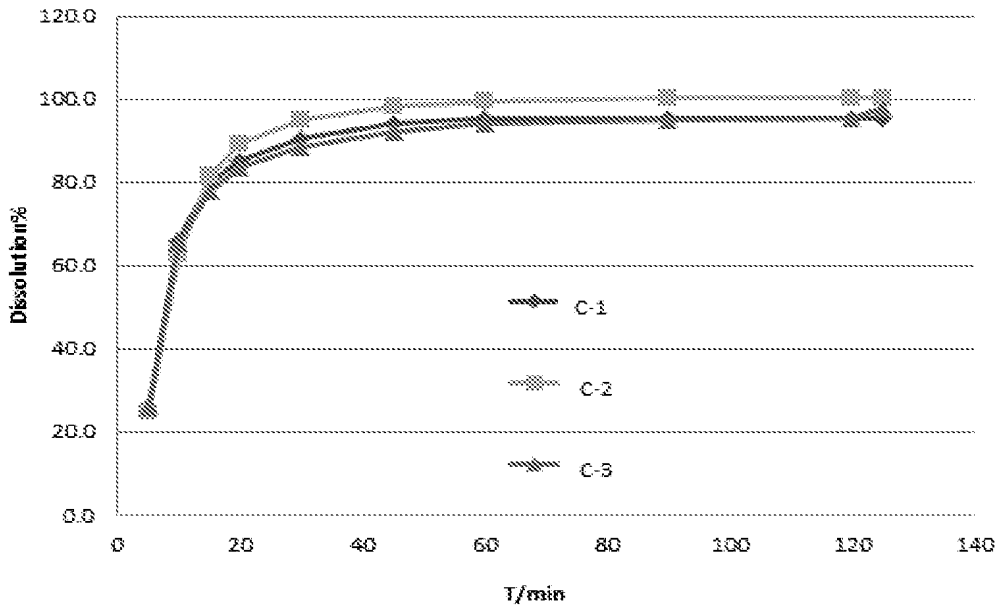

120.0
100.0
80.0
60.0
40.0
20.0
0.0
Dissolution%
C-1
C-2
C-3
0
20
40
60
80
100
120
140
T/min

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/46707

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07D 471/04 (2018.01)
CPC - A61P 35/00, C07D 471/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/0222339 A1 (Chen et al.) 02 September 2010 (02.09.2010); Abstract, para[0055], para[0056], para[0144]	1-3, 31-33
A	US 2011/0166345 A1 (Rizzo et al.) 07 July 2011 (07.07.2011); entire document	1-3, 31-33
A	US 2010/0022560 A1 (Chen et al.) 28 January 2010 (28.01.2010); entire document	1-3, 31-33
A	Hodgetts et al. 'Discovery of N-(1-Ethylpropyl)-[3-methoxy-5-(2-methoxy-4-trifluoromethoxyphenyl)-6-methyl-pyrazin-2-yl]amine 59 (NGD 98-2): An Orally Active Corticotropin Releasing Factor-1 (CRF-1) Receptor Antagonist', Journal of Medicinal Chemistry, 07 May 2011 (07.05.2011), Vol.54, pages4187-4206; entire document	1-3, 31-33

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 October 2018	24 OCT 2018

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/46707

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-30, 34-62
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Bibliographic data

Title:	ANTITUMOR AGENT
Pub/Pat no:	JPH0971528A
Pub/Issue Date:	1997-03-18
Inventor(s):	ARAI NARIYUKI[JP]| NISHIZAKI TAIJI[JP]| KIMOTO TETSUO[JP]| KURIMOTO MASASHI[JP]|ARAI NARIYUKI |NISHIZAKI TAIJI |KIMOTO TETSUO |KURIMOTO MASASHI
Applicant(s):	HAYASHIBARA BIOCHEM LAB[JP]
Classification:	A61K35/64AI; A61K31/19AI; A61K36/28AI; A61P35/00AI; C07C59/52AI
Application number:	JP19960177237 1996-06-19
Priority number:	JP19960177237 19960619; JP19950191015 19950705;

Abstract of JPH0971528A

PROBLEM TO BE SOLVED: To obtain a safe antitumor agent exhibiting excellent antitumor action on malignant tumor and causing little side actions. SOLUTION: This agent contains >=0.01wt.% of 3-[4-hydroxy-3,5-bis(3-methyl-2- butenyl)phenyl]-2-propenoic acid of formula (X is a cation) or its salt as an active component. It can be administered by oral or parenteral administration at a daily dose of 10m to 10mg, preferably 10g to 1mg for adult in 1-4 or 1-5 divided doses. The agent exhibits remarkable antitumor action on solid cancers such as colon cancer, rectal cancer, gastric cancer, thyroid cancer, lingual cancer, bladder cancer, chorionic carcinoma, hepatic cancer, uterine cancer, prostate cancer, laryngeal cancer, pulmonary cancer, mammary cancer, malignant melanoma, Kaposi's sarcoma, cerebral tumor, neuroblastoma, ovarian tumor, testicular tumor, osteogenic sarcoma, pancreatic cancer, renal cancer, hypernephroma and hemangioendothelioma and corpuscular malignant tumors such as leukemia and lymphoma.

(19)日本国特許庁（ＪＰ）　(12) **公開特許公報（Ａ）**　(11)特許出願公開番号

特開平9－71528

(43)公開日　平成９年(1997)３月18日

(51)Int.Cl.[6]		識別記号	庁内整理番号	ＦＩ			技術表示箇所
Ａ６１Ｋ	31/19	ＡＤＵ		Ａ６１Ｋ	31/19	ＡＤＵ	
	35/64				35/64		
	35/78				35/78	Ｔ	
// Ｃ０７Ｃ	59/52		9450－4H	Ｃ０７Ｃ	59/52		

審査請求　未請求　請求項の数４　ＦＤ　（全　７　頁）

(21)出願番号　特願平8－177237

(22)出願日　平成８年(1996)６月19日

(31)優先権主張番号　特願平7－191015

(32)優先日　平７(1995)７月５日

(33)優先権主張国　日本（ＪＰ）

(71)出願人　000155908
株式会社林原生物化学研究所
岡山県岡山市下石井１丁目２番３号

(72)発明者　新井　成之
岡山県岡山市奥田１丁目７番10－403号

(72)発明者　西▲ざき▼　泰司
岡山県玉野市八浜町八浜1330番地の22

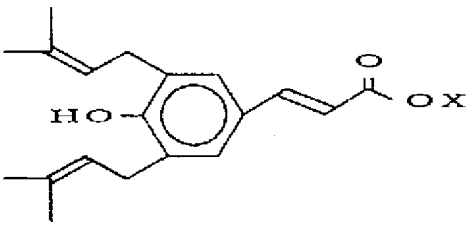
(72)発明者　木本　哲夫
岡山県岡山市山崎311番地の９

(72)発明者　栗本　雅司
岡山県岡山市学南町２丁目７番25号

(54)【発明の名称】　抗腫瘍剤

(57)【要約】
【課題】　著明な抗腫瘍作用を示しつつも、副作用を起こし難い、安全な抗腫瘍剤を提供することを課題とする。
【解決手段】　有効成分として３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸又はその生理学的に許容される塩を含んでなる抗腫瘍剤により解決する。

【特許請求の範囲】
【請求項１】　有効成分として３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸又はその生理学的に許容される塩を含んでなる抗腫瘍剤。
【請求項２】　有効成分として３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸又はその生理学的に許容される塩を０．０１重量％以上含んでなる請求項１に記載の抗腫瘍剤。
【請求項３】　３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸又はその生理学的に許容される塩がプロポリスから採取されたものである請求項１又は２に記載の抗腫瘍剤。
【請求項４】　生理学的に許容される塩がナトリウム塩、カリウム塩、カルシウム塩、マグネシウム塩又はアンモニウム塩である請求項１、２又は３に記載の抗腫瘍剤。
【発明の詳細な説明】
【０００１】
【発明の属する技術分野】この発明は抗腫瘍剤、とりわけ、有効成分として３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸又はその生理学的に許容される塩を含んでなる抗腫瘍剤に関する。
【０００２】
【従来の技術】悪性腫瘍を治療する現在の医療現場においては、悪性腫瘍の早期発見と外科的切除に主眼が置かれている。悪性腫瘍が外科的に切除し難い部位に発生したり、原発部位以外の部位への転移や浸潤が考えられる場合には、患部に放射線を照射したり、抗腫瘍剤を定期的に投与する治療法が行なわれる。
【０００３】後者の治療法の場合、現在頻用されている抗腫瘍剤は、（ｉ）免疫賦活作用を利用するタイプ、（ｉｉ）代謝拮抗作用を利用するタイプ及び（ｉｉｉ）腫瘍細胞を直接殺傷する作用を利用するタイプに大別することができる。このうち、（ｉｉ）及び（ｉｉｉ）のタイプ、とりわけ、人工的に創成したものは抗腫瘍作用が強い半面、副作用が強く、長期連用の難しいことがある。（ｉ）のタイプは副作用は弱いものの、抗腫瘍作用が充分でないものが多く、その多くは他の薬剤と併用するか、末期の悪性腫瘍患者で、他に有効な治療方法がない場合に適用されることが多い。
【０００４】
【発明が解決しようとする課題】斯かる状況に鑑み、この発明の目的は著明な抗腫瘍作用を示しつつも、副作用を起こし難い、安全な抗腫瘍剤を提供することにある。
【０００５】
【課題を解決するための手段】斯かる課題を解決すべく、本発明者が種々の天然抽出物、とりわけ、プロポリス抽出物中の抗腫瘍性物質について鋭意研究したところ、カルボン酸の一種である３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸及びその塩に著明な抗腫瘍作用のあることが判明した。
【０００６】プロポリス抽出物は、抗菌剤、抗炎症剤、抗腫瘍剤などとして古くから民間伝承薬として用いられている。最近になってプロポリス抽出物の生物作用に近代科学のメスが入るようになり、例えば、松田忍『フーズ・アンド・フード・イングレディエンツ・ジャーナル・オブ・ジャパン』、１９９４年、第１６０号、第６４乃至７３頁には、プロポリス抽出物に含まれるケルセチン、カフェイン酸フェネチルエステル及びクロレダン系ジテルペンに分類される新規化合物がヒトの悪性腫瘍に対して抗腫瘍作用を発揮したことが記載されている。
【０００７】本発明者がプロポリス抽出物中に存在と抗腫瘍作用を確認した３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸は自体公知の化合物であり、例えば、本特許出願人は特開平６－２５６１７７号公報において同化合物をプロポリス抽出物から単離し得ることを開示した。しかしながら、本発明者が知るかぎりにおいて、同化合物の抗腫瘍作用を教示又は示唆する刊行物等は存在せず、それを有効成分として含んでなる抗腫瘍剤はこの発明をもって嚆矢とするものである。
【０００８】
【作用】有効成分として３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸又はその生理学的に許容される塩を含んでなるこの発明の抗腫瘍剤は、ヒトに発生する種々の悪性腫瘍に対して著明な抗腫瘍作用を発揮する。実験動物を用いる急性毒性試験の結果に依ると、それら化合物は毒性が極めて低い。
【０００９】以下、この発明の抗腫瘍剤について詳細に説明すると、この発明の抗腫瘍剤で用いる３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸及びその塩は下記の化１に示す構造を有している。化１において、Ｘは生理学的に許容される陽イオンを表わし、斯かる陽イオンとしては、例えば、水素イオン、ナトリウムイオン、カリウムイオン、カルシウムイオン、マグネシウムイオン、アンモニウムイオンなどが挙げられる。
【００１０】
【化１】



【００１１】この発明で用いる３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸及びその塩は天然の給源から採取しても化学合成してもよく、所期の効果を発揮するかぎり、出所・由来は問わない。天然の給源としてはプロポリスや、例えば、カワラヨモギなどのキク科植物の葉茎が挙げられ、例えば、特開平６－２５６１７７号公報、シー・ツェロら『フィトケミストリー』、第２５巻、第１２号、第２，８４１乃至２，８５５頁（１９８６年）及びアイ・オクノら『ケミカル・ファーマシューティカル・ブレティン』、第３６巻、第２号、第７６９乃至７７５頁（１９８８年）には、それら給源から３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸を単離する方法が詳述されている。化学合成する場合には、例えば、特開昭６０－１６３８４１号公報に開示された方法が有利に適用できる。

【００１２】天然の給源から単離する方法について概説すれば、まず、原料のプロポリスやキク科植物の葉茎を破砕し、水若しくはメタノール、エタノール、アセトン、ジエチルエーテル、酢酸エチルなどの有機溶媒又はそれらの混液により抽出し、得られた抽出物を濃縮し、精製する。抽出物の濃縮・精製には類似の化合物を濃縮・精製するための通常の方法を適用すればよく、斯かる方法としては、例えば、塩析、透析、沪過、分液、分別沈澱、結晶化、ゲル沪過クロマトグラフィー、イオン交換クロマトグラフィー、ガスクロマトグラフィー、高速液体クロマトグラフィーなどが挙げられ、これらは必要に応じて適宜組合せて適用される。なお、３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸の生理学的に許容される塩を得るには、３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸に、例えば、水酸化ナトリウム、水酸化カリウム、水酸化カルシウム、水酸化マグネシウム、水酸化アンモニウムなどの水酸化物を作用させればよい。斯かる塩は３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸に比べて水溶性良好であり、製剤化や高用量投与の際の取扱いに優れている。

【００１３】この発明でいう抗腫瘍剤とは、３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸又はその塩単独の形態はもとより、これらと製剤化に際して通常用いられる、例えば、担体、賦形剤、希釈剤、安定剤、さらには、必要に応じて、インターフェロン－α、インターフェロン－β、インターフェロン－γ、インターロイキン２、インターロイキン１２、ＴＮＦ－α、ＴＮＦ－β、シクロフォスファミド、アドリアマイシン、α－ジフルオロメチルオルニチン、メルファラン、５－フルオロウラシル、ドキソルビシン、クロラムブシル、ビンブラスチン、１，３－ビス（２－クロロエチル）－１－ニトロソウレア、シスプラチン、レバミゾール、Ｄ－ペニシラン、金製剤、ＢＣＧ、クレスチン、ピシバニール（ＯＫ－４３２）、丸山ワクチン及びレンチナンを始めとする他の生理活性物質の１種又は２種以上との組成物としての形態をも包含する。

【００１４】さらに、この発明の抗腫瘍剤は投薬単位形態の薬剤としての形態であってもよく、その投薬単位形態の薬剤とは、この発明の抗腫瘍剤における有効成分である３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸又はその塩の１回当りの用量又はその整数倍（４倍まで）若しくはその約数（１／４０まで）に相当する量を含有し、投与に適した物理的に分離した一体の剤形にある形態を意味する。斯かる投薬単位形態の薬剤とは、例えば、注射剤、液剤、散剤、顆粒剤、錠剤、カプセル剤、舌下剤、点眼剤、点鼻剤、坐剤などが挙げられる。剤形にも依るが、この発明の抗腫瘍剤は、通常、３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸又はその塩を０．０１重量%以上含有する。

【００１５】この発明の抗腫瘍剤の使用方法について説明すると、この発明の抗腫瘍剤は経口的に投与しても非経口的に投与しても、著明な抗腫瘍作用を発揮する。悪性腫瘍の種類と症状にも依るが、具体的には、患者の症状や投与後の経過を観察しながら、成人当たり約１μｇ乃至１０ｍｇ、望ましくは、約１０μｇ乃至１ｍｇを１乃至４回／日又は１乃至５回／週の用量で経口投与するか、皮内、皮下、筋肉内若しくは静脈内又は経皮的に非経口投与すればよい。

【００１６】この発明の抗腫瘍剤は、ヒトに発生する各種の悪性腫瘍に対して著明な抗腫瘍作用を発揮する。個々の悪性腫瘍としては、例えば、結腸癌、直腸癌、胃癌、甲状腺癌、舌癌、膀胱癌、絨毛癌、肝癌、子宮癌、前立腺癌、喉頭癌、肺癌、乳癌、悪性黒色腫、カポジ肉腫、脳腫瘍、神経芽細胞腫、卵巣腫瘍、睾丸腫瘍、骨肉腫、膵臓癌、腎癌、副腎腫、血管内皮腫などの固形腫瘍及び白血病、リンパ腫などの血球系悪性腫瘍が挙げられる。

【００１７】次の実験例では、齧歯類を用いる動物実験に基づきこの発明の抗腫瘍剤の有効性と毒性を説明するが、齧歯類における後記悪性腫瘍がヒトにおける悪性腫

瘍の信頼できるモデルであることはすでに知られている。

【００１８】

【実験例１】

〈３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸及びその塩の調製〉

【００１９】

【実験例１－１】

〈３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸の調製〉特開平６－２５６１７７号公報に記載された方法にしたがって、プラジル産プロポリスから３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸（以下、「化合物１」と言う。）を調製した。

【００２０】すなわち、プラジル産プロポリス塊１５３．３重量部を破砕し、酢酸エチルを加えて抽出後、メタノールを加え、生じた沈澱部を遠心分離により除去する一方、エバポレータにより上清部を濃縮し、エタノールを加えた。新たに生じた沈澱部を遠心分離により除去し、残る上清部を上記と同様に濃縮後、メタノールを加えて抽出液６３．９重量部を得た。

【００２１】この抽出液を固形物換算で３５重量部とり、片山化学工業製『シリカゲル６０Ｇ６５０』のカラムに負荷し、濃度勾配下、ヘキサンと酢酸エチルの混液を通液した。そして、ヘキサンと酢酸エチルの濃度比が５９：４１乃至５７：４３の範囲で溶出した画分を採取し、これをファルマシア製『セファデックスＬＨ－２０』のカラムに負荷し、メタノールをＳＶ０．１６で通液し、ゲル容量の１．１倍付近で溶出した画分を採取し、濃縮したところ、結晶が析出した。この結晶をヘキサンで洗浄後、乾燥したところ、結晶性固状物が０．２８重量部得られた。

【００２２】この結晶性固状物の一部を採り、常法にしたがって元素分析法、質量分析法、紫外線分光法、赤外線分光法及び核磁気共鳴分光法により分析し、分析結果をアイ・オクノら『ケミカル・ファーマシューティカル・ブレティン』、第３６巻、第２号、第７６９乃至７７５頁（１９８８年）に報告されている３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸の分析結果と比較したところ、いずれも良好な一致を示した。斯くして、上記操作によりプロポリスから得られた結晶状の化合物は３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸と同定された。

【００２３】

【実験例１－２】

〈３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸塩の調製〉実験例１－１の方法で調製した化合物１を約１０重量％になるようにエタノールに溶解し、等モルの水酸化ナトリウム、水酸化カルシウム、水酸化マグネシウム又は水酸化カリウムを加えた後、遠心濃縮機により濃縮・乾燥して固状物を得た。実験例１－１の方法により調製した化合物１及び本例において調製したそれらナトリウム塩（以下、「化合物２」と言う。）、カルシウム塩（以下、「化合物３」と言う。）、マグネシウム塩（以下、「化合物４」と言う。）及びカリウム塩（以下、「化合物５」と言う。）を適宜濃度の水溶液とし、常法により滅菌沪過した後、次に述べる動物実験に供した。

【００２４】

【実験例２】

〈担癌マウスにおける抗腫瘍作用〉８週齢の雄ＢＡＬＢ／ｃマウスの腹腔内にＭｅｔｈ　Ａ腹水腫瘍細胞を約1×10^{6}個／匹注射移植した。翌日よりマウスを１群１０匹とし、毎週２回、化合物１乃至５のいずれかを０．０００５μｇ、０．００５μｇ、０．０５μｇ又は０．５μｇ含む生理食塩水０．２ｍｌを腹腔内に注射投与し、４０日間観察を続けた。対照群には、同じ投与スケジュールで薬剤無含有の生理食塩水を０．２ｍｌ／回腹腔内注射投与した。

【００２５】上記の条件における投与群の平均生存日数（Ｔ）と対照群の平均生存日数（Ｃ）に基づき、下記の数１により延命率（Ｔ／Ｃ　％）を算出した。結果を表１に示す。

【００２６】

【数１】

$$\text{延命率（Ｔ／Ｃ％）}=\frac{\text{投与群の平均生存日数}}{\text{対照群の平均生存日数}}\times 100$$

【００２７】

【表１】

化合物	投与量（μg/0.2ml/匹）	平均生存日数	４０日後生存匹数	延命率（T/C%）
1	0.0005	33.5±0.6	3	119.2
	0.005	35.0±0.9	5	124.6
	0.05	37.3±0.4	7	132.7*
	0.5	39.8±0.1	9	141.6*
2	0.0005	32.3±0.8	2	114.9
	0.005	36.5±0.5	6	129.9
	0.05	38.1±0.1	8	135.6*
	0.5	40.0 <	10	142.3*
3	0.0005	31.1±0.6	2	110.7
	0.005	35.9±0.3	6	127.8
	0.05	38.9±0.2	8	138.4*
	0.5	39.0±0.2	9	138.8*
4	0.0005	34.9±0.3	3	124.2
	0.005	35.7±0.4	5	127.0
	0.05	37.5±0.2	7	133.5*
	0.5	40.0 <	10	142.3*
5	0.0005	32.6±0.6	2	116.0
	0.005	36.1±0.5	6	128.5
	0.05	38.2±0.4	8	135.9*
	0.5	39.2±0.3	9	139.5*
対照	－	28.1±0.2	0	100

註：(*)危険率５％以下で、対照に対して統計学的に有意差あり。

【００２８】表１の結果から明らかなように、化合物１乃至５はいずれも０．０５μｇ／匹／回以上の用量で同系腫瘍担癌マウスに対して著明な延命効果を発揮した。

【００２９】

【実験例３】

〈腫瘍増殖抑制による抗腫瘍作用〉実験例２の結果より、ヌードマウスにおける化合物１乃至５の最少有効用量を０．０５μｇ／匹／回前後と推定し、ヒトの悪性腫瘍を移植したヌードマウスに同用量の化合物１乃至５を投与したときの抗腫瘍作用について試験した。

【００３０】すなわち、８週齢の雄ＢＡＬＢ／ｃヌードマウスの背部皮下に表２に示すヒトの悪性腫瘍細胞を５×10^7 個／匹注射移植した。その後、着生の確認された個体を１群７匹とし、週２回、化合物２を０．０５μｇ含む生理食塩水０．２ｍｌを背部皮下に注射投与し、さらに２５日間観察を続けた。対照群には、同じ投与スケジュールで薬剤無含有の生理食塩水を０．２ｍｌ／回投与した。

【００３１】移植から２７日目に、腫瘍湿重量を『人癌とヌードマウス』、１９８２年、医歯薬出版発行、第３２１乃至３２３頁に記載されている方法に準じて測定するとともに、３５日目の生存率（％）を計算した。結果を表２に示す。

【００３２】

【表２】

腫瘍細胞	平均腫瘍湿重量（ｇ）	判定時生存率（％）
HLC-1細胞（肺癌）	0.124±0.06（1.25±0.81）	100（71.4）
HGC-27細胞（胃癌）	0.35 ±0.09（1.86±0.64）	100（85.7）
Hep G2細胞（肝癌）	0.18 ±0.02（2.06±0.85）	100（42.8）
HeLa細胞（子宮癌）	0.54 ±0.06（1.36±0.61）	100（100）
RPMI-4788細胞（大腸癌）	0.21 ±0.03（2.95±1.36）	100（71.4）
WiDr細胞（結腸癌）	0.36 ±0.02（1.83±0.85）	100（85.7）
KB細胞（鼻咽腔癌）	0.54 ±0.03（2.12±0.96）	100（42.8）
HEp-2細胞（喉頭癌）	0.81 ±0.65（2.16±1.31）	100（42.8）
G-361細胞（悪性黒色腫）	0.36 ±0.02（1.96±0.98）	100（57.1）
HL-60細胞（白血病）	0.18 ±0.03（1.43±0.08）	100（100）
U-937細胞（リンパ腫）	0.25 ±0.02（0.99±0.81）	100（85.7）

註：括弧内の数字は、対照系における平均腫瘍湿重量及び判定時生存率を示す。

【００３３】表２の結果から明らかなように、化合物２は比較的低用量を投与したにも拘らず、ヒトの肺癌、胃癌、肝癌、子宮癌、大腸癌、結腸癌、鼻咽腔癌、喉頭癌、悪性黒色腫、白血病、リンパ腫などのヒトの悪性腫瘍に対して著明な抗腫瘍作用を発揮し、移植後３５日目におけるマウスの生存率も高かった。なお、データは示していないものの、化合物１及び化合物３乃至５について同様の試験をしたところ、ほぼ同様の結果が得られた。

【００３４】

【実験例４】

〈急性毒性試験〉体重１０乃至２０ｇのｄｄｙマウス１０匹を１群とし、その腹腔内に実験例１－１及び１－２の方法により得た適宜濃度の化合物１乃至５と５重量％アラビアガムを含む生理食塩水を注射投与するか、胃ゾンデにより経口投与し、その後７日間観察して死亡数を調べ、Ｖａｎ　ｄｅｒ　Ｖａｅｒｄｅｎの面積法によりＬＤ５０値を求めた。

【００３５】その結果、化合物１乃至５のＬＤ５０値はいずれの投与経路によっても２００ｍｇ／ｋｇ以上と著しく高く、これは、３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸及びその塩が、通常、成人において数μｇ／回以上の用量で有効であることを考え併せれば、この発明の抗腫瘍剤が極めて安全性の高いことを裏付けていると言える。

【００３６】以下、実施例によりこの発明を具体的に説明する。

【００３７】

【実施例１】

〈錠剤〉常法により、１錠当たり実験例１－１及び１－２の方法により得た化合物１乃至５のいずれかを０．１ｍｇ、乳糖７９．９ｍｇ、コーンスターチ６２．５ｍｇ及び蔗糖脂肪酸エステル７．５ｍｇ含有する５種類の錠剤を調製した。なお、錠剤が胃溶性剤の場合には、５重量％のヒドロキシプロピルメチルセルロースでコーティングした後、糖衣を施し、腸溶性剤の場合には、１０重量％のヒドロキシプロピルメチルセルロースでコーティングした後、糖衣を施した。

【００３８】本例の錠剤は、いずれも胃癌、肺癌、肝癌、子宮癌、乳癌、大腸癌、結腸癌及び悪性黒色腫を始めとするヒトの悪性腫瘍の治療剤として有用である。

【００３９】

【実施例２】

〈カプセル剤〉常法により、１錠当たり実験例１－１及び１－２の方法により得た化合物１乃至５のいずれかを０．２ｍｇ、乳糖１４６．８ｍｇ及び蔗糖脂肪酸エステル３．０ｍｇ含有する５種類のカプセル剤を調製した。

【００４０】本例のカプセル剤は、いずれも胃癌、肺癌、肝癌、子宮癌、乳癌、大腸癌、結腸癌及び悪性黒色腫を始めとするヒトの悪性腫瘍の治療剤として有用である。

【００４１】

【実施例３】

〈注射剤〉常法により、重炭酸ナトリウム５ｇに実験例１－１及び１－２の方法により得た化合物１乃至５のいずれかを５ｍｇ混和し、滅菌したガラス容器に０．１ｇずつ充填して５種類の注射剤を調製した。

【００４２】本例の注射剤は、いずれも用時溶解型の注射剤であり、胃癌、肺癌、肝癌、子宮癌、乳癌、大腸癌、結腸癌及び悪性黒色腫を始めとするヒトの悪性腫瘍の治療剤として有用である。

【００４３】

【実施例４】

〈外用剤〉常法により、実験例１－１及び１－２の方法により得た化合物１乃至５のいずれかを有効成分として少量の流動パラフィンに研和した後、ワセリンを加えて有効成分を５ｍｇ／ｇ含有する５種類の外用剤を調製した。

【００４４】本例の外用剤は、いずれも皮膚癌、乳癌及びリンパ腫を始めとするヒトの悪性腫瘍の治療剤として有用である。

【００４５】

【実施例５】

〈散剤〉常法により、１回用量当たり実験例１－１及び１－２の方法により得た化合物１乃至５のいずれかを０．３ｍｇ及び重炭酸ナトリウム４９９．７ｍｇを含有する５種類の散剤を調製した。

【００４６】本例の散剤は、いずれも胃癌、肺癌、肝臓癌、子宮癌、乳癌、大腸癌、結腸癌及び悪性黒色腫を始めとするヒトの悪性腫瘍の治療剤として有用である。

【００４７】

【実施例６】

〈坐剤〉常法により、１回用量当たり実験例１－１及び１－２の方法により得た化合物１乃至５のいずれかを０．３ｍｇ、ポリエチレングリコール＃１０００を１，２８０ｍｇ、ポリエチレングリコール＃４０００を３１９．７ｍｇ含有する５種類の坐剤を調製した。

【００４８】本例の坐剤は、いずれも大腸癌及び結腸癌を始めとするヒトの悪性腫瘍の治療剤として有用である。

【００４９】

【発明の効果】以上説明のごとく、この発明は３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸及びその生理学的に許容される陽イオンとの塩がヒトの悪性腫瘍に対して著明な抗腫瘍作用を発揮するという独自の知見に基づくものである。これら化合物は自然界の生物から単離された物質であり、人工的に創成されたものと比べて安全性が高い。したがって、有効成分としてこれら化合物を含んでなるこの発明の抗腫瘍剤は重篤な副作用を惹起することなく、ヒトの悪性腫瘍の治療に安全に用いることができる実益がある。

【００５０】斯くも顕著な作用効果を発揮するこの発明は、斯界に貢献すること誠に多大な、意義のある発明であるといえる。

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(71) Applicant *(for all designated States except US)*: **ANGIOGENE PHARMACEUTICALS LIMITED** [GB/GB]; 14 Plowden Park, Aston Rowant, Watlington, Oxfordshire OX9 5SX (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **ARNOULD, Jean, Claude** [FR/FR]; ZI La Pompelle BP 1050, 51689 Reims Cedex 2 (FR). **LAMORLETTE, Maryannick, Andree** [FR/FR]; ZI La Pompelle BP 1050, 51689 Reims Cedex 2 (FR).

(74) Agents: **BRYANT, Tracey** et al.; Astrazeneca, Global Intellectual Property, P.O. Box 272, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4GR (US).

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(54) Title: COLCHINOL DERIVATIVES AS VASCULAR DAMAGING AGENTS

(57) Abstract: The invention relates to colchinol derivatives of the formula 1: wherein the substituents are as defined in the description or a pharmaceutically-acceptable salt, solvate or pro-drug thereof. The invention also relates to processes for preparing compounds of formula (1), pharmaceutical compositions of compounds of formula (1) and the use of compounds of formula (1), in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm blooded animal.

COLCHINOL DERIVATIVES AS VASCULAR DAMAGING AGENTS

The present invention relates to vascular damaging agents, to the use of compounds of the invention in the manufacture of medicaments for use in the production of antiangiogenic effects in warm-blooded animals such as humans, to processes for the preparation of such compounds, to pharmaceutical compositions containing such compounds as active ingredient, to methods for the treatment of disease states associated with angiogenesis and to the use of such compounds as medicaments.

Normal angiogenesis plays an important role in a variety of processes including embryonic development, wound healing and several components of female reproductive function. Undesirable or pathological angiogenesis has been associated with disease states including diabetic retinopathy, psoriasis, cancer, rheumatoid arthritis, atheroma, Kaposi's sarcoma and haemangioma (Fan et al, 1995, Trends Pharmacol. Sci. 16: 57-66; Folkman, 1995, Nature Medicine 1: 27-31). Formation of new vasculature by angiogenesis is a key pathological feature of several diseases (J. Folkman, New England Journal of Medicine 333, 1757-1763 (1995)). For example, for a solid tumour to grow it must develop its own blood supply upon which it depends critically for the provision of oxygen and nutrients; if this blood supply is mechanically shut off the tumour undergoes necrotic death. Neovascularisation is also a clinical feature of skin lesions in psoriasis, of the invasive pannus in the joints of rheumatoid arthritis patients and of atherosclerotic plaques. Retinal neovascularisation is pathological in macular degeneration and in diabetic retinopathy.

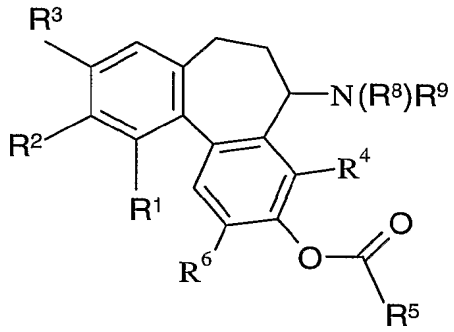
Reversal of neovascularisation by damaging the newly-formed vascular endothelium is expected to have a beneficial therapeutic effect. The present invention is based on the discovery of tricyclic compounds that surprisingly specifically damage newly formed vasculature without affecting the normal, established vascular endothelium of the host species, a property of value in the treatment of disease states associated with angiogenesis such as cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation, endometriosis, dysfunctional uterine bleeding and ocular diseases with retinal vessel proliferation.

Compounds of the present invention are colchinol derivatives. Colchinol derivatives for example *N*-acetyl-colchinol are known. Anti-tumour effects have been noted on animal

models (see for example - Jnl. Natl. Cancer Inst. 1952, 13, 379-392). However, the effect studied was that of gross damage (haemorrhage, softening and necrosis) and there is no suggestion of treatment of inappropriate angiogenesis by destruction of neovasculature.

It is believed, though this is not limiting on the invention, that the use of compounds of the invention damages newly-formed vasculature, for example the vasculature of tumours, thus effectively reversing the process of angiogenesis as compared to known anti-angiogenic agents which tend to be less effective once the vasculature has formed.

According to one aspect of the present invention there is provided a compound of the formula I:



(I)

wherein:

R^1, R^2 and **R^3** are each independently hydroxy, phosphoryloxy ($-OPO_3H_2$), C_{1-4}alkoxy or an in vivo hydrolysable ester of hydroxy; with the proviso that at least two of R^1, R^2 and R^3 are C_{1-4}alkoxy;

R^4 and **R^6** are each independently selected from:

hydrogen, nitro, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, fluoro, C_{1-4}alkoxy and C_{1-4}alkyl;

R^5 is selected from one of the following groups:

1) of the formula -A -X^1-Y^1 -B, wherein:

A is C_{1-4}alkylene or $-(CH_2)_p$-Q- (wherein **p** is 0, 1 or 2 and **Q** is phenylene or thienylene);

X^1 is -O-, -CO-, -C(O)O-, $-CON(R^{10})$-, $-N(R^{10})$-, $-N(R^{10})CO$-, $N(R^{10})C(O)O$-, $-N(R^{10})CON(R^{11})$-, $-N(R^{10})SO_2$-, $-SO_2N(R^{10})$- or $OC(O)N(R^{10})$-

(wherein **R^{10}** is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

Y^1 is C_{1-3}alkylene;

B is carboxy, sulpho, phosphoryloxy, hydroxy, amino, N-(C_{1-4}alkyl)amino, N,N-di(C_{1-3}alkyl)amino (wherein the C_{1-}alkyl group in the alkylated amino groups is optionally substituted by hydroxy or amino), -R^{12} or -NHC(R^{13})COOH;

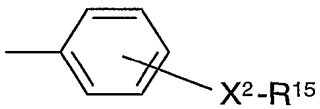
(wherein $\mathbf{R^{12}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, aminoC_{1-4}alkyl, N,N-di(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein $\mathbf{R^{14}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl);

R^{13} is an amino acid side chain;

2) of the formula:



wherein: the phenyl ring is substituted by -$\mathbf{X^2}$-$\mathbf{R^{15}}$ in the 3- or 4-position;

$\mathbf{X^2}$ is -CO- or of the formula -$(CH_2)_r$- (wherein **r** is 0, 1, 2 or 3) and $\mathbf{R^{15}}$ is a 5-6 membered saturated heterocyclic group (linked via a ring carbon or nitrogen atom) containing 1 or 2 ring heteroatoms selected from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, C_{1-4}aminoalkyl, N,N-di(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein $\mathbf{R^{14}}$ is as hereinabove defined);

provided that the heterocyclic group (R^{15}) is substituted by at least one substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N,N-di(C_{1-4}alkyl)carbamoyl;

3) -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15} (wherein **a** is 0, 1, 2, 3 or 4; **b** is 0, 1, 2, 3 or 4; $\mathbf{Y^2}$ is a direct bond, -O-, -C(O)-, -N(R^{16})-, -N(R^{16})C(O)- or -C(O)N(R^{16})- (wherein $\mathbf{R^{16}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and wherein 1 or 2 of the $(CH_2)_a$ or $(CH_2)_b$ groups are optionally substituted by 1 or 2 substituents selected from hydroxy and amino and $\mathbf{R^{15}}$ is as hereinabove defined; provided that when a is 0, then Y^2 is a single direct bond;

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl- (wherein the alkyl groups are independently optionally substituted by 1 or 2 substituents selected from:

amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkanoyl, carboxy, sulpho and phosphoryloxy);

provided that:

a) when A is C_{1-4}alkylene and X^1 is of the formula -CO-, -N(R^{10})-, -N(R^{10})CO- or -CON(R^{10})- then when B is R^{12}, R^{12} is as defined hereinabove for R^{15} ;

b) when A is C_{1-4}alkylene and X^1 is of the formula -N(R^{10})CO-, -CON(R^{10})- , or -C(O)O-, then B is not carboxy;

c) when A is C_{1-4}alkylene and X^1 is -CONH- or -NHCO-, then B is not carboxy, hydroxy, phosphoryloxy, amino, N-C_{1-4}alkylamino or N,N-di-C_{1-4}alkylamino;

$\mathbf{R^8}$ is a group -Y^3R^{17} (wherein $\mathbf{Y^3}$ is a direct bond, -C(O)-, -C(O)O-, -N(R^{18})-, -C(O)N(R^{18})-, -SO_2- or -SO_2NR^{18}- (wherein $\mathbf{R^{18}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and $\mathbf{R^{17}}$ is selected from one of the following 4 groups:

1) hydrogen, C_{1-4}alkyl, phenyl, C_{1-4}alkylY^4C_{1-4}alkyl (wherein $\mathbf{Y^4}$ is -C(O)-, -NR^{19}C(O)- or -C(O)NR^{19}- (wherein $\mathbf{R^{19}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl));

[which alkyl, alkylY^4alkyl or phenyl group is optionally substituted by 1 or 2 substituents selected from:

halogeno, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, carboxy, -CON(R^{23})R^{24} (wherein $\mathbf{R^{23}}$ **and** $\mathbf{R^{24}}$ are independently selected from hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl and C_{1-3}alkoxyC_{2-3}alkyl), C_{1-4}alkoxy, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, sulpho, phosphoryloxy, R^{12} (wherein $\mathbf{R^{12}}$ is as hereinabove defined), and a group -$\mathbf{Y^5R^{20}}$ [wherein $\mathbf{Y^5}$ is -NR^{21}C(O)- or -OC(O)- (wherein

$\mathbf{R^{21}}$ represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and $\mathbf{R^{20}}$ is C_{1-4}alkyl or a group R^{22} (wherein $\mathbf{R^{22}}$ is a 5 or 6 membered aromatic heterocyclic group containing 1 to 4, inclusive, ring heteroatoms selected independently from O, N and S, which aromatic heterocyclic group is optionally substituted by 1 or 2 substituents selected from hydroxy, amino, C_{1-4}alkyl, aminoC_{1-4}alkyl, N-C_{1-4}alkylaminoC_{1-4}alkyl, N, N-di(C_{1-4}alkyl)aminoC_{1-4}alkyl, carboxy, -$CONR^{25}R^{26}$ and -$NR^{25}COR^{27}$ (wherein $\mathbf{R^{25}}$ **and** $\mathbf{R^{26}}$, which may be the same or different, are hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl and $\mathbf{R^{27}}$ is C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))];

2) R^{22} (wherein R^{22} is as hereinabove defined);

3) R^{22} -C_{1-4}alkyl- (wherein R^{22} is as hereinabove defined); or

4) $R^{12}Y^7C_{1-4}$alkyl- (wherein R^{12} is as hereinabove defined and Y^7 is -C(O)-, -NR^{23}C(O)-, -NR^{23}C(O)C_{1-4}alkyl-, -C(O)NR^{23}- or -C(O)$NR^{23}C_{1-4}$alkyl- (wherein R^{23} is as hereinabove defined))];

and $\mathbf{R^9}$ is hydrogen or C_{1-3}alkyl;

or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

In another aspect, the invention relates to a compound of the formula (I) as hereinabove defined or to a pharmaceutically-acceptable salt thereof.

In this specification the generic term "alkyl" includes both straight-chain and branched-chain alkyl groups. However references to individual alkyl groups such as "propyl" are specific for the straight-chain version only and references to individual branched-chain alkyl groups such as "isopropyl" are specific for the branched-chain version only. An analogous convention applies to other generic terms.

R^{13} is an amino acid side chain. This includes amino acid side chains from natural and non-natural amino acids and includes the possibility of R^{13} joining to the NH group so as to form a ring as in the amino acid proline. It includes α-amino acids β-amino acids and γ-amino acids. In addition, the amino acids may be L-isomers or D-isomers, but preferably L-isomers. Preferred amino acids include glycine, alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tryptophan, serine, threonine, cysteine, tyrosine, asparaginine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, β-alanine and ornithine. More preferred amino acids include glutamic acid, serine, threonine, arginine, glycine, alanine, β-alanine and lysine. Especially preferred amino acids include glutamic acid, serine, threonine, arginine, alanine and β-alanine. Specific values for R^{12} include hydrogen,

C_{1-4}alkyl, C_{1-4}alkylthioC_{1-4}alkyl, hydroxyC_{1-4}alkyl, thioC_{1-4}alkyl, phenylC_{1-4}alkyl (optionally substituted by hydroxy), guanidinoC_{1-4}alkyl, carboxyC_{1-4}alkyl, carbamoylC_{1-4}alkyl, aminoC_{1-4}alkyl and imidazolyl C_{1-4}alkyl and R^{12} forming a pyrrolidinyl ring with the NH group. Preferred values for R^{13} include hydrogen, C_{1-4}alkyl, C_{1-4}alkylthioC_{1-4}alkyl, hydroxyC_{1-4}alkyl, thioC_{1-4}alkyl, guanidinoC_{1-4}alkyl, carboxyC_{1-4}alkyl, carbamoylC_{1-4}alkyl and aminoC_{1-4}alkyl.

In this specification, the term heteroaryl is used to describe fully saturated heterocyclic rings. Examples of 5- or 6-membered heteroaryl rings include pyrrolyl, imidazolyl, pyrazolyl, isothiazolyl, isoxazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazolyl, tetrazolyl, thiazolyl, thiadiazolyl, thienyl, furyl and oxazolyl.

It is to be understood that, insofar as certain of the compounds of Formula I defined above may exist in optically active or racemic forms by virtue of one or more asymmetric carbon atoms, the invention includes in its definition any such optically active or racemic form which possesses vascular damaging activity. The synthesis of optically active forms may be carried out by standard techniques of organic chemistry well known in the art, for example by synthesis from optically active starting materials or by resolution of a racemic form. Similarly, the above-mentioned activity may be evaluated using the standard laboratory techniques referred to hereinafter.

Suitable values for the generic radicals referred to above include those set out below. Within the present invention it is to be understood that a compound of the formula I or a salt thereof may exhibit the phenomenon of tautomerism and that the formulae drawings within this specification can represent only one of the possible tautomeric forms. It is to be understood that the invention encompasses any tautomeric form which has vascular damaging activity and is not to be limited merely to any one tautomeric form utilised within the formulae drawings.

It is also to be understood that certain compounds of the formula I and salts thereof can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms which have vascular damaging activity.

The present invention relates to the compounds of formula I as hereinbefore defined as well as to the salts thereof. Salts for use in pharmaceutical compositions will be pharmaceutically acceptable salts, but other salts may be useful in the production of the

compounds of formula I and their pharmaceutically acceptable salts. Pharmaceutically acceptable salts of the invention may, for example, include acid addition salts of the compounds of formula I as hereinbefore defined which are sufficiently basic to form such salts. Such acid addition salts include for example salts with inorganic or organic acids affording pharmaceutically acceptable anions such as with hydrogen halides (especially hydrochloric or hydrobromic acid of which hydrochloric acid is particularly preferred) or with sulphuric or phosphoric acid, or with trifluoroacetic, citric or maleic acid. Suitable salts include hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates and tartrates. In addition where the compounds of formula I are sufficiently acidic, pharmaceutically acceptable salts may be formed with an inorganic or organic base which affords a pharmaceutically acceptable cation. Such salts with inorganic or organic bases include for example an alkali metal salt, such as a sodium or potassium salt, an alkaline earth metal salt such as a calcium or magnesium salt, an ammonium salt or for example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

Various forms of prodrugs are known in the art. For examples of such prodrug derivatives, see:

a) Design of Prodrugs, edited by H. Bundgaard, (Elsevier, 1985) and Methods in Enzymology, Vol. 42, p. 309-396, edited by K. Widder, *et al.* (Academic Press, 1985);

b) A Textbook of Drug Design and Development, edited by Krogsgaard-Larsen and H. Bundgaard, Chapter 5 "Design and Application of Prodrugs", by H. Bundgaard p. 113-191 (1991);

c) H. Bundgaard, Advanced Drug Delivery Reviews, 8, 1-38 (1992);

d) H. Bundgaard, *et al.*, Journal of Pharmaceutical Sciences, 77, 285 (1988); and

e) N. Kakeya, *et al.*, Chem. Pharm. Bull., 32, 692 (1984).

Examples of such pro-drugs may be used to form in-vivo-cleavable esters of a compound of the Formula I. An in-vivo-cleavable ester of a compound of the Formula I containing a carboxy group is, for example, a pharmaceutically-acceptable ester which is cleaved in the human or animal body to produce the parent acid. Suitable pharmaceutically-acceptable esters for carboxy include C_{1-6}alkoxymethyl esters, for example

methoxymethyl; C_{1-6}alkanoyloxymethyl esters, for example pivaloyloxymethyl; phthalidyl esters; C_{3-8}cycloalkoxycarbonyloxy C_{1-6}alkyl esters, for example 1-cyclohexylcarbonyloxyethyl; 1,3-dioxolan-2-ylmethyl esters, for example 5-methyl-1,3-dioxolan-2-ylmethyl; and C_{1-6}alkoxycarbonyloxyethyl esters, for example 1-methoxycarbonyloxyethyl; and may be formed at any carboxy group in the compounds of this invention.

Suitable values for R^1, R^2, R^3 R^4, R^5, R^6, R^7, R^8, R^9, R^{10} or R^{16} or for various substituents on D, R^{12}, R^{14} or R^{15} include:

for halogeno	fluoro, chloro, bromo and iodo;
for C_{1-3}alkyl:	methyl, ethyl, propyl, and isopropyl;
for C_{1-4}alkyl:	methyl, ethyl, propyl, isopropyl and tert-butyl;
for N-C_{1-4}alkylamino:	methylamino, ethylamino, propylamino, isopropylamino and butylamino;
for N,N-di-(C_{1-4}alkyl)amino:	dimethylamino, diethylamino, N-ethyl-N-methylamino and diisopropylamino;
for C_{2-4}alkanoyl:	acetyl and propionyl;
for C_{2-4}alkanoylamino:	acetamido and propionamido;
for C_{1-4}alkoxy:	methoxy and ethoxy;
for C_{1-3}alkoxyC_{2-3}alkyl:	methoxyethyl and ethoxypropyl;
for cyanoC_{1-4}alkyl:	cyanomethyl and 2-cyanoethyl;
for N-C_{1-4}alkylcarbamoyl:	N-methylcarbamoyl, N-ethylcarbamoyl and N-propylcarbamoyl;
for N,N-di-(C_{1-4}alkyl)carbamoyl:	N,N-dimethylcarbamoyl, N-ethyl-N-methylcarbamoyl and N,N-diethylcarbamoyl;
for C_{1-4}alkylsulphonylalkyl:	methylsulphonylmethyl and ethylsulphonylmethyl;
for hydroxyC_{1-4}alkyl:	hydroxymethyl, 2-hydroxyethyl, 1-hydroxyethyl and 3-hydroxypropyl as appropriate;
for hydroxyC_{2-3}alkyl:	2-hydroxyethyl, 1-hydroxyethyl and 3-hydroxypropyl as appropriate;
for C_{1-4}alkoxyC_{1-4}alkyl:	methoxymethyl, ethoxymethyl, 1-methoxyethyl, 2-methoxyethyl, 2-ethoxyethyl and 3-methoxypropyl as appropriate;

for aminoC_{1-4}alkyl or	aminomethyl, 2-aminoethyl, 1-aminoethyl and 3-aminopropyl as appropriate;
for aminoC_{2-3}alkyl or	2-aminoethyl, 1-aminoethyl and 3-aminopropyl as appropriate;
for N-C_{1-4}alkylaminoC_{1-4}alkyl:	methylaminomethyl, ethylaminomethyl, 1-methylaminoethyl, 2-methylaminoethyl, 2-ethylaminoethyl and 3-methylaminopropyl as appropriate;
for N,N-di-(C_{1-4}alkyl)aminoC_{1-4}alkyl:	dimethylaminomethyl, diethylaminomethyl, 1-dimethylaminoethyl, 2-dimethylaminoethyl and 3-dimethylaminopropyl as appropriate:
for carboxyC_{1-4}alkyl:	carboxymethyl, 1-carboxyethyl, 2-carboxyethyl, 3-carboxypropyl and 4-carboxybutyl;
for C_{1-4}alkoxycarbonylC_{1-4}alkyl:	methoxycarbonylmethyl, ethoxycarbonylmethyl, tert-butoxycarbonylmethyl, 1-methoxycarbonylethyl, 1-ethoxycarbonylethyl, 2-methoxycarbonylethyl, 2-ethoxycarbonylethyl, 3-methoxycarbonylpropyl and 3-ethoxycarbonylpropyl;
for C_{1-4}alkoxycarbonylamino:	methoxycarbonylamino and ethoxycarbonylamino;
for carbamoylC_{1-4}alkyl:	carbamoylmethyl, 1-carbamoylethyl, 2-carbamoylethyl and 3-carbamoylpropyl;

Examples of 5- or 6-membered saturated heterocyclic ring ring systems include pyrrolidinyl, imidazolidinyl, pyrazolidinyl, piperidyl, piperazinyl and morpholinyl.

Preferably at least 2 of R^1, R^2, and R^3 are methoxy.

Preferably R^1 , R^2, and R^3 are all C_{1-4}alkoxy.

Most preferably R^1, R^2, and R^3 are all methoxy.

Preferably A is ethylene, propylene, benzylene or phenylene. More preferably A is ethylene or phenylene.

More preferably A is phenylene.

Most preferably A is 1,4-phenylene.

Preferably X^1 is -CO-, -CON(R^{10})-, -N(R^{10}) -, -N(R^{10})CO- or -OC(O)N(R^{10})-.

Most preferably X^1 is -CO- or -N(R^{10})CO-.

Preferably R^{10} is hydrogen or methyl. Most preferably R^{10} is hydrogen.

Preferably Y^1 is propylene or ethylene.

More preferably Y^1 is ethylene.

Preferably B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} wherein R^{12} is as hereinabove defined.

Yet more preferably B is phosphoryloxy or -R^{12}. Most preferably, B is -R^{12}.

Preferably R^{12} is a 5 or 6 membered saturated heterocyclic ring containing 1 or 2 ring heteratoms selected from N and O.

Preferably R^{12} is a 6 membered saturated heterocyclic ring containing 1 or 2 ring heteratoms selected from N and O.

Preferably R^{12} contains at least 1 ring nitrogen atom.

Preferably -R^{12} is piperazinyl, morpholinyl, pyrrolidiyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents mentioned above for -R^{12}.

Preferably -R^{12} is linked via a ring nitrogen atom.

More preferably -R^{12} is piperazino or morpholino, each ring being optionally substituted by 1 or 2 of the substituents mentioned hereinabove for -R^{12}.

The saturated heterocyclic ring may be substituted on ring carbon or ring nitrogen atoms, providing this does not result in quaternisation.

When the saturated heterocyclic ring contains a ring nitrogen atom which is not linked to Y^1, preferably this ring nitrogen atom is substituted.

Preferred substituents for the saturated heterocyclic ring in R^{12} include C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl.

More preferred substituents for the saturated heterocyclic ring in -R^{12} include C_{1-3}alkyl, C_{2-3}alkanoyl, carbamoyl and hydroxyC_{2-3}alkyl.

Yet more preferred substituents for the saturated heterocyclic ring in $-R^{12}$ include methyl, ethyl, acetyl, propionyl, carbamoyl and 2-hydroxyethyl.

The most preferred substituents for the saturated heterocyclic ring include methyl, acetyl and carbamoyl.

Preferably the saturated heterocyclic ring in $-R^{12}$ is unsubstituted or substituted by 1 substituent.

When the saturated heterocyclic ring in $-R^{12}$ is morpholino, preferably it is unsubstituted. When the saturated heterocyclic ring in $-R^{12}$ is piperazino, preferably it is unsubstituted or substituted by 1 substituent on a ring nitrogen atom.

Most preferably R^{12} is morpholino, 4-methylpiperazin-l-yl or 4-acetylpiperazin-1-yl.

Preferably X^2 is $-(CH_2)_r-$.

Preferably r is 0, 1 and 2.

Most preferably r is 1.

In another aspect r is 0.

In one aspect the $-X^2-R^{15}$ substituent is in the 3-position of the phenyl ring in R^5.

In another aspect the $-X^2-R^{15}$ substituent is in the 4-position of the phenyl ring in R^5.

Preferably R^{15} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted as hereinabove defined for R^{12}, and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N,N-di(C_{1-4}alkyl)carbamoyl.

More preferably R^{15} is morpholinyl, piperazinyl or piperidinyl linked by either a ring carbon or nitrogen atom, optionally substituted as hereinabove defined for R^{12} and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N,N-di (C_{1-4}alkyl)carbamoyl.

More preferably, R^{15} is morpholino or piperazino, each ring being substituted by 1 substituent selected from C_{2-3}alkanoyl, carbamoyl, N-C_{1-3}alkylcarbamoyl and N,N-di (C_{1-3}alkyl)carbamoyl.

Yet more preferably R^{15} is piperazin-1-yl which is substituted in the 4-position by 1 substituent selected from acetyl, carbamoyl, N-methylcarbamoyl and N,N-dimethylcarbamoyl.

Preferably $-X^2-R^{15}$ is 4-carbamoylpiperazin-1-ylmethyl or 4-acetylpiperazin-1-ylmethyl.

Preferably a is 0, 1, 2 or 3.

More preferably a is 2 or 3.

Most preferably a is 2.

Preferably b is 0, 1, or 2.

More preferably b is 0 or 1

Most preferably b is 0.

Preferably Y^2 is -C(O)-, -N(R^{16})C(O)- or -C(O)N(R^{16})-

More preferably Y^2 is -C(O)- or -N(R^{16})C(O)-.

Most preferably Y^2 is -C(O)-.

Preferably R^{16} is hydrogen.

Preferably the alkyl groups in N,N-di (C_{1-4} alkyl)carbamoylC_{1-4}alkyl in R^5 are optionally substituted by 1 or 2 substituents selected from amino, N-methylamino, N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy.

More preferably the alkyl groups in N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl in R^5 are optionally substituted by substituents selected from amino, hydroxy and phosphoryloxy.

Yet more preferably the alkyl groups in N,N-di (C_{1-4}alkyl)carbamoylC_{1-4}alkyl are optionally substituted by 1 hydroxy substituent.

In one aspect R^5 is selected from group 1).

In another aspect R^5 is selected from group 2).

In yet another aspect R^5 is selected from group 3).

In yet another aspect R^5 is selected from group 4).

When R^5 is selected from group 1), it is preferably 4-[2-(4-methylpiperazin-1-yl)ethylcarbonylamino]phenyl, 4-[2-(4-acetylpiperazin-1-yl)ethylcarbonylamino]phenyl, 4-[2-(4-methylpiperazin-1-yl)methylcarbonylamino]phenyl or 4-[2-(4-acetylpiperazin-1-yl)methylcarbonylamino]phenyl.

When R^5 is selected from group 2), it is preferably 4-(4-acetylpiperazin-1-ylmethyl)phenyl or 3-(4-acetylpiperazin-1-ylmethyl)phenyl.

When R^5 is selected from group 3), it is preferably, 2-(4-acetylpiperazin-1-ylcarbonyl)ethyl or 3-(4-acetylpiperazin-1-ylcarbonyl)propyl.

Preferably when R^5 is selected from group 4), it is N-N-di-(2-hydroxyethyl)carbamoylC_{1-4}alkyl.

Most preferably when R^5 is selected from group 4), it is 2-[N-N-di-(2-hydroxyethyl)carbamoyl]ethyl or 3-[N,N-di-(2-hydroxyetnyl)carbamoyl]propyl.

Preferably Y^3 is -C(O)-, -C(O)O- or -C(O)N(R^{18})- . More preferably Y^3 is -C(O)- or -C(O)O-.

Most preferably Y^3 is -C(O)-.

Preferably R^{18} is hydrogen, methyl, 2-hydroxyethyl or 2-aminoethyl.

Most preferably R^{18} is hydrogen.

Preferably R^{19} is hydrogen or methyl. Most preferably R^{19} is hydrogen.

Preferably Y^4 is -NHCO- or -CONH-.

Preferred optional substituents for alkyl, alkylY^4 alkyl and phenyl groups in R^{17} include: halogeno, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, C_{1-4}alkoxy, C_{1-4}alkoxycarbonylamino, C_{1-4} alkanoyl, phosphoryloxy, R^{12} (wherein R^{12} is as hereinabove defined), -Y^5R^{20} [wherein Y^5 is -NHCO-; and R^{20} is C_{1-4}alkyl or R^{22} (wherein R^{22} is as hereinabove defined)].

More preferred optional substituents for alkyl, alkylY^4alkyl and phenyl groups include: fluoro, chloro, bromo, amino, methoxy, methoxycarbonylamino, acetyl, phosphoryloxy, R^{12} (wherein R^{12} is as hereinabove defined), - Y^5R^{20} [wherein Y^5 is -NHCO-; and R^{20} is methyl, ethyl or R^{22} (wherein R^{22} is as hereinabove and hereinbelow defined)].

Yet more preferably, optional substituents for alkyl, alkylY^4 alkyl and phenyl groups in R^{17} include fluoro, chloro and bromo. Most preferably alkyl and alkylY^4 alkyl groups in R^{17} are unsubstituted.

Preferably R^{21} is hydrogen.

Preferably R^{22} is optionally substituted: imidazolyl, pyridyl, pyrimidyl, thiazolyl or pyrazinyl.

More preferably R^{22} is optionally substituted: imidazolyl.

A preferred optional substituent for the aromatic heterocyclic group in R^{22} is C_{1-4}alkyl.

A more preferred optional substituent for the aromatic heterocyclic group in R^{22} methyl.

The aromatic heterocyclic group in R^{22} may be unsubstituted.

Preferably R^{23} and R^{24} are independently hydrogen or methyl.

More preferably R^{23} and R^{24} are hydrogen.

Preferably R^{25} and R^{26} are independently selected from hydrogen and methyl. More Preferably R^{25} and R^{26} are hydrogen.

Preferably R^{27} is C_{1-3}alkyl.

More preferably R^{27} is methyl.

Preferably R^{22}-C_{1-4} alkyl in group 3) of R^8 is R^{22}-methylene, R^{22}-propylene.

More preferably R^{22}- C_{1-4} alkyl is R^{22}- ethylene.

Preferably Y^7 is -$N(R^{23})C(O)$- or -$CON(R^{23})$-.

More preferably Y^7 is -$N(R^{23})C(O)$- or -$CON(R^{23})$-.

More preferably Y^7 is -NHC(O)- or -CONH-.

Preferably R^{17} is methyl, fluoromethyl, difluoromethyl or trifluoromethyl.

More preferably R^{17} is methyl.

Most preferably R^8 is acetyl.

Most preferably R^9 is hydrogen.

A preferred class of compound is of the formula (I) wherein:

R^1 , R^2, and R^3 are all C_{1-4}alkoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, C_{1-3} alkoxy, and C_{1-3}alkyl;

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

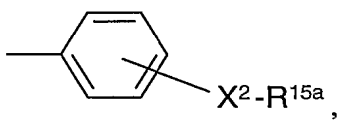
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, -$CON(R^{10})$-, -$N(R^{10})$ -, -$N(R^{10})CO$- or -$OC(O)N(R^{10})$-;

B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the -X^2-R^{15a} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 0, 1 and 2; and

R^{15a} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula $-(CH_2)_a-Y^2-(CH_2)_b-R^{15b}$, wherein:

a is 2 or 3;

b is 0, 1, or 2; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; and

R^{15b} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino, N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy; and

$\mathbf{R^8}$ is a group $-Y^3R^{17}$ (wherein Y^3 is -C(O)-, -C(O)O- or -C(O)NH-;

and $\mathbf{R^{17}}$ is selected from one of the following 4 groups:

1) hydrogen, C_{1-4}alkyl, phenyl or C_{1-4}alkylY^4C_{1-4}alkyl (wherein Y^4 is -NHCO- or -CONH-); [which alkyl, alkylY^4alkyl or phenyl group is optionally substituted by 1 or 2 substituents selected from:

halogeno, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, C_{1-4}alkoxy, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, phosphoryloxy, R^{12a} (wherein R^{12a} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl), $-Y^5-R^{20}$ [wherein Y^5 is -NHCO-; and R^{20} is C_{1-4}alkyl or R^{22a} (wherein R^{22a} is imidazolyl, pyridyl, pyrimidyl, thiazolyl or pyrazinyl, each of which is optionally substituted by C_{1-4}alkyl)];

2) R^{22a} (wherein R^{22a} is as hereinabove defined);

3) R^{22a} -C_{1-4}alkyl- (wherein R^{22a} is as hereinabove defined); or

4) $R^{12a}Y^7C_{1-4}$alkyl- (wherein R^{12a} is as hereinabove defined and Y^7 is Y^7 is -NHC(O)- or -CONH-)];

and $\mathbf{R^9}$ is hydrogen;

or a pharmaceutically-aceeptable salt, solvate or pro-drug thereof.

Another preferred class of compound is of the formula (I) wherein:

R^1 , R^2, and R^3 are all methoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, methoxy and methyl;

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

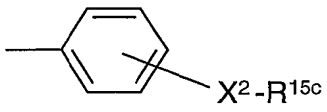
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, -CON(R^{10})-, -N(R^{10}) -, -N(R^{10})CO- or -OC(O)N(R^{10})-;

B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the -X^2-R^{15c} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 0, 1 and 2; and

R^{15c} is morpholinyl, piperazinyl or piperidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15d}, wherein:

a is 2 or 3;

b is 0 or 1; and

Y^2 is a single direct bond, -C(O)- or -NHC(O)-; and

R^{15d} is morpholinyl, piperazinyl or piperidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino, N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy; and

R^8 is a group -Y^3R^{17} (wherein Y^3 is -C(O)- or -C(O)O-;

and **R^{17}** is selected from one of the following 4 groups:

1) C_{1-4}alkyl [which alkyl, group is optionally substituted by 1 or 2 substituents selected from: fluoro, chloro and bromo;

2) R^{22b} (wherein R^{22b} is imidazolyl, pyridyl, pyrimidyl, thiazolyl or pyrazinyl, each of which is optionally substituted by C_{1-4}alkyl);

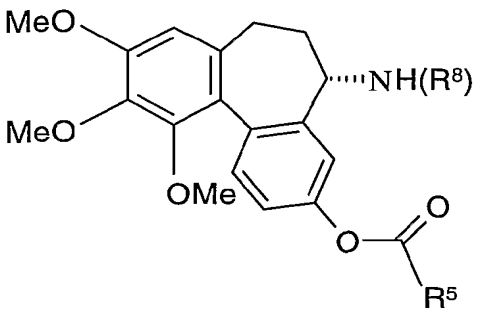
3) R^{22b} -C_{1-4}alkyl- (wherein R^{22b} is as hereinabove defined); or

4) $R^{12b}Y^7C_{1-4}$alkyl- (wherein R^{12b} is morpholinyl, piperidinyl or piperazinyl each of which is optionally substituted by methyl, ethyl, acetyl, propionyl, carbamoyl or 2-hydroxyethyl; and Y^7 is -NHC(O)- or -CONH-)];

and **R^9** is hydrogen;

or a pharmaceutically-aceeptable salt, solvate or pro-drug thereof.

A preferred compound of the present invention is of the formula (II):

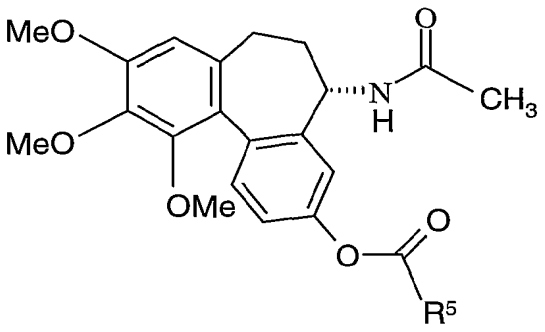


(II)

wherein R^5 and R^8 are as hereinabove defined;

or a pharmacetically-acceptable salt, solvate or pro-drug thereof.

Another preferred class of compounds is that of the formula (III) wherein:



(III)

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

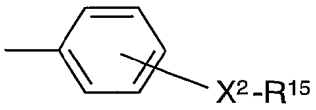
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, -CONH-, -NH-, -NHCO- or -OC(O)NH-;

B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the -X^2-R^{15} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 0, 1 and 2; and

R^{15} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted as immediately hereinabove defined for R^{12}, and substituted by at least 1 substituent selected from C_{2-4}alkanyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15}, wherein:

a is 2 or 3;

b is 0, 1, or 2; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino, N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Another preferred class of compounds is that of the formula (III) wherein:

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

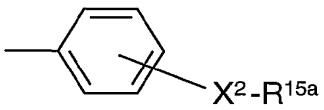
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, -NHCO-;

B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the -X^2-R^{15a} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 1 and 2; and

R^{15a} is as hereinabove defined;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15b}, wherein:

a is 2 or 3;

b is 0; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; and

R^{15b} is as hereinabove defined; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, hydroxy and phosphoryloxy;

or a pharmaceutically acceptable salt, solvate or pro-drug thereof.

Another preferred class of compounds is that of the formula (III) wherein:

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

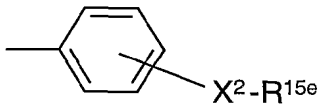
A is phenylene;

X^1 is -CO-, -NHCO-;

Y^1 is methylene or ethylene;

B is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group;

2) of the formula



wherein:

the -X^2-R^{15e} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 1; and

R^{15e} is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15f}, wherein:

a is 2 or 3;

b is 0; and

Y^2 is -C(O)-;

R^{15f} is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group; or

4) 2-[N,N-di(C_{1-4}alkyl)carbamoyl]ethyl- or 3-[N,N-di(C_{1-4}alkyl)carbamoyl]propyl-, wherein the C_{1-4}alkyl group is optionally substituted by 1 hydroxy group;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Yet another preferred class of compounds is that of the formula (III) wherein:

R^5 is 2-[N,N-di(C_{1-4}alkyl)carbamoyl]ethyl- or 3-[N,N-di(C_{1-4}alkyl)carbamoyl]propyl-, wherein the C_{1-4}alkyl group is optionally substituted by 1 hydroxy group;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Particular compounds of the present invention include:

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 5-(4-acetylpiperazin-l-yl)-5-oxopentanoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c}cyclohepten-3-yl 4-(4-acetylpiperazin-1-yl)-4-oxobutanoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-acetylpiperazin-1-ylmethyl)benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[3-(4-methylpiperazin-l-yl)propionylamino]benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-carbamoylpiperazin-1-ylmethyl)benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl N-acetylpiperidin-1-ylcarboxylate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-[N,N-di-(2-hydroxyethyl)carbamoyl]propanoate; and

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[N,N-di(2-hydroxyethyl)carbamoyl]butanoate;

and pharmaceutically-acceptable salts, solvates or pro-drugs thereof.

Synthesis of Compounds of the Formula I

Compounds of Formula I may be prepared by a number of processes as generally described herein below and more specifically in the Examples hereinafter. Processes for the preparation of novel compounds of formula I, are provided as a further feature of the invention and are as described hereinafter. Necessary starting materials may be obtained by standard procedures of organic chemistry. The preparation of such starting materials is described within the accompanying non-limiting Examples. Alternatively necessary starting materials are obtainable by analogous procedures to those illustrated which are within the ordinary skill of an organic chemist.

Thus according to another aspect of the invention, a compound of the formula (I) may be formed by deprotecting a compound of the formula (I) wherein at least 1 functional group is protected. For example, amino, hydroxy, carboxy or phosphoryloxy groups may be protected during the reaction sequence used to prepare a compound of the formula (I).

Protecting groups may in general be chosen from any of the groups described in the literature or known to the skilled chemist as appropriate for the protection of the group in question, and may be introduced by conventional methods.

Protecting groups may be removed by any convenient method as described in the literature or known to the skilled chemist as appropriate for the removal of the protecting group in question, such methods being chosen so as to effect removal of the protecting group with minimum disturbance of groups elsewhere in the molecule.

A suitable protecting group for a hydroxy group is, for example, an arylmethyl group (especially benzyl), a tri-(1-4C)alkylsilyl group (especially trimethylsilyl or tert-butyldimethylsilyl), an aryldi-(1-4C)alkylsilyl group (especially dimethylphenylsilyl), a diaryl-(1-4C)alkylsilyl group (especially tert-butyldiphenylsilyl), a (1-4C)alkyl group (especially methyl), a (2-4C)alkenyl group (especially allyl), a (1-4C)alkoxymethyl group (especially methoxymethyl) or a tetrahydropyranyl group (especially tetrahydroyran-2-yl). The deprotection conditions for the above protecting groups will necessarily vary with the choice of protecting group. Thus, for example, an arylmethyl group such as a benzyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-charcoal. Alternatively a trialkylsilyl or an aryldialkylsilyl group such as a tert-butyldimethylsilyl or a dimethylphenylsilyl group may be removed, for example, by treatment with a suitable acid such as hydrochloric, sulphuric, phosphoric or trifluoroacetic acid, or with an alkali metal or ammonium fluoride such as sodium fluoride or, preferably, tetrabutylammonium fluoride. Alternatively an alkyl group may be removed, for example, by treatment with an alkali metal (1-4C)alkylsulphide such as sodium thioethoxide or, for example, by treatment with an alkali metal diarylphosphide such as lithium diphenylphosphide or, for example, by treatment with a boron or aluminium trihalide such as boron tribromide. Alternatively a (1-4C)alkoxymethyl group or tetrahydropyranyl group may be removed, for example, by treatment with a suitable acid such as hydrochloric or trifluoroacetic acid.

Alternatively a suitable protecting group for a hydroxy group is, for example, an acyl group, for example a (2-4C)alkanoyl group (especially acetyl) or an aroyl group (especially benzoyl). The deprotection conditions for the above protecting groups will necessarily vary with the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl or an aroyl group may be removed, for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide.

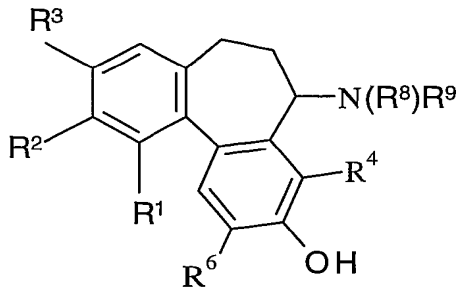
A suitable protecting group for an amino, imino or alkylamino group is, for example, an acyl group, for example a (2-4C)alkanoyl group (especially acetyl), a (1-4C)alkoxycarbonyl group (especially methoxycarbonyl, ethoxycarbonyl or tert-butoxycarbonyl), an arylmethoxycarbonyl group (especially benzyloxycarbonyl) or an aroyl group (especially benzoyl). The deprotection conditions for the above protecting groups necessarily vary with the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl, alkoxycarbonyl or aroyl group may be removed for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide. Alternatively an acyl group such as a tert-butoxycarbonyl group may be removed, for example, by treatment with a suitable acid such as hydrochloric, sulphuric or phosphoric acid or trifluoroacetic acid, and an arylmethoxycarbonyl group such as a benzyloxycarbonyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-charcoal.

A suitable protecting group for a carboxy group is, for example, an esterifying group, for example a (1-4C)alkyl group (especially methyl or ethyl) which may be removed, for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide; or, for example, a tert-butyl group which may be removed, for example, by treatment with a suitable acid such as hydrochloric, sulphuric or phosphoric acid or trifluoroacetic acid.

In the following process description (the symbols R^1-R^7, A, B, D, R^a, R^b, a and b are to be understood to represent those groups described above in relation to formulae (I), (II) and (III) unless otherwise stated.

A compound of the formula (I), or a compound of the formula (I) wherein at least 1 functional group is protected, may be prepared using one of the following processes:

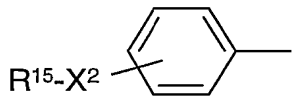
(a) reacting a compound of the formula (X):



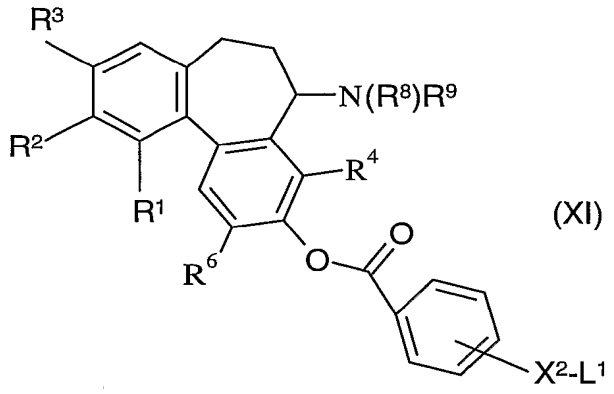
(X)

with a compound of the formula R^5-COOH or an activated derivative thereof;

(b) when R^5 is of the formula:



reacting a compound of the formula (XI):



(XI)

with R^{15} (wherein L^1 is a leaving group) ;

(c) introducing substituents onto a ring nitrogen atom in R^{12} or R^{15};

(d) converting one compound of the formula (I) into another compound of the formula (I);

(e) when a phosphoryloxy group is desired, reacting the corresponding hydroxy compound with a phosphoramidite;

wherein any functional groups are optionally protected.

and thereafter if necessary:

i) converting a compound of formula (I) into another compound of formula (I);

ii) removing any protecting groups;

iii) forming a pharmaceutically acceptable salt, solvate or pro-drug thereof.

The reaction between a compound of the formula (X) and a compound of the formula R^5-COOH or an activated derivative thereof is performed under standard coupling conditions. For example, in the presence of a coupling agent such as dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, and optionally a base, for example an organic base such as triethylamine or DMAP. The reaction is conveniently carried out in a solvent such as an aprotic solvent, for example dimethylformamide, or in a chlorinated solvent, for example trichloromethane or dichloromethane, and at a temperature in the range of about -30°C to about 60°C. Conveniently at or near ambient temperature.

When X^2 is -(CH_2)r, the reaction between a compound of the formula (XI) and R^{15} is conveniently carried out in an inert organic solvent such as acetonitrile, in a temperature range of 0°C to 60°C, normally at ambient temperature. Suitable leaving groups (L^1) include halogeno, mesyloxy and tosyloxy. Preferably halogeno, and particularly chloro or iodo.

When X^2 is -CO-, L^1 is usually chloro and the reaction is normally carried out in a chlorinated solvent such as dichloromethane. The reaction is carried out in the presence of a base such as triethylamine and in a temperature range of 0 to 60°C, normally about ambient temperature.

Substituents, such as C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl and alkylated carbamoyl groups, can be in introduced onto a ring nitrogen atom in R^{12} or R^{15}, using standard conditions known in the art for alkylation and acylation of an amino group. Alkylations are normally carried out by reacting a ring-nitrogen containing R^{12} or R^{15} with the appropriate alkylating agent, such as an alkyl halide, an alkyl toluenesulphonate, an alkyl methanesulphonate or an alkyl triflate. The alkylation reaction can be carried out in the presence of a base for example an inorganic base such as a carbonate e.g. caesium or potassium carbonate, a hydride such as sodium hydride or an alkoxide such as potassium tert-butoxide in a suitable solvent such as an aprotic solvent e.g. dimethylformamide or an ether solvent such as tetrahydrofuran at a temperature of around -10°C to 80°C.

Acylation of a ring nitrogen in R^{12} or R^{15} is carried out by reacting the saturated heterocyclic ring with an acylating agent, for example, an acyl halide or anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for example, a solvent such as a hydrocarbon solvent e.g. dichloromethane, at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

A carbamoyl group can be introduced by reacting the saturated heterocyclic ring with a tri(alkyl)silyl isocyanate in an inert organic solvent such as dichloromethane.

A compound of formula I may also be prepared from another compound of formula I by chemical modification. Examples of such chemical modifications include standard alkylation, arylation, heteroarylation, acylation, sulphonylation, phosphorylation, aromatic halogenation and coupling reactions. These reactions may be used to add new substituents or to modify existing substituents. Alternatively, existing substituents in compounds of formula I may be modified by, for example, oxidation, reduction, elimination, hydrolysis or other cleavage reaction to yield other compounds of formula I. For example a substituent can be introduced onto a ring nitrogen atom in R^{12} using similar processes to those described above, for the alkylation or acylation of a ring nitrogen.

In another general example an alkoxy group may be cleaved to the corresponding hydroxy group by reaction with boron tribromide, in a solvent such as a chlorinated solvent e.g. dichloromethane, at a low temperature e.g. around -78°C.

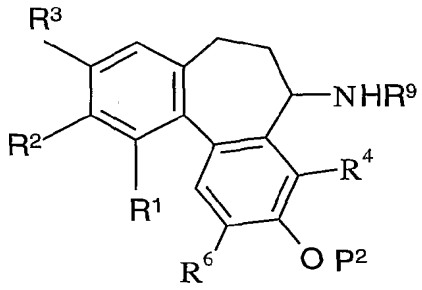
A amino group can be alkylated or acylated using similar reaction conditions to those described above for alkylation or acylation of a ring nitrogen atom in R^{12} or R^{15}.

A compound containing a hydroxy group can be converted into the corresponding phosphoryloxy compound by treatment with for example di-tert-butyl diisopropylphoramidite or di-tert-butyl diethylphosphoramidite, in the presence of a suitable catalyst for example tetrazole. A solvent, such as an ether solvent, for example tetrahydrofuran can be used at a temperature in the range of -40°C to 40°C, conveniently at or near ambient temperature, followed by treatment with an oxidising agent, such as 3-chloroperoxy benzoic acid. The reaction is carried out at a temperature in the range -78°C to 40°C, preferably -40°C to 10°C. The resulting intermediate phosphate triester is treated with an acid for example trifluoroacetic acid in a solvent such as a chlorinated solvent e.g. dichloromethane at a temperature in the range -30°C to 40°C conveniently at or near 0°C to give the phosphoryloxy compound.

Synthesis of Intermediates

A compound of the formula (X) may be known in the art or may be prepared from by

(f) reacting a compound of the formula (XII):



(XII)

wherein P^2 is an hydroxy-protecting group, with a compound of the formula L^2-R^8 , wherein L^2 is a leaving group;

The reaction between a compound of the formula (XII) and a compound of the formula L^2-R^8 is conveniently performed under standard acylation or sulphonylation conditions. L^1 is usually halogeno, for example chloro or bromo, hydroxy, mesyloxy or tosyloxy or an 'activated' hydroxy group. The precise conditions depending largely upon the nature of R^8.

For example, when Y^3 is -CO-, L^2 may be hydroxy and the reaction is normally carried out in the presence of coupling agent such as dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide. Optionally, a base may be used, for example an organic base such as triethylamine. Suitable solvents are usually aprotic solvents, for example dimethylformamide, or chlorinated solvents, for example trichloromethane or dichloromethane. The temperature is usually in the range of about -30°C to about 60°C, conveniently at or near ambient temperature.

When Y^3 is -C(O)O-, L^2 is usually an 'activated' hydroxy group. That is a group which acts as a leaving group in the same way as hydroxy, but is more labile. It can be formed in situ. An example, of an activated hydroxy group is 4-nitrophenoxy, in which case the compound R^8-L^2 can be formed by reacting a hydroxy group (R^{17}-OH) with 4-nitrophenylchloroformate. The reaction is usually carried out in an organic solvent such as dichloromethane, acetonitrile or tetrahydrofuran, in a temperature range of about -20°C to the reflux temperature of the solvent. In addition an organic base such as triethylamine or N-methylmorpholine is normally present. Alternatively, a compound of the formula (XII) can be reacted with 4-nitrophenylchloroformate and the resulting intermediate reacted with R^{17}-OH under similar conditions to those described above for the reaction of a compound of the formula (XII) with a compound of the formula R^8-L^2 wherein L^2 is 4-nitrophenoxy.

When Y^3 is -CON(R^{18})-, L^2 is preferably halogeno, particularly chloro. Alternatively when -A- is -CONH-, a compound of the formula (XII) can be reacted with an isocyanate of

the formula $C\equiv N\text{-}R^{17}$. These reactions are conveniently carried out in the presence of a base, particularly an organic base, such as triethylamine, pyridine or N-methylmorpholine, triethylamine, pyridine or N-methylmorpholine in a solvent such as an ether solvent for example tetrahydrofuran or in a chlorinated solvent for example dichloromethane at a temperature in the range from about -20°C to the reflux temperature of the solvent. Alternatively, a compound of the formula (XII) can be reacted with 4-nitrophenylchloroformate and the resulting intermediate reacted with $R^{17}\text{-}NH_2$ under similar conditions to those described above for the reaction of a compound of the formula (XII) with a compound of the formula $R^8\text{-}L^2$ wherein L^2 is 4-nitrophenoxy.

When $-X^1-$ is of the formula $-SO_2N(R^8)-$, L^2 is preferably halogeno, for example chloro. The reaction is conveniently carried out in the presence of a base such as dimethylaniline, in a chlorinated solvent such as trichloromethane and at a temperature in the range from about -20°C to about 60°C. More preferably in pyridine, at a temperature in the range from about -20°C to about 60°C. A compound of the formula (XI) may be prepared by reacting a compound of the formula (X) with a compound of $L^1\text{-}X^2\text{-phenyl-COOH}$ in which L^1 is protected or a precursor of L^1 is used, using similar conditions to those described for the formation of a compound of the formula (I) from a compound of the formula (X).

L^1 can be deprotected or the precursor converted to L^1 following the coupling with the compound of the formula (X).

A compound of the formula (XII) can be formed from a compound of the formula (X) or (XI) wherein R^8 is hydrogen, using similar conditions to those described above for the formation of a compound of the formula (I).

Compounds of the formula $R^5\text{-COOH}$ may be known in the art or prepared by methods known in the art or analogous to those illustrated in the specific examples. The following description gives some general processes for preparing some compounds of the formula $R^5\text{-COOH}$.

When R^5 is of the formula $-A\text{-}X^1Y^1\text{-}B$, and X^1 is $-N(R^{10})CO-$ or $-CON(R^{10})-$ then a compound of the formula $R^5\text{-COOH}$ can be formed by reacting a compound of the formula $P^1OOC\text{-}A\text{-}NHR^{19}$, wherein P^1 is a carboxy-protecting group, with a compound of the formula $HOOC\text{-}Y^1\text{-}B$, or of the formula $P^1OOC\text{-}A\text{-}COOH$ with a compound of the formula $NH(R^{10})\text{-}Y^1\text{-}B$, under standard amide forming conditions. Similarly when X^1 is of the

formula $-N(R^{10})SO^2-$ or $SO^2N(R^{10})-$ a compound of the formula R^5-COOH can be formed by reacting the appropriate amine and sulphonyl chloride under conditions known for the formation of a sulphonamide. Likewise a compound of the formula R^5-COOH wherein X^1 is -C(O)O- can be formed by reacting together the appropriate carboxylic acid and alcohol and when X^1 is $-N(R^{10})C(O)O-$, by reacting together the appropriate amine and ROC(O)OR compound.

When R^5 is of the formula R^{15}-X^2-phenyl, a compound of the formula R^5-COOH can be formed by reacting together the appropriate L^1-$(CH_2)_r$- or L^1-CO-substituted benzoic acid, wherein the carboxylic group in the benzoic acid is protected during the course of the reaction, under similar conditions to that described for the reaction between a compound of the formula (XI) with R^{15}.

When R^5 is of the formula $-(CH_2)a-Y^2-(CH_2)b$ and $-R^{15}$ a is 2 or 3 and b is 0, a compound of the formula R^5-COOH can be formed by reacting R^{15} with succinic anhydride or glutaric anhydride, as appropriate. The reaction is normally carried out in an inert organic solvent such as dichloromethane, in a temperature range of 0° to 60°C, usually around ambient temperature.

When R^5 is N,N-di-(C_{1-4}alkyl)carbamoylC_{1-4}alkyl and C_{1-4}alkyl is ethyl or propyl, a compound of the formula R^5-COOH can be formed by reacting the $HN(C_{1-4}alkyl)_2$ compound with succinic anhydride or glutaric anhydride as appropriate. The reaction is normally carried out in an inert organic solvent such as dichloromethane, in a temperature range of 0° to 60°C, usually around ambient temperature.

Acid addition salts of the compounds of formula I are prepared in a conventional manner by treating a solution or suspension of the free base I with about one equivalent of a pharmaceutically acceptable acid. Salts of compounds of formula I derived from inorganic or organic bases are prepared in a conventional manner by treating a solution or suspension of the free acid I with about one equivalent of a pharmaceutically acceptable organic or inorganic base. Alternatively both acid addition salts and salts derived from bases may be prepared by treatment of the parent compound with the appropriate ion-exchange resin in a standard fashion. Conventional concentration and recrystallistion techniques are employed in isolating the salts.

Compounds according to the invention are able to destroy vasculature that has been newly formed such as tumour vasculature while leaving unaffected normal, mature

vasculature. The identification of compounds which selectively, and preferably potently, damage newly-formed vasculature is desirable and is the subject of the present invention. The ability of the compounds to act in this way may be assessed, for example, using one or more of the procedures set out below:

(a) Activity against tumour vasculature measured by radioactive tracer

This assay demonstrates the ability of compounds to damage selectively tumour vasculature.

Subcutaneous CaNT tumours were initiated by injecting 0.05ml of a crude tumour cell suspension, approximately 10^6 cells, under the skin overlying the rear dorsum of 12-16 week-old mice. The animals were selected for treatment after approximately 3-4 weeks, when their tumours reached a geometric mean diameter of 5.5-6.5 mm. Compounds were dissolved in sterile saline and injected intraperitoneally in a volume of 0.1 ml per 10g body weight. Tumour perfusion was measured 6 hours after intraperitoneal administration in tumour, kidney, liver, skin, muscle, gut and brain by the ^{86}RbCl extraction technique (Sapirstein, Amer. Jnl. Physiol., 1958, 193, 161-168). Tissue radioactivity measured 1 minute after an intravenous injection of ^{86}RbCl was used to calculate relative blood flow as a proportion of cardiac output (Hill and Denekamp, Brit. Jnl. Radiol., 1982, 55, 905-913). Five animals were used in control and treated groups. Results were expressed as a percentage of the blood flow in the corresponding tissues in vehicle treated animals.

(b) Activity against tumour vasculature measured by fluorescent dye

This assay demonstrates the ability of compounds to damage tumour vasculature.

Tumour functional vascular volume in CaNT tumour-bearing mice was measured using the fluorescent dye Hoechst 33342 according to the method of Smith et al (Brit. Jnl. Cancer 1988, 57, 247-253). Five animals were used in control and treated groups. The fluorescent dye was dissolved in saline at 6.25mg/ml and injected intravenously at 10mg/kg 24 hours after intraperitoneal drug treatment. One minute later, animals were killed and tumours excised and frozen; 10μm sections were cut at 3 different levels and observed under UV illumination using an Olympus microscope equipped with epifluorescence. Blood vessels were identified by their fluorescent outlines and vascular volume was quantified using a point scoring system based on that described by Chalkley, (Jnl. Natl. Cancer Inst., 1943, 4, 47-53). All estimates were based on counting a minimum of 100 fields from sections cut at the 3 different levels.

The ability of the compounds to bind to preparations of mammalian tubulin can be evaluated by a number of methods available in the literature, for example by following temperature initiated tubulin polymerisation by turbidity in the absence and presence of the compound (for example O.Boye *et al* Med. Chem. Res., 1991, 1, 142-150).

The activity of *N*-[3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide, (V. Fernholz Justus Liebigs Ann., 1950, 568, 63-72), against tumour vasculature was measured by the fluorescent dye method described above. This compound decreased perfused vascular volume by 88% relative to control when dosed at 50mg/kg intraperitoneally. The IC_{50} of this compound in a tubulin polymerisation assay was 58 micromolar (O.Boye *et al* Med. Chem. Res., 1991, 1, 142-150).

(c) HUVEC detachment assay

This assay examined the effects of compounds on the adherence of HUVECs to tissue culture plasticware.

HUVECs were plated in 0.2% gelatin-coated 12 well tissue culture plates at a concentration of $3x10^4$ cells per well in 1ml TCS medium. After 24 hours, when the cells were at ~30% confluency, the cells were dosed with compound for 40 minutes at 37°C, 5% CO_2. After this incubation the medium containing drug was pipetted off, and the cells were then gently washed in 2mls of HBSS (Hanks' Balanced Salt Solution purchased from Life Technologies Ltd, Paisley UK; Catalogue # 24020-083) to remove any detached cells. The washing solution was then removed, and the adherent cells remaining were trypsinised using 300µl of 1x Trypsin-EDTA solution (Life Technologies Ltd, Paisley, UK; Catalogue # 43500-019) at ambient temperature for 2 minutes. The trypsinised cells were then made up to 1ml with TCS Biologicals medium, then centrifuged at 2000rpm for 2 minutes. The cell pellet was then resuspended in a volume of 50µl of TCS Biologicals medium. Total cell counts were obtained by counting the cells on a haemocytometer. The amount of cell detachment was calculated by comparing the number of cells remaining attached following treatment with the number in undosed control wells.

(d) Hras5 necrosis model

NIH 3T3 fibroblasts transfected with Harvey ras, clone 5, (Hras5 cells) were kept in continual passage in Dulbecco's modifed Eagles medium (DMEM) containing 10% foetal bovine serum (FBS) and 1% glutamine, at 37°C in a humidified incubator gassed with 7.5%

carbon dioxide and 92.5% oxygen. Cells were implanted subcutaneously into the left flank of male nude mice (8-10weeks of age) at an inoculum of 2 x 10^5 cells/mouse. Tumours were measured using calipers and randomised into groups of 2-4 mice between days 9-14 after implant. Mice were dosed with compounds, either intravenously or intraperitoneally, once on day of randomisation and culled 24 hours after dosing. Compounds were dissolved in 20% hydroxypropyl beta cyclodextrin in physiological saline at pH 7 and dosed in a volume of 0.1ml per 10g body weight. Tumours were excised, weighed and placed in buffered formalin. Area of necrosis in individual tumours was assessed from a haematoxylin/eosin stained-slide by a pathologist and scored from 0, meaning no significant change, to 10, meaning 91-100% necrosis. The activity of examples 5 and 7 (described hereinafter) against tumour vasculature was measured by the fluorescent dye method described hereinabove. Example 1 scored 6.6 at 25mg/kg.

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises a compound of the formula I as defined hereinbefore or a pharmaceutically acceptable salt, solvate or pro-drug thereof, in association with a pharmaceutically acceptable excipient or carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for nasal administration or administration by inhalation, for example as a powder or solution, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) for example as a sterile solution, suspension or emulsion, for topical administration for example as an ointment or cream or for rectal administration for example as a suppository. In general the above compositions may be prepared in a conventional manner using conventional excipients.

The compositions of the present invention are advantageously presented in unit dosage form. The compound will normally be administered to a warm-blooded animal at a unit dose within the range 5-5000mg per square metre body area of the animal, i.e. approximately 0.1-100mg/kg. A unit dose in the range, for example, 1-100mg/kg, preferably 1-50mg/kg is envisaged and this normally provides a therapeutically-effective dose. A unit dose form such as a tablet or capsule will usually contain, for example 1-250mg of active ingredient.

As stated above the size of the dose required for the therapeutic or prophylactic treatment of a particular disease state will necessarily be varied depending on the host treated,

the route of administration and the severity of the illness being treated. Preferably a daily dose in the range of 1-50mg/kg is employed. However the daily dose will necessarily be varied depending upon the host treated, the particular route of administration, and the severity of the illness being treated. Accordingly the optimum dosage may be determined by the practitioner who is treating any particular patient.

According to a further aspect of the present invention there is provided a compound of the formula I or a pharmaceutically acceptable salt, solvate or pro-drug thereof as defined hereinbefore for use in a method of treatment of the human or animal body by therapy.

A further feature of the present invention is a compound of formula I, or a pharmaceutically acceptable salt, solvate or pro-drug thereof, for use as a medicament, conveniently a compound of formula I, or a pharmaceutically acceptable salt, solvate or pro-drug thereof, for use as a medicament for producing a vascular damaging effect in a warm-blooded animal such as a human being.

Thus according to a further aspect of the invention there is provided the use of a compound of the formula I, or a pharmaceutically acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm-blooded animal such as a human being.

According to a further feature of the invention there is provided a method for producing a vascular damaging effect in a warm-blooded animal, such as a human being, in need of such treatment which comprises administering to said animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt, solvate or pro-drug thereof as defined hereinbefore.

According to a further aspect of the present invention there is provided a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition, when dosed in divided doses (also known as split doses) produces a greater anti-tumour effect than when a single dose is given.

Anti-tumour effects of a method of treatment of the present invention include but are not limited to, inhibition of tumour growth, tumour growth delay, regression of tumour, shrinkage of tumour, increased time to re-growth of tumour on cessation of treatment, slowing of disease progression. It is expected that when a method of treatment of the present invention is administered to a warm-blooded animal such as a human, in need of treatment for cancer involving a solid tumour, said method of treatment will produce an effect, as measured

by, for example, one or more of: the extent of the anti-tumour effect, the response rate, the time to disease progression and the survival rate.

According to a further aspect of the present invention there is provided a method for the production of a vascular damaging effect in a warm-blooded animal such as a human, which comprises administering to said animal in divided doses an effective amount of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition.

According to a further aspect of the present invention there is provided a method for the treatment of a cancer involving a solid tumour in a warm-blooded animal such as a human, which comprises administering to said animal in divided doses an effective amount of a compound of formula (I) or pharmaceutically-acceptable salt or solvate thereof, preferably in the form of a pharmaceutical composition.

According to a further aspect of the present invention there is provided a medicament comprising two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition, which together add up to a total daily dose, for administration in divided doses for use in a method of treatment of a human or animal body by therapy.

According to a further aspect of the present invention there is provided a kit comprising two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition, which together add up to a total daily dose, for administration in divided doses.

According to a further aspect of the present invention there is provided a kit comprising:

a) two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, which together add up to a total daily dose, in unit dosage forms for administration in divided doses; and

b) container means for containing said dosage forms.

According to a further aspect of the present invention there is provided a kit comprising:

a) two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, which together add up to a total daily dose, together with a pharmaceutically acceptable excipient or carrier, in unit dosage forms; and

b) container means for containing said dosage forms.

According to a further aspect of the present invention there is provided the use of compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for administration in divided doses for use in the production of a vascular damaging effect in a warm-blooded animal such as a human.

According to a further aspect of the present invention there is provided the use of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for administration in divided doses for use in the production of an anti-cancer effect in a warm-blooded animal such as a human.

According to a further aspect of the present invention there is provided the use of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for administration in divided doses for use in the production of an anti-tumour effect in a warm-blooded animal such as a human.

Divided doses, also called split doses, means that the total dose to be administered to a warm-blooded animal, such as a human, in any one day period (for example one 24 hour period from midnight to midnight) is divided up into two or more fractions of the total dose and these fractions are administered with a time period between each fraction of about greater than 0 hours to about 10 hours, preferably about 1 hour to about 6 hours, more preferably about 2 hours to about 4 hours. The fractions of total dose may be about equal or unequal.

Preferably the total dose is divided into two parts which may be about equal or unequal.

The time intervals between doses may be for example selected from:

about 1 hour, about 1.5 hours, about 2 hours, about 2.5 hours, about 3 hours, about 3.5 hours, about 4 hours, about 4.5 hours, about 5 hours, about 5.5 hours and about 6 hours.

The time intervals between doses may be any number (including non-integers) of minutes between greater than 0 minutes and 600 minutes, preferably between 45 and 375 minutes inclusive. If more than two doses are administered the time intervals between each dose may be about equal or unequal.

Preferably two doses are given with a time interval in between them of greater than or equal to 1 hour and less than 6 hours.

More preferably two doses are given with a time interval in between them of greater than or equal to two hours and less than 5 hours.

Yet more preferably two doses are given with a time interval in between them of greater than or equal to two hours and less than or equal to 4 hours.

Particularly the total dose is divided into two parts which may be about equal or unequal with a time interval between doses of greater than or equal to about two hours and less than or equal to about 4 hours.

More particularly the total dose is divided into two parts which may be about equal with a time interval between doses of greater than or equal to about two hours and less than or equal to about 4 hours.

For the avoidance of doubt the term 'about' in the description of time periods means the time given plus or minus 15 minutes, thus for example about 1 hour means 45 to 75 minutes, about 1.5 hours means 75 to 105 minutes. Elsewhere the term 'about' has its usual dictionary meaning.

The antiangiogenic treatment defined hereinbefore may be applied as a sole therapy or may involve, in addition to a compound of the invention, one or more other substances and/or treatments. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate administration of the individual components of the treatment. In the field of medical oncology it is normal practice to use a combination of different forms of treatment to treat each patient with cancer. In medical oncology the other component(s) of such conjoint treatment in addition to the antiangiogenic treatment defined hereinbefore may be: surgery, radiotherapy or chemotherapy. Such chemotherapy may include the following categories of therapeutic agent:

(i) other antiangiogenic agents that work by different mechanisms from those defined hereinbefore (for example linomide, inhibitors of integrin $\alpha v\beta 3$ function, angiostatin, endostatin, razoxin, thalidomide) and including vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitors (RTKIs) (for example those described in International Patent Applications Publication Nos. WO 97/22596, WO 97/30035, WO 97/32856 and WO 98/13354 the entire disclosure of which documents is incorporated herein by reference);

(ii) cytostatic agents such as antioestrogens (for example tamoxifen,toremifene, raloxifene, droloxifene, iodoxyfene), progestogens (for example megestrol acetate), aromatase inhibitors (for example anastrozole, letrazole, vorazole, exemestane), antiprogestogens, antiandrogens (for example flutamide, nilutamide, bicalutamide, cyproterone acetate), LHRH agonists and antagonists (for example goserelin acetate, luprolide), inhibitors of testosterone 5α-

dihydroreductase (for example finasteride), anti-invasion agents (for example metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator receptor function) and inhibitors of growth factor function, (such growth factors include for example epidermal growth factor (EGF), platelet derived growth factor and hepatocyte growth factor such inhibitors include growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors and serine/threonine kinase inhibitors);

(iii) biological response modifiers (for example interferon);

(iv) antibodies (for example edrecolomab); and

(v) antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as antimetabolites (for example antifolates like methotrexate, fluoropyrimidines like 5-fluorouracil, purine and adenosine analogues, cytosine arabinoside); antitumour antibiotics (for example anthracyclines like doxorubicin, daunomycin, epirubicin and idarubicin, mitomycin-C, dactinomycin, mithramycin); platinum derivatives (for example cisplatin, carboplatin); alkylating agents (for example nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide, nitrosoureas, thiotepa); antimitotic agents (for example vinca alkaloids like vincristine and taxoids like taxol, taxotere); enzymes (for example asparaginase); thymidylate synthase inhibitors (for example raltitrexed); topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan, irinotecan).

As stated above the compounds defined in the present invention are of interest for their vascular damaging effects. Such compounds of the invention are expected to be useful in the prophylaxis and treatment of a wide range of disease states where inappropriate angiogenesis occurs including cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation, endometriosis, dysfunctional uterine bleeding and ocular diseases with retinal vessel proliferation. In particular such compounds of the invention are expected to slow advantageously the growth of primary and recurrent solid tumours of, for example, the colon, breast, prostate, lungs and skin.

In addition to their use in therapeutic medicine, the compounds of formula I and their pharmaceutically acceptable salts, solvates and pro-drugs are also useful as pharmacological tools in the development and standardisation of in vitro and in vivo test systems for the evaluation of the effects of vascular damaging agents in laboratory animals

such as cats, dogs, rabbits, monkeys, rats and mice, as part of the search for new therapeutic agents.

It is to be understood that where the term "ether" is used anywhere in this specification it refers to diethyl ether.

The invention will now be illustrated in the following non-limiting Examples in which, unless otherwise stated:

(i) evaporations were carried out by rotary evaporation in vacuo and work-up procedures were carried out after removal of residual solids such as drying agents by filtration;

(ii) operations were carried out at ambient temperature, that is in the range 18-25°C and under an atmosphere of an inert gas such as argon or nitrogen;

(iii) yields are given for illustration only and are not necessarily the maximum attainable;

(iv) the structures of the end-products of the formula I were confirmed by nuclear (generally proton) magnetic resonance (NMR) and mass spectral techniques; proton magnetic resonance chemical shift values were measured on the delta scale and peak multiplicities are shown as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; q, quartet, quin, quintet;

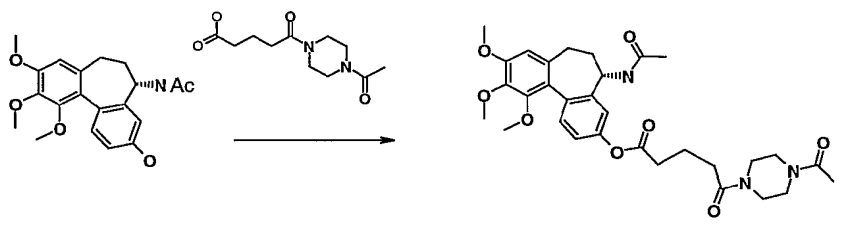
(v) intermediates were not generally fully characterised and purity was assessed by thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), infra-red (IR) or NMR analysis;

Abbreviations

4-Dimethylaminopyridine	DMAP
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride	EDCI
Dimethyl sulphoxide	DMSO
Trifluoroacetic acid	TFA

Example 1

(5S)-5-Acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 5-(4-acetylpiperazin-1-yl)-5-oxopentanoate



A solution of 5-(4-acetylpiperazin-1-yl)-5-oxopentanoic acid (0.308 g ; 1.27 mmol), EDCI (0.244 g ; 1.27 mmol), DMAP (0.036 g ; 0.29 mmol) in dichloromethane (30 ml) was stirred under argon atmosphere for 30 minutes. N-acetyl colchinol [International Patent Application No. PCT/GB98/01977] (0.350 g ; 0.98 mmol) was then added and the mixture was stirred overnight. After evaporation to dryness, the residue was purified by flash chromatography, eluting with dichloromethane/methanol (95/5) to give the title compound after evaporation of the appropriate fractions and trituration in ether / pentane.

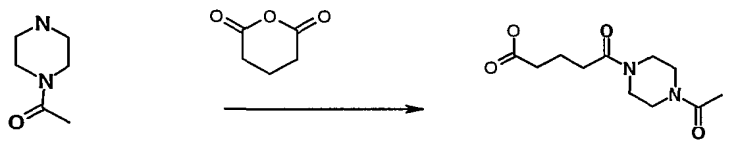
Yield : 82 %

^{1}H NMR (DMSO-d_6) : 1.45-1.50 (m, 1H) ; 1.51-1.75 (m, 1H) ; 1.87 (s, 3H) ; 1.79-1.94 (m, 1H) ; 1.94-2.11 (m, 2H) ; 2.02 (s, 3H) ; 2.10-2.24 (m, 1H) ; 2.52-2.62 (m, 1H, signal partially obscured by DMSO peak) ; 2.74-2.85 (m, 1H) ; 2.88-2.98 (m, 1H) ; 3.14-3.24 (m, 1H) ; 3.28-3.33 (m, 1H) ; 3.51 (s, 3H) ; 3.78 (s, 3H) ; 3.78-3.89 (m, 1H) ; 3.85 (s, 3H) ; 4.24-4.33 (m, 1H) ; 4.49-4.59 (m, 1H) ; 6.80 (s, 1H) ; 7.07 (s, 1H) ; 7.09 (dd, 1H) ; 7.35 (d, 1H) ; 3.39 (d, 1H).

MS-ESI : 582 $[MH]^+$

Elemental analysis	Found	C 62.54	H 6.92	N 6.93
$C_{31}H_{39}N_3O_8$, 0.8 H_2O	Requires	C 62.47	H 6.87	N 7.05

The starting material as follows :



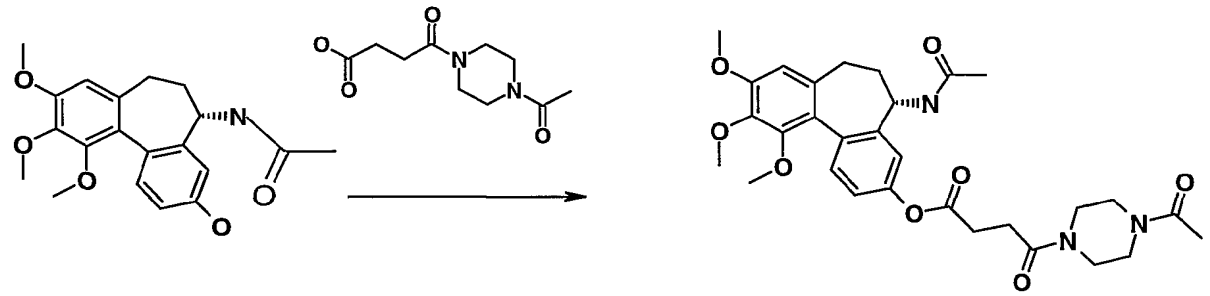
A solution of glutaric anhydride (1.6 g ; 14 mmol) and N-acetylpiperazine (1.5 g ; 12 mmol) in dichloromethane (20 ml) was stirred overnight. The resulting precipitate was filtered, washed with ether and dried to give 5-(4-acetylpiperazin-l-yl)-5-oxopentanoic acid as a white solid.

Yield : 83 %

^{1}H NMR ($CDCl_3$) : 1.98 (m, 2H) ; 2.13 (s, 3H) ; 2.46 (m, 4H) ; 3.47 (m, 4H) ; 3.64 (m, 4H).

Example 2

(5S)-5-Acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c}cyclohepten-3-yl 4-(4-acetylpiperazin-1-yl)-4-oxobutanoate



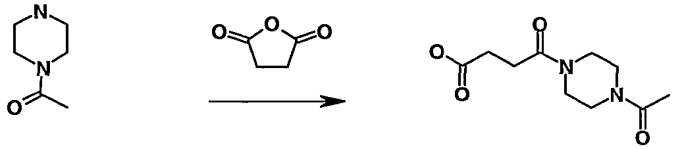
The compound was prepared using a similar method to that described in Example 1, but using 4-(4-acetylpiperazin-1-yl)-4-oxobutanoic acid in place of 5-(4-acetylpiperazin-l-yl)-5-oxopentanoic acid.

Yield : 67 %

^{1}H NMR (DMSO-d_6) : 1.81-1.93 (m, 1H) ; 1.86 (s, 3H) ; 1.98-2.10 (m, 1H) ; 2.02 (d, 3H) ; 2.11-2.23 (m, 1H) ; 2.52-2.59 (m, 1H, signal partially obscured by DMSO peak) ; 2.71-2.85 (m, 4H) ; 3.27-3.54 (m, 8H) ; 3.51 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.47-4.58 (m, 1H) ; 6.80 (s, 1H) ; 7.04 (dd, 1H) ; 7.06 (d, 1H) ; 7.34 (d, 1H) ; 8.41 (d, 1H).

MS-ESI : 568 $[MH]^+$

The starting material was prepared as follows:



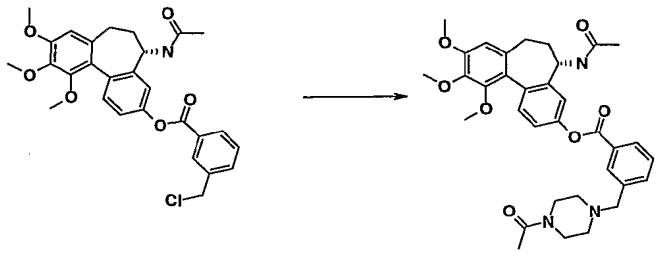
A solution of succinic anhydride (1.72 g ; 17 mmol) and N-acetyl piperazine (2 g ; 15.6 mmol) in dichloromethane (40 ml) was stirred overnight. After evaporation to dryness, the residue was triturated in ether / pentane to give 4-(4-acetylpiperazin-1-yl)-4-oxobutanoic acid as a solid.

Yield : 95 %

^{1}H NMR ($CDCl_3$) : 2.13 (s, 3H) ; 2.70 (m, 4H) ; 3.48 (m, 4H) ; 3.66 (m, 4H).

Example 3

(5S)-5-Acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-acetylpiperazin-1-ylmethyl)benzoate



A solution of (5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-chloromethylbenzoate (0.408 g ; 0.8 mmol), N-acetylpiperazine (0.144 g ; 1.12 mmol) and sodium iodide (0.06 g ; 0.4 mmol) in acetonitrile (6 ml) was stirred under argon atmosphere at room temperature overnight. After evaporation to dryness, the mixture was purified by flash chromatography eluting with dichloromethane/ethanol (92/8) to give the title compound as a white solid.

Yield : 69 %

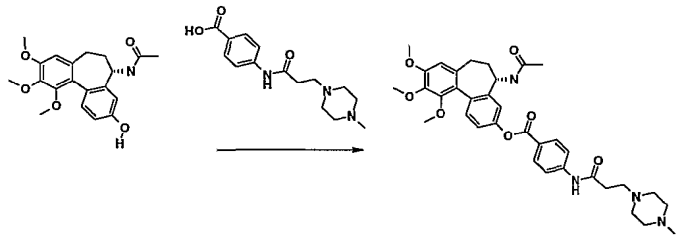
^{1}H NMR (DMSO-d_6) : 1.81-1.97 (m, 1H) ; 1.87 (s, 3H) ; 1.99 (s, 3H) ; 2.04-2.28 (m, 2H) ; 2.28-2.38 (m, 2H) ; 2.39-2.48 (m, 2H) ; 2.48-2.55 (m, 1H, signal partially obscured by DMSO peak) ; 3.42-3.51 (m, 4H) ; 3.56 (s, 3H) ; 3.64 (s, 2H) ; 3.81 (s, 3H) ; 3.87 (s, 3H) ; 4.54-4.67 (m, 1H) ; 6.84 (s, 1H) ; 7.25 (s, 1H) ; 7.26 (dd, 1H) ; 7.43 (d, 1H) ; 7.52 (dd, 1H) ; 7.72 (d, 1H) ; 8.10 (d, 1H) ; 8.12 (s, 1H) ; 8.40 (d, 1H).

MS-ESI : 602 $[MH]^+$

Elemental analysis	Found	C 67.00	H 6.76	N 6.81
$C_{34}H_{39}N_3O_7$, 0.3 H_2O	Requires	C 67.27	H 6.57	N 6.92

Example 4

(5S)-5-Acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[3-(4-methylpiperazin-1-yl)propionylamino]benzoate



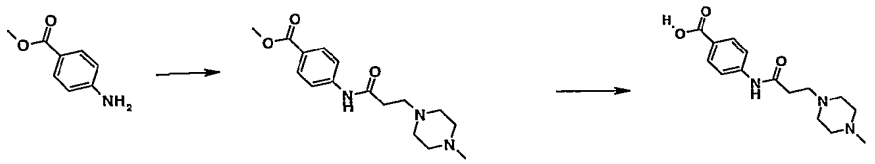
The title compound was prepared using a similar method to that in Example 1 but replacing 5-(4-acetylpiperazin-1-yl)-5-oxopentanoic acid by 4-{3-(4-methylpiperazin-1-yl)propionylamino]benzoic acid.

Yield : 55 %

^{1}H NMR (DMSO-d_6) : 1.78-2.75 (m, 14H) ; 1.88 (s, 3H) ; 2.17 (s, 3H) ; 2.64 (t, 2H) ; 3.56 (s, 3H) ; 3.81 (s, 3H) ; 3.83 (s, 3H) ; 4.51-4.65 (m, 1H) ; 6.82 (s, 1H) ; 7.19-7.24 (m, 2H) ; 7.40 (d, 1H) ; 7.82 (d, 2H) ; 8.12 (d, 2H) ; 8.39 (d, 1H) ; 10.53 (s, 1H).

MS-ESI : 631 $[MH]^+$

The starting material was prepared as follows:



A solution of methyl 4-aminobenzoate (0.76 g ; 5 mmol), EDCI (1.25 g ; 6.5 mmol), DMAP (0.13 g ; 1 mmol), 3-(4-methylpiperazin-1-yl)propanoic acid (1.49 g ; 7.5 mmol) and triethylamine (1.05 ml ; 7.5 mmol) in dichloromethane (20 ml) was stirred under argon atmosphere for 2 days. The mixture was extracted with ethyl acetate, evaporated and purified by flash chromatography eluting with dichloromethane/methanol to give methyl 4-[3-(4-methylpiperazin-1-yl) propionylamino]benzoate.

Yield : 46 %

^{1}H NMR (DMSO-d_6) : 2.14 (s, 3H) ; 2.07-2.70 (m, 10H) ; 2.62 (t, 2H) ; 3.82 (s, 3H) ; 7.71 (d, 2H) ; 7.91 (d, 2H) ; 10.41 (s, 1H).

MS-ESI : 306 $[MH]^+$

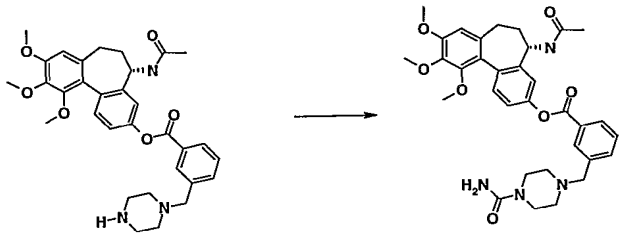
A solution of methyl 4-[3-(4-methylpiperazin-1-yl) propionylamino]benzoate (0.69 g ; 2.26 mmol) in methanol (10 ml) was treated with 2N sodium hydroxide solution (1.25 ml ; 2.48 mmol) at 60°C for 6 hours. After evaporation to dryness, the residue was triturated in acetone. The insoluble material was taken-up in water and the pH adjusted to 6.5 with 2N HCl. After evaporation, the residue was triturated in acetone to give 4-[3-(4-methylpiperazin-1-yl)propionylamino]benzoic acid as a solid.

Yield : 98 %.

^{1}H NMR (DMSO-d_6) : 2.17 (s, 3H) ; 2.10-2.70 (m, 10H) ; 2.64 (t, 2H) ; 7.69 (d, 2H) ; 7.88 (d, 2H).

Example 5

(5S)-5-Acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-carbamoylpiperazin-1-ylmethyl)benzoate



A solution (5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(piperazin-1-ylmethyl)benzoate (0.2 g ; 0.357 mmol) and trimethylsilyl isocyanate (0.290 ml ; 2.14 mmol) in dichloromethane (3 ml) was stirred at ambient temperature overnight. After evaporation to dryness, the residue was purified by flash chromatography, eluting with dichloromethane/ethanol (85/15) to give the title compound.

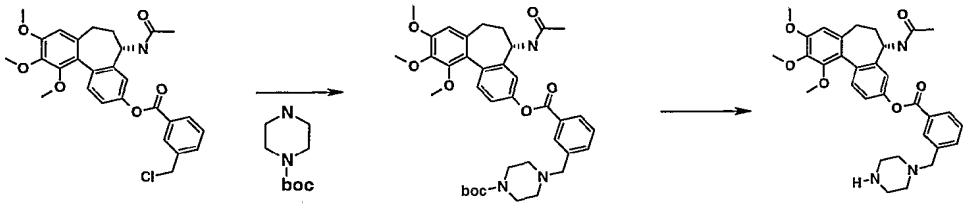
Yield : 87 %

^{1}H NMR (DMSO-d_6 : 1.80-1.96 (m, 1H) ; 1.88 (s, 3H) ; 2.00-2.28 (m, 2H) ; 2.35 (m, 4H) ; 2.52-2.59 (m, 1H, signal partially obscured by DMSO peak) ; 3.30 (m, 4H) ; 3.56 (s, 3H) ;

3.63 (s, 2H) ; 3.81 (s, 3H) ; 3.87 (s, 3H) ; 4.55-4.54 (m, 1H) ; 5.94 (s, 2H) ; 6.82 (s, 1H) ; 7.23 (s, 1H) ; 7.25 (dd, 1H) ; 7.41 (d, 1H) ; 7.59 (t, 1H) ; 7.70 (d, 1H) ; 8.07 (d, 1H) ; 8.10 (s, 1H) ; 8.39 (d, 1H).

MS-ESI : 603 $[MH]^+$

The starting material was prepared as follows :



A solution of (5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-chloromethylbenzoate (0.714 g ; 1.4 mmol), N-tert-butoxycarbonyl piperazine (0.417 g ; 2.24 mmol) and sodium iodide (0.21 g ; 1.4 mmol) in dichloromethane (20 ml) was stirred at 45°C, under argon atmosphere for 24 hours. After evaporation to dryness, the residue was purified by flash chromatography eluting with dichloromethane/ethanol (95/5) to give (5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-tert-butoxycarbonylpiperazin-l-ylmethyl)benzoate.

Yield: 55%

^1H NMR (DMSO-d_6) : 1.39 (s, 9H) ; 1.81-1.96 (m, 1H) ; 1.86 (s, 3H) ; 2.02-2.26 (m, 2H) ; 2.36 (t, 4H) ; 2.53-2.60 (m, 1H, signal partially obscured by DMSO peak) ; 3.34 (m, 8H) ; 3.54 (s, 3H) ; 3.61 (s, 2H) ; 3.80 (s, 3H) ; 3.85 (s, 3H) ; 4.53-4.64 (m, 1H) ; 6.82 (s, 1H) ; 7.23 (s, 1H) ; 7.24 (dd, 1H) ; 7.41 (d, 1H) ; 7.59 (t, 1H) ; 7.70 (d, 1H) ; 8.07 (d, 1H) ; 8.10 (s, 1H) ; 8.38 (d, 1H).

MS-ESI: 660 $[MH]^+$

A solution of (5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-tert-butoxycarbonylpiperazin-1-ylmethyl)benzoate (0.711 g; 1.07 mmol) in dichloromethane (15 ml) was treated with 2.5N HCl / ether (3.5 ml) at ambient temperature for 1 hour. After evaporation, the residue was taken-up in water, the pH was adjusted to 5 with 2N sodium hydroxide solution and the solution purified on reverse phase silica, eluting with a gradient of 40-50 % methanol / ammonium carbonate buffer (2 g/l

pH 7) to give (5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(piperazin-l-ylmethyl)benzoate.

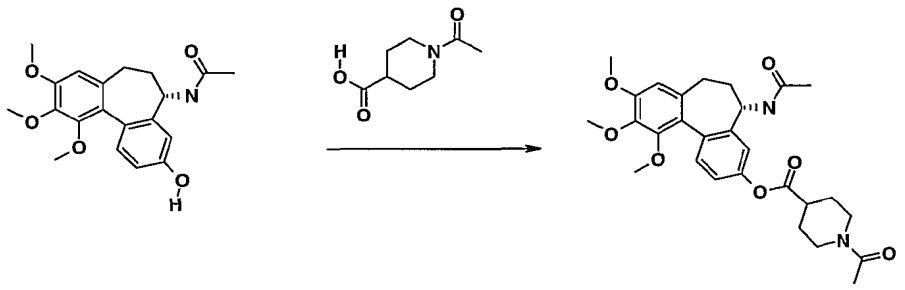
Yield : 55 %

^{1}H NMR (DMSO-d_6) : 1.82 -1.97 (m, 1H) ; 1.88 (s, 3H) ; 2.02-2.26 (m, 2H) ; 2.33 (bs, 4H) ; 2.51-2.60 (m, 1H) ; 2.71 (m, 4H) ; 3.56 (s, 3H) ; 3.82 (s, 3H) ; 3.87 (s, 3H) ; 4.54-4.64 (m, 1H) ; 6.84 (s, 1H) ; 7.24 (s, 1H) ; 7.25 (dd, 1H) ; 7.41 (d, 1H) ; 7.58 (t, 1H) ; 7.69 (d, 1H) ; 8.05 (d, 1H) ; 8.08 (s, 1H) ; 8.38 (d, 1H).

MS-ESI : 560 $[MH]^+$

Example 6

(5S)-5-Acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl N-acetylpiperidin-1-ylcarboxylate



The title compound was prepared using a similar method to that described in Example 1, but 4-acetylpiperidin-1-ylcarboxylic acid was used in place of 5-(4-acetylpiperazin-l-yl)-5-oxopentanoic acid :

Yield : 79 %

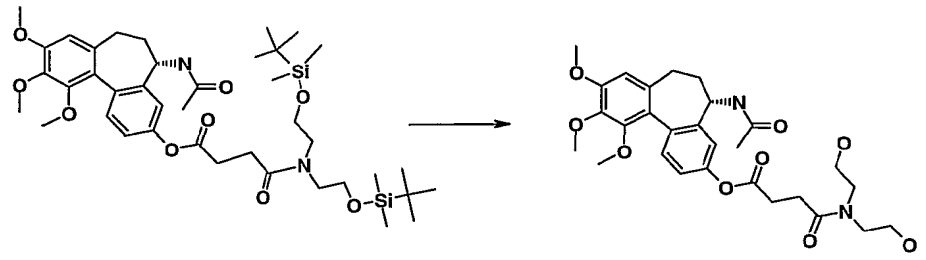
^{1}H NMR (DMSO-d_6) : 1.45-1.60 (m, 1H) ; 1.61-1.75 (m, 1H) ; 1.88-1.93 (m, 2H) ; 1.87 (s, 3H) ; 1.94-2.09 (m, 2H) ; 2.02 (s, 3H) ; 2.10-2.24 (m, 1H) ; 2.53-2.60 (m , 1H, signal partially obscured by DMSO peak) ; 2.75-2.85 (m, 1H) ; 2.88-2.98 (m, 1H) ; 3.14-3.25 (m, 1H) ; 3.26 (bs, 1H) ; 3.50 (s, 3H) ; 3.78 (s, 3H) ; 3.81 (bs, 1H) ; 3.84 (s, 3H) ; 4.23-4.33 (m, 1H) ; 4.49-4.59 (m, 1H) ; 6.80 (s, 1H) ; 7.06 (s, 1H) ; 7.08 (dd, 1H) ; 7.35 (d, 1H) ; 8.39 (d, 1H).

MS-ESI : 511 $[MH]^+$

Elemental analysis	Found	C 64.65	H 6.85	N 5.43
$C_{28}H_{33}N_2O_7$, 0.5 H_2O	Requires	C 64.85	H 6.61	N 5.40

Example 7

(5S)-5-Acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-[N,N-di-(2-hydroxyethyl)carbamoyl]propanoate



A 2.4 N solution of sulphuric acid in methanol (4 ml) was added at 3°C under argon atmosphere, to a solution of (5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-{N-N-di-[2-(tert-butyldimethylsilyloxy)ethyl]carbamoyl} propanoate (0.7 g ; 0.906 mmol) in methanol (15 ml). After stirring at 3° C for 45 mins, a mixture of ethyl acetate (100 ml) and saturated aqueous sodium hydrogen carbonate solution (75 ml) was added. The organic phase was washed with water, dried and was purified by flash chromatography eluting dichloromethane/methanol (92/8) to give the title compound.

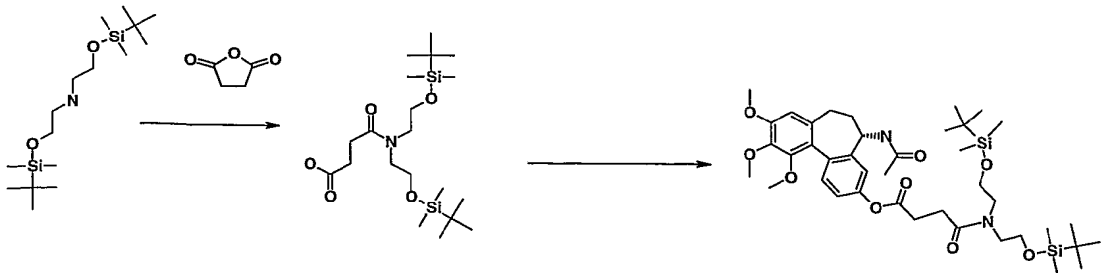
Yield : 66 %

^{1}H NMR (DMSO-d_6) : 1.81-1.96 (m, 1H) ; 1.89 (s, 3H) ; 1.98-2.56 (m, 2H) ; 2.55-2.65 (m, 1H, signal partially obscured by DMSO peak) ; 2.80 (bs, 4H) ; 4.37-4.63 (m, 8H) ; 3.53 (s, 3H) ; 3.80 (s, 3H) ; 3.86 (s, 3H) ; 4.49-4.60 (m, 1H) ; 4.69 (t, 1H) ; 4.88 (t, 1H) ; 6.80 (s, 1H) ; 7.02-7.07 (m, 2H) ; 7.34 (d, 1H). 8.40 (d, 1H).

MS-ESI : 545 $[MH]^+$

Elemental analysis	Found	C 59.27	H 6.62	N 4.89
$C_{28}H_{36}N_2O_9$, 1.2 H_2O	Requires	C 59.40	H 6.84	N 4.95

The starting material was prepared as follows :



Succinic anhydride (2.64 g ; 0.026 mol) was added under argon atmosphere to a solution of N-N-di-[2-(tert-butyldimethylsilyloxy)ethyl]amino [Synthesis (19997), 6, 643-648] (8 g ; 0.024 mol) in dichloromethane. The mixture was stirred at ambient temperature overnight. After filtration of the insoluble, the filtrate was evaporated and dried to give 3-{N,N-di[2-(tert-butyldimethylsilyloxy)ethyl] carbamoyl}propanoic acid.

Yield : 96 %

^{1}H NMR ($CDCl_3$) : 0.04 (s, 6H) ; 0.05 (s, 6H) ; 0.87 (s, 9H) ; 0.88 (s, 9H) ; 2.62-2.69 (m, 2H) ; 2.83-2.90 (m, 2H) ; 3.52 (t, 2H) ; 3.58 (t, 2H) ; 3.75 (t, 2H) ; 3.78 (t, 2H).

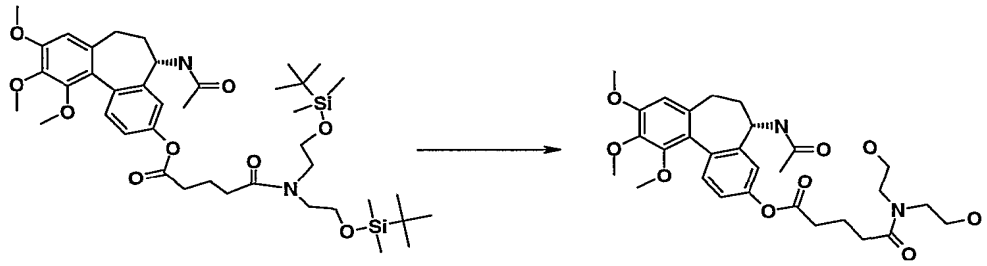
A solution of 3-{N,N-di[2-(tert-butyldimethylsilyloxy)ethyl]carbamoyl}propanoic acid (0.552 g ; 1.27 mmol), EDCI (0.244 g ; 1.27 mmol), DMAP (0.036 g ; 0.294 mmol) in dichloromethane (30 ml) was stirred under argon atmosphere for 30 mins. N-acetyl colchinol (0.35 g ; 0.98 mmol) was added and the mixture was stirred at ambient temperature overnight. After evaporation to dryness, the residue was purified by flash chromatography, eluting with ethyl acetate/ petroleum ether (70/30) to give 5(S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3{N-N-di[2-(tert-butyl-dimethylsilyloxy]ethyl}carbamoyl}propanoate.

Yield : 74 %

^{1}H NMR (DMSO-d_6) : 0.03 (s, 6H) ; 0.04 (s, 6H) ; 0.86 (s, 9H) ; 0.87 (s, 9H) ; 1.83-1.94 (m, 1H) ; 1.80 (s, 3H) ; 2.00-2.11 (m, 1H) ; 2.12-2.24 (m, 1H) ; 2.53-2.60 (m 1H, signal partially obscured by DMSO peak) ; 2.72-2.78 (m, 2H) ; 2.78-2.86 (m, 2H) ; 3.42 (t, 2H) ; 3.52 (s, 3H) ; 3.53 (t, 2H) ; 3.57 (t, 2H) ; 3.75 (t, 2H) ; 3.80 (s, 3H) ; 3.85 (s, 3H) ; 4.50-4.60 (m, 1H) ; 6.81 (s, 1H) ; 7.03 (dd, 1H) ; 7.07 (d, 1H) ; 7.34 (d, 1H) ; 8.41 (d, 1H).

Example 8

(5S)-5-Acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[N,N-di(2-hydroxyethyl)carbamoyl]butanoate



The title compound was prepared using a similar method to that described in Example 7, but using (5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c}cyclohepten-3-yl 4-N,N-di[2-(tert-butyldimethylsilyloxy)ethyl]carbamoyl}butanoate in place of (5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3{N,N-di[2-(tert-butyl-dimethylsilyloxy)ethyl]carbamoyl}propanoate.

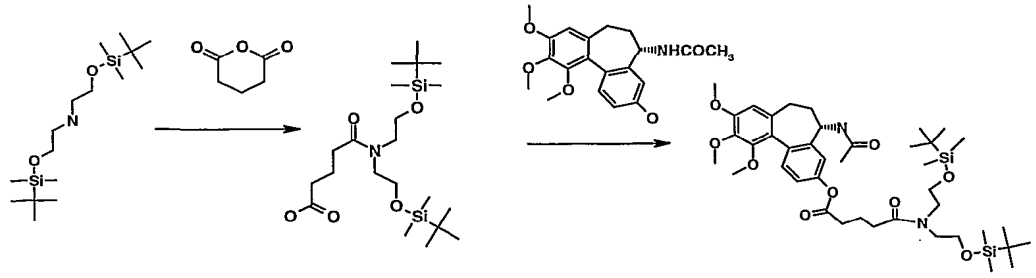
Yield : 37 %

^{1}H NMR (DMSO-d_6) : 1.83-1.92 (m, 2H) ; 1.87 (s, 3H) ; 1.98-2.10 (m, 1H) ; 2.11-2.22 (m, 1H) ; 2.48 (t , 2H, signal partially obscured by DMSO peak) ; 2.52-2.56 (m, 1H, signal partially obscured by DMSO peak) ; 2.63 (t, 2H) ; 3.35 (t, 2H) ; 3.41 (t, 2H) ; 3.44-3.50 (m, 2H) ; 3.50-3.57 (m, 2H) ; 3.51 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.50-4.60 (m, 1H) ; 4.67 (t, 1H) ; 4.85 (t, 1H) ; 6.80 (s, 1H) ; 7.07 (s, 1H) ; 7.09 (dd, 1H) ; 7.34 (d, 1H). 8.38 (d, 1H).

MS - ESI : 581 $[MNa]^+$

Elemental analysis	Found	C 61.22	H 7.21	N 4.89
$C_{29}H_{38}N_2O_9$, 0.5 H_2O	Required	C 61.36	H 6.93	N 4.94

The starting material was prepared as follows :



A solution of glutaric anhydride (0.753 g ; 6.6 mmol) and N,N-di{2-(tert-butyldimethylsilyloxy)ethyl]amino (2 g ; 6.6 mmol) in dichloromethane (40 ml) was stirred overnight. After evaporation to dryness the residue was triturated in pentane to give a solid which was filtered. The filtrate was evaporated to give 4-{N,N-di[2-(tert-butyldimethylsilyloxy)ethyl]carbamoyl}butanoic acid as an oil.

Yield : 95 %

^{1}H NMR (DMSO-d_6) : 0.03 (s, 6H) ; 0.04 (s, 6H) ; 0.86 (s, 9H) ; 0.87 (s, 9H) ; 1.70 (m, 2H) ; 2.23 (t, 2H) ; 2.38 (t, 2H) ; 3.38 (t, 2H) ; 3.46 (t, 2H) ; 3.65 (t, 2H) ; 3.70 (t, 2H).

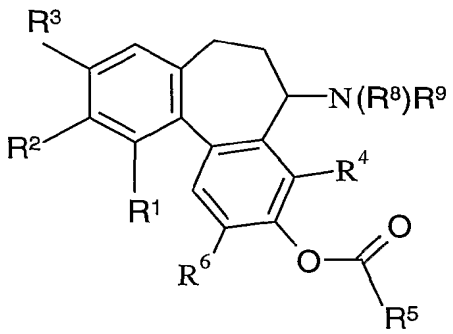
4-{N,N-di[2-(tert-Butyldimethylsilyloxy)ethyl]carbamoyl}butanoic acid was reacted with N-acetyl colchinol using the similar conditions to that in Example 7, but using 4-{N,N-di[2-(tert-butyldimethylsilyloxy)ethyl]carbamoyl}butanoic acid in place of 3-{N,N-di[2-(tert-butyldimethylsilyloxy)ethyl]carbamoyl}propanoic acid.

Yield : 92 %

^{1}H NMR (DMSO-d_6) : 0.04 (s, 12H) ; 0.86 (s, 9H) ; 0.87 (s, 9H) ; 1.83-1.94 (m, 2H) ; 1.88 (s, 3H) ; 2.00-2.11 (m, 1H) ; 2.12-2.24 (m, 1H) ; 2.48 (t , 2H, signal partially obscured by DMSO peak) ; 2.51-2.54 (m, 1H, signal partially obscured by DMSO peak) ; 2.65 (t, 2H) ; 3.41 (t, 2H) ; 3.50 (t, 2H) ; 3.53 (s, 3H) ; 3.64-3.76 (m, 2H) ; 3.80 (s, 3H) ; 3.86 (s, 3H) ; 4.51-4.61 (m, 1H) ; 6.81 (s, 1H) ; 7.08 (dd, 1H) ; 7.09 (d, 1H) ; 7.36 (d, 1H) ; 7.99 (s, 1H) ; 8.40 (d, 1H).

Claims

1. A compound of the formula I:



(I)

wherein:

R^1, R^2 and **R^3** are each independently hydroxy, phosphoryloxy ($-OPO_3H_2$), C_{1-4}alkoxy or an in vivo hydrolysable ester of hydroxy; with the proviso that at least two of R^1, R^2 and R^3 are C_{1-4}alkoxy;

R^4 and **R^6** are each independently selected from: hydrogen, nitro, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, fluoro, C_{1-4}alkoxy and C_{1-4}alkyl;

R^5 is selected from one of the following groups:

1) of the formula -A -X^1-Y^1 -B, wherein:

A is C_{1-4}alkylene or $-(CH_2)_p$-Q- (wherein **p** is 0, 1 or 2 and **Q** is phenylene or thienylene);

X^1 is -O-, -CO-, -C(O)O-, $-CON(R^{10})$-, $-N(R^{10})$-, $-N(R^{10})CO$-, $N(R^{10})C(O)O$-, $-N(R^{10})CON(R^{11})$-, $-N(R^{10})SO_2$-, $-SO_2N(R^{10})$- or $OC(O)N(R^{10})$- (wherein **R^{10}** is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

Y^1 is C_{1-3}alkylene;

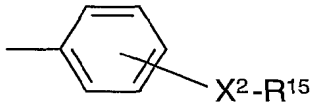
B is carboxy, sulpho, phosphoryloxy, hydroxy, amino, N-(C_{1-4}alkyl)amino, N,N-di (C_{1-3}alkyl)amino, $-R^{12}$ or $-NHC(R^{13})COOH$; (wherein **R^{12}** is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N,

which heterocyclic group is optionally substituted by 1 or 2 substituents selected from: oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, aminoC_{1-4}alkyl, N,N-di(C_{1-4}alkyl)amino-C_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein $\mathbf{R^{14}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkoxy-C_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl);

$\mathbf{R^{13}}$ is an amino acid side chain;

2) of the formula:



wherein:

the phenyl ring is substituted by $-\mathbf{X^2}-\mathbf{R^{15}}$ in the 3- or 4-position;

$\mathbf{X^2}$ is -CO- or of the formula $-(CH_2)_r-$ (wherein **r** is 0, 1, 2 or 3) and

$\mathbf{R^{15}}$ is a 5-6 membered saturated heterocyclic group (linked via a ring carbon or nitrogen atom) containing 1 or 2 ring heteroatoms selected from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, C_{1-4}aminoalkyl, N,N-di(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein $\mathbf{R^{14}}$ is as hereinabove defined); provided that the heterocyclic group (R^{15}) is substituted by at least one substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N,N-di(C_{1-4}alkyl)carbamoyl;

3) -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15}

(wherein **a** is 0, 1, 2, 3 or 4; **b** is 0, 1, 2, 3 or 4; $\mathbf{Y^2}$ is a direct bond, -O-, -C(O)-, -N(R^{16})-, -N(R^{16})C(O)- or -C(O)N(R^{16})- (wherein $\mathbf{R^{16}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and wherein 1 or 2 of the $(CH_2)_a$ or $(CH_2)_b$ groups are optionally substituted by 1 or 2 substituents selected from hydroxy and amino and $\mathbf{R^{15}}$ is as hereinabove defined; provided that when a is 0, then Y^2 is a single direct bond;

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-

(wherein the alkyl groups are independently optionally substituted by 1 or 2 substituents selected from: amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkanoyl, carboxy, sulpho and phosphoryloxy);

provided that:

a) when A is C_{1-4}alkylene and X^1 is of the formula -CO-, -N(R^{10})-, -N(R^{10})CO- or -CON(R^{10})- then when B is R^{12}, $R^{12}$is defined hereinabove for R^{15} ;

b) when A is C_{1-4}alkylene and X^1 is of the formula -N(R^{10})CO-, -CON(R^{10})-, or -C(O)O-, then B is not carboxy;

c) when A is C_{1-4}alkylene and X^1 is -CONH- or -NHCO-, then B is not carboxy, hydroxy, phosphoryloxy, amino, N-C_{1-4}alkylamino or N,N-di-C_{1-4}alkylamino;

$\mathbf{R^8}$ is a group -Y^3R^{17}

(wherein $\mathbf{Y^3}$ is a direct bond, -C(O)-, -C(O)O-, -N(R^{18})-, -C(O)N(R^{18})-, -SO_2- or -SO_2NR^{18}- (wherein $\mathbf{R^{18}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and $\mathbf{R^{17}}$ is selected from one of the following 4 groups:

1) hydrogen, C_{1-4}alkyl, phenyl, C_{1-4}alkylY^4C_{1-4}alkyl (wherein $\mathbf{Y^4}$ is -C(O)-, -NR^{19}C(O)- or -C(O)NR^{19}- (wherein $\mathbf{R^{19}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl));

[which alkyl, alkylY^4alkyl or phenyl group is optionally substituted by 1 or 2 substituents selected from: halogeno, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, carboxy, -CON(R^{23})R^{24} (wherein $\mathbf{R^{23}}$ **and** $\mathbf{R^{24}}$ are independently selected from hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl and C_{1-3}alkoxyC_{2-3}alkyl), C_{1-4}alkoxy, C_{1-4}alkoxycarbonylamino,

C_{1-4}alkanoyl, sulpho, phosphoryloxy, R^{12} (wherein $\mathbf{R^{12}}$ is as hereinabove defined), and a group $\mathbf{-Y^5R^{20}}$ [wherein $\mathbf{Y^5}$ is $-NR^{21}C(O)-$ or $-OC(O)-$ (wherein $\mathbf{R^{21}}$ represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and $\mathbf{R^{20}}$ is C_{1-4}alkyl or a group R^{22} (wherein $\mathbf{R^{22}}$ is a 5 or 6 membered aromatic heterocyclic group containing 1 to 4, inclusive, ring heteroatoms selected independently from O, N and S, which aromatic heterocyclic group is optionally substituted by 1 or 2 substituents selected from hydroxy, amino, C_{1-4}alkyl, aminoC_{1-4}alkyl, N-C_{1-4}alkylaminoC_{1-4}alkyl, N, N-di(C_{1-4}alkyl)aminoC_{1-4}alkyl, carboxy, $-CONR^{25}R^{26}$ and $-NR^{25}COR^{27}$ (wherein $\mathbf{R^{25}}$ **and** $\mathbf{R^{26}}$, which may be the same or different, are hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl and $\mathbf{R^{27}}$ is C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))];

2) R^{22} (wherein R^{22} is as hereinabove defined);

3) R^{22} -C_{1-4}alkyl- (wherein R^{22} is as hereinabove defined); or

4) $R^{12}Y^7C_{1-4}$alkyl- (wherein R^{12} is as hereinabove defined and Y^7 is -C(O)-, $-NR^{23}C(O)-$, $-NR^{23}C(O)C_{1-4}$alkyl-, $-C(O)NR^{23}-$ or $-C(O)NR^{23}C_{1-4}$alkyl- (wherein R^{23} is as hereinabove defined))];

and $\mathbf{R^9}$ is hydrogen or C_{1-3}alkyl;

or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

2. A compound according to claim 1 where R^1, R^2 and R^3 are all methoxy or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

3. A compound according to claim 1 wherein:

R^1 , R^2, and R^3 are all C_{1-4}alkoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, C_{1-3} alkoxy, and C_{1-3}alkyl;

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

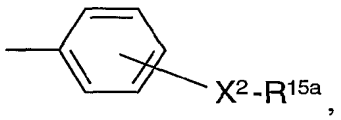
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, $-CON(R^{10})-$, $-N(R^{10})-$, $-N(R^{10})CO-$ or $-OC(O)N(R^{10})-$;

B is carboxy sulpho, phosphoryloxy or of the formula $-R^{12}$ (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the $-X^2-R^{15a}$ substituent is in the 3, or 4-position of the phenyl ring;

X^2 is $-(CH_2)_r-$;

r is 0, 1 and 2; and

R^{15a} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula $-(CH_2)_a-Y^2-(CH_2)_b-R^{15b}$, wherein:

a is 2 or 3;

b is 0, 1, or 2; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; and

R^{15b} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino,

N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy; and

$\mathbf{R^8}$ is a group $-Y^3R^{17}$ (wherein Y^3 is -C(O)-, -C(O)O- or -C(O)NH-; and

$\mathbf{R^{17}}$ is selected from one of the following 4 groups:

1) hydrogen, C_{1-4}alkyl, phenyl or C_{1-4}alkylY^4C_{1-4}alkyl (wherein Y^4 is -NHCO- or -CONH-); [which alkyl, alkylY^4alkyl or phenyl group is optionally substituted by 1 or 2 substituents selected from: halogeno, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, C_{1-4}alkoxy, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, phosphoryloxy, R^{12a} (wherein R^{12a} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl), $-Y^5-R^{20}$ [wherein Y^5 is -NHCO-; and R^{20} is C_{1-4}alkyl or R^{22a} (wherein R^{22a} is imidazolyl, pyridyl, pyrimidyl, thiazolyl or pyrazinyl, each of which is optionally substituted by C_{1-4}alkyl)];

2) R^{22a} (wherein R^{22a} is as hereinabove defined);

3) R^{22a} -C_{1-4}alkyl- (wherein R^{22a} is as hereinabove defined); or

4) $R^{12a}Y^7C_{1-4}$alkyl- (wherein R^{12a} is as hereinabove defined and Y^7 is Y^7 is -NHC(O)- or -CONH-)];

and $\mathbf{R^9}$ is hydrogen;

or a pharmaceutically-aceeptable salt, solvate or pro-drug thereof.

4. A compound according to claim 1 wherein:

R^1 , R^2, and R^3 are all methoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, methoxy and methyl;

R^5 is selected from one of the following groups:

1) of the formula $-A-X^1-Y^1-B$, wherein:

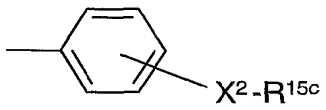
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, $-CON(R^{10})-$, $-N(R^{10})$ -, $-N(R^{10})CO-$ or $-OC(O)N(R^{10})-$, wherein R^{10} is as defined in claim 1;

B is carboxy sulpho, phosphoryloxy or of the formula $-R^{12}$ (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the $-X^2-R^{15c}$ substituent is in the 3, or 4-position of the phenyl ring;

X^2 is $-(CH_2)_r-$;

r is 0, 1 and 2; and

R^{15c} is morpholinyl, piperazinyl or piperidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula $-(CH_2)_a-Y^2-(CH_2)_b-R^{15d}$, wherein:

a is 2 or 3;

b is 0 or 1; and

Y^2 is a single direct bond, -C(O)- or -NHC(O)-; and

R^{15d} is morpholinyl, piperazinyl or piperidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino, N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy; and

$\mathbf{R^8}$ is a group $-Y^3R^{17}$ (wherein Y^3 is -C(O)- or -C(O)O-; and

$\mathbf{R^{17}}$ is selected from one of the following 4 groups:

1) C_{1-4}alkyl [which alkyl, group is optionally substituted by 1 or 2 substituents selected from: fluoro, chloro and bromo;

2) R^{22b} (wherein R^{22b} is imidazolyl, pyridyl, pyrimidyl, thiazolyl or pyrazinyl, each of which is optionally substituted by C_{1-4}alkyl);

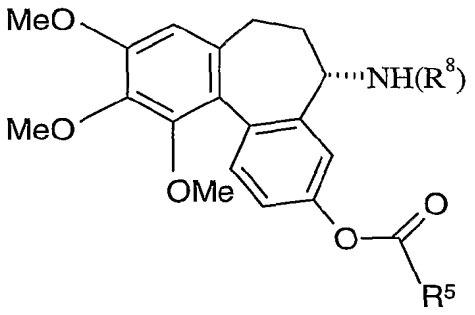
3) R^{22b} -C_{1-4}alkyl- (wherein R^{22b} is as hereinabove defined); or

4) $R^{12b}Y^7C_{1-4}$alkyl- (wherein R^{12a} is morpholinyl, piperidinyl or piperazinyl each of which is optionally substituted by methyl, ethyl, acetyl, propionyl, carbamoyl or 2-hydroxyethyl; and Y^7 is -NHC(O)- or -CONH-)];

and $\mathbf{R^9}$ is hydrogen;

or a pharmaceutically-aceeptable salt, solvate or pro-drug thereof.

5. A compound of formula (II):

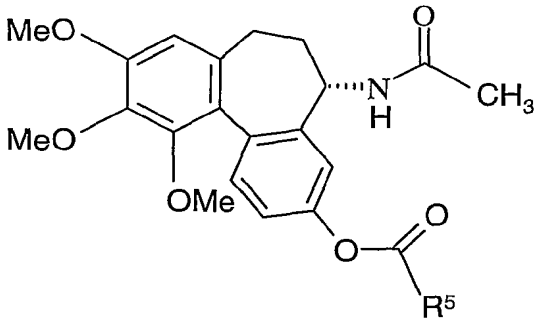


(II)

wherein R^5 and R^8 are as defined in claim 1;

or a pharmacetically-acceptable salt, solvate or pro-drug thereof.

6. A compound of the formula (III) wherein:



(III)

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

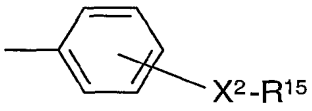
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, -CONH-, -NH-, -NHCO- or -OC(O)NH-;

B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the -X^2-R^{15} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 0, 1 and 2; and

R^{15} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted as immediately hereinabove defined for R^{12}, and substituted by at least 1 substituent selected from C_{2-4}alkanyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15}, wherein:

a is 2 or 3;

b is 0, 1, or 2; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino, N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

7. A compound according to Claim 6 wherein:

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

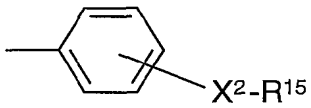
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, -NHCO-;

B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the -X^2-R^{15} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 1 and 2; and

R^{15a} is as hereinabove defined;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15b}, wherein:

a is 2 or 3;

b is 0; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; and

R^{15b} is as hereinabove defined; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, hydroxy and phosphoryloxy;

or a pharmaceutically acceptable salt, solvate or pro-drug thereof.

8. A compound according to Claim 6 wherein:

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

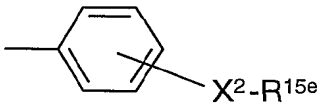
A is phenylene;

X^1 is -CO-, -NHCO-;

Y^1 is methylene or ethylene;

B is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group;

2) of the formula



wherein:

the -X^2-R^{15e} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 1; and

R^{15e} is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15f}, wherein:

a is 2 or 3;

b is 0; and

Y^2 is -C(O)-;

R^{15f} is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group; or

4) 2-[N,N-di(C_{1-4}alkyl)carbamoyl]ethyl- or 3-[N,N-di(C_{1-4}alkyl)carbamoyl]propyl-, wherein the C_{1-4}alkyl group is optionally substituted by 1 hydroxy group;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

9. A compound according to Claim 6 wherein:

R^5 is 2-[N,N-di(C_{1-4}alkyl)carbamoyl]ethyl-, or

3-[N,N-di(C_{1-4}alkyl)carbamoyl]propyl-,

wherein the C_{1-4}alkyl group is optionally substituted by 1 hydroxy group;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

10. A compound selected from:

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 5-(4-acetylpiperazin-l-yl)-5-oxopentanoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c}cyclohepten-3-yl 4-(4-acetylpiperazin-1-yl)-4-oxobutanoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-acetylpiperazin-1-ylmethyl)benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[3-(4-methylpiperazin-l-yl)propionylamino]benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-carbamoylpiperazin-1-ylmethyl)benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl N-acetylpiperidin-1-ylcarboxylate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-[N,N-di-(2-hydroxyethyl)carbamoyl]propanoate; and

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[N,N-di(2-hydroxyethyl)carbamoyl]butanoate;

and pharmaceutically-acceptable salts, solvates and pro-drugs thereof.

11. A pharmaceutical composition comprising a compound according to any one of claims 1 to 10 or pharmaceutically acceptable salt, solvate or pro-drug thereof, in association with a pharmaceutically acceptable carrier.

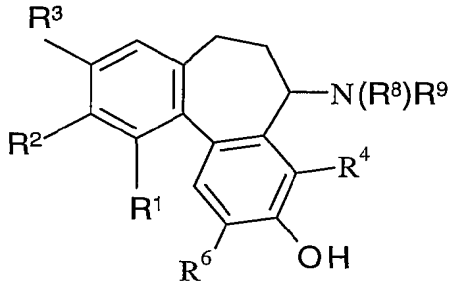
12. The use of a compound according any one of claims 1 to 10, or a pharmaceutically-acceptable salt, solvate or pro-drug thereof, in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm-blooded animal.

13. The use of a compound according to any one of claim 1 to 10 or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for

administration in divided doses for use in the production of a vascular damaging effect in a warm-blooded animal.

14. A process for preparing a compound of the formula (I), or a compound of the formula (I) wherein at least 1 functional group is protected, wherein R^1, R^2, R^3 R^4, R^5, R^6, R^7, R^8, R^9, R^{10} R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}, R^{21}, R^{22}, R^{23}, R^{24}, R^{25}, R^{26}, R^{27}, A, B, Q, X^1, X^2, Y^1, Y^2, Y^3, Y^4, Y^5, Y^7, p and r are as defined in claim 1, comprising:

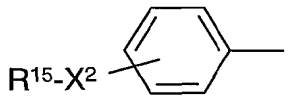
(a) reacting a compound of the formula (X):



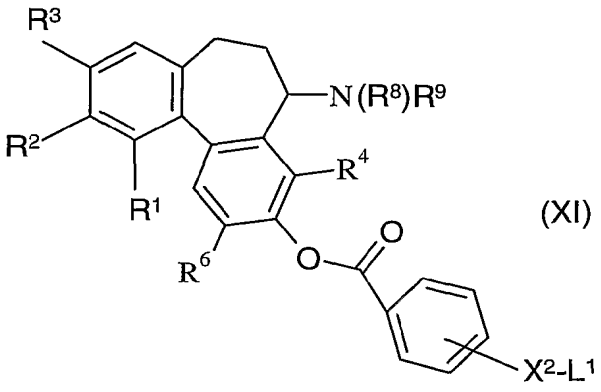
(X)

with a compound of the formula R^5-COOH or an activated derivative thereof;

(b) when R^5 is of the formula:



reacting a compound of the formula (XI):



(XI)

with R^{15} (wherein L^1 is a leaving group) ;

(c) introducing substituents onto a ring nitrogen atom in R^{12} or R^{15};

(d) converting one compound of the formula (I) into another compound of the formula (I);

(e) when a phosphoryloxy group is desired, reacting the corresponding hydroxy compound with a phosphoramidite;

wherein any functional groups are optionally protected.

and thereafter if necessary:

i) converting a compound of formula (I) into another compound of formula (I);

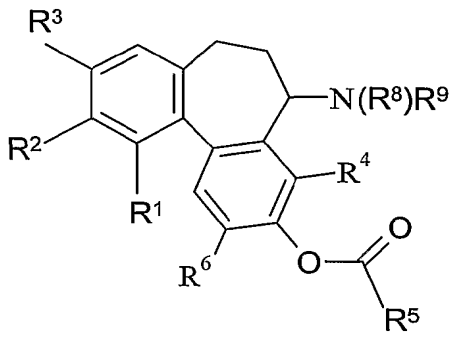
ii) removing any protecting groups;

iii) forming a pharmaceutically acceptable salt, solvate or pro-drug thereof.

AMENDED CLAIMS

[received by the International Bureau on 11 December 2001 (11.12.01);
original claims 1-14 replaced by amended claims 1-14 (14 pages)]

1. A compound of the formula I:



(I)

wherein:

R^1, R^2 and **R^3** are each independently hydroxy, phosphoryloxy ($-OPO_3H_2$), C_{1-4}alkoxy or an in vivo hydrolysable ester of hydroxy; with the proviso that at least two of R^1, R^2 and R^3 are C_{1-4}alkoxy;

R^4 and **R^6** are each independently selected from: hydrogen, nitro, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, fluoro, C_{1-4}alkoxy and C_{1-4}alkyl;

R^5 is selected from one of the following groups:

1) of the formula -A -X^1-Y^1 -B, wherein:

A is C_{1-4}alkylene or -$(CH_2)_p$-Q- (wherein **p** is 0, 1 or 2 and **Q** is phenylene or thienylene);

X^1 is -O-, -CO-, -C(O)O-, -CON(R^{10})-, -N(R^{10})-, -N(R^{10})CO-, N(R^{10})C(O)O-, -N(R^{10})CON(R^{11})-, -N(R^{10})SO_2-, -SO_2N(R^{10})- or OC(O)N(R^{10})- (wherein **R^{10}** is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

Y^1 is C_{1-3}alkylene;

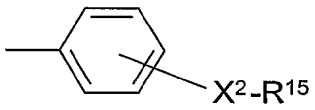
B is carboxy, sulpho, phosphoryloxy, hydroxy, amino, N-(C_{1-4}alkyl)amino, N,N-di (C_{1-3}alkyl)amino, -R^{12} or -NHC(R^{13})COOH; (wherein **R^{12}** is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N,

which heterocyclic group is optionally substituted by 1 or 2 substituents selected from: oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, aminoC_{1-4}alkyl, N,N-di(C_{1-4}alkyl)amino-C_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein **R^{14}** is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkoxy-C_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl));

R^{13} is an amino acid side chain;

2) of the formula:



wherein:

the phenyl ring is substituted by -**X^2**-**R^{15}** in the 3- or 4-position;

X^2 is -CO- or of the formula -$(CH_2)_r$- (wherein **r** is 0, 1, 2 or 3) and

R^{15} is a 5-6 membered saturated heterocyclic group (linked via a ring carbon or nitrogen atom) containing 1 or 2 ring heteroatoms selected from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, C_{1-4}aminoalkyl, N,N-di(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein **R^{14}** is as hereinabove defined); provided that the heterocyclic group (R^{15}) is substituted by at least one substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N,N-di(C_{1-4}alkyl)carbamoyl;

3) -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15}

(wherein **a** is 0, 1, 2, 3 or 4; **b** is 0, 1, 2, 3 or 4; $\mathbf{Y^2}$ is a direct bond, -O-, -C(O)-, -N(R^{16})-, -N(R^{16})C(O)- or -C(O)N(R^{16})- (wherein $\mathbf{R^{16}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and wherein 1 or 2 of the $(CH_2)_a$ or $(CH_2)_b$ groups are optionally substituted by 1 or 2 substituents selected from hydroxy and amino and $\mathbf{R^{15}}$ is as hereinabove defined; provided that when a is 0, then Y^2 is a single direct bond);

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-

(wherein the alkyl groups are independently optionally substituted by 1 or 2 substituents selected from: amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkanoyl, carboxy, sulpho and phosphoryloxy);

provided that:

a) when A is C_{1-4}alkylene and X^1 is of the formula -CO-, -N(R^{10})-, -N(R^{10})CO- or -CON(R^{10})- then when B is R^{12}, $R^{12}$is defined hereinabove for R^{15} ;

b) when A is C_{1-4}alkylene and X^1 is of the formula -N(R^{10})CO-, -CON(R^{10})-, or -C(O)O-, then B is not carboxy;

c) when A is C_{1-4}alkylene and X^1 is -CONH- or -NHCO-, then B is not carboxy, hydroxy, phosphoryloxy, amino, N-C_{1-4}alkylamino or N,N-di-C_{1-4}alkylamino;

$\mathbf{R^8}$ is a group -Y^3R^{17}

(wherein $\mathbf{Y^3}$ is a direct bond, -C(O)-, -C(O)O-, -N(R^{18})-, -C(O)N(R^{18})-, -SO_2- or -SO_2NR^{18}- (wherein $\mathbf{R^{18}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and $\mathbf{R^{17}}$ is selected from one of the following 4 groups:

1) hydrogen, C_{1-4}alkyl, phenyl, C_{1-4}alkylY^4C_{1-4}alkyl (wherein $\mathbf{Y^4}$ is -C(O)-, -NR^{19}C(O)- or -C(O)NR^{19}- (wherein $\mathbf{R^{19}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl));

[which alkyl, alkylY^4alkyl or phenyl group is optionally substituted by 1 or 2 substituents selected from: halogeno, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, carboxy, -CON(R^{23})R^{24} (wherein $\mathbf{R^{23}}$ **and** $\mathbf{R^{24}}$ are independently selected from hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl and C_{1-3}alkoxyC_{2-3}alkyl), C_{1-4}alkoxy, C_{1-4}alkoxycarbonylamino,

C_{1-4}alkanoyl, sulpho, phosphoryloxy, R^{12} (wherein $\mathbf{R^{12}}$ is as hereinabove defined), and a group $-\mathbf{Y^5R^{20}}$ [wherein $\mathbf{Y^5}$ is $-NR^{21}C(O)-$ or $-OC(O)-$ (wherein $\mathbf{R^{21}}$ represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and $\mathbf{R^{20}}$ is C_{1-4}alkyl or a group R^{22} (wherein $\mathbf{R^{22}}$ is a 5 or 6 membered aromatic heterocyclic group containing 1 to 4, inclusive, ring heteroatoms selected independently from O, N and S, which aromatic heterocyclic group is optionally substituted by 1 or 2 substituents selected from hydroxy, amino, C_{1-4}alkyl, aminoC_{1-4}alkyl, N-C_{1-4}alkylaminoC_{1-4}alkyl, N, N-di(C_{1-4}alkyl)aminoC_{1-4}alkyl, carboxy, $-CONR^{25}R^{26}$ and $-NR^{25}COR^{27}$ (wherein $\mathbf{R^{25}}$ **and** $\mathbf{R^{26}}$, which may be the same or different, are hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl and $\mathbf{R^{27}}$ is C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))]];

2) R^{22} (wherein R^{22} is as hereinabove defined);

3) R^{22} -C_{1-4}alkyl- (wherein R^{22} is as hereinabove defined); or

4) $R^{12}Y^7C_{1-4}$alkyl- (wherein R^{12} is as hereinabove defined and Y^7 is -C(O)-, $-NR^{23}C(O)-$, $-NR^{23}C(O)C_{1-4}$alkyl-, $-C(O)NR^{23}-$ or $-C(O)NR^{23}C_{1-4}$alkyl- (wherein R^{23} is as hereinabove defined)));

and $\mathbf{R^9}$ is hydrogen or C_{1-3}alkyl;

or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

2. A compound according to claim 1 where R^1, R^2 and R^3 are all methoxy or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

3. A compound according to claim 1 wherein:

R^1 , R^2, and R^3 are all C_{1-4}alkoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, C_{1-3} alkoxy, and C_{1-3}alkyl;

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

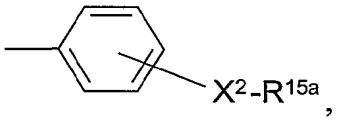
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, $-CON(R^{10})-$, $-N(R^{10})$ -, $-N(R^{10})CO-$ or $-OC(O)N(R^{10})-$;

B is carboxy sulpho, phosphoryloxy or of the formula $-R^{12}$ (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the $-X^2-R^{15a}$ substituent is in the 3, or 4-position of the phenyl ring;

X^2 is $-(CH_2)_r-$;

r is 0, 1 and 2; and

R^{15a} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula $-(CH_2)_a-Y^2-(CH_2)_b-R^{15b}$, wherein:

a is 2 or 3;

b is 0, 1, or 2; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; and

R^{15b} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1_4}alkyl)carbamoyl;

or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino,

N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy; and

$\mathbf{R^8}$ is a group $-Y^3R^{17}$ (wherein Y^3 is -C(O)-, -C(O)O- or -C(O)NH-; and

$\mathbf{R^{17}}$ is selected from one of the following 4 groups:

1) hydrogen, C_{1-4}alkyl, phenyl or C_{1-4}alkylY^4C_{1-4}alkyl (wherein Y^4 is -NHCO- or -CONH-); [which alkyl, alkylY^4alkyl or phenyl group is optionally substituted by 1 or 2 substituents selected from: halogeno, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, C_{1-4}alkoxy, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, phosphoryloxy, R^{12a} (wherein R^{12a} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl), $-Y^5-R^{20}$ [wherein Y^5 is -NHCO-; and R^{20} is C_{1-4}alkyl or R^{22a} (wherein R^{22a} is imidazolyl, pyridyl, pyrimidyl, thiazolyl or pyrazinyl, each of which is optionally substituted by C_{1-4}alkyl)]];

2) R^{22a} (wherein R^{22a} is as hereinabove defined);

3) R^{22a} -C_{1-4}alkyl- (wherein R^{22a} is as hereinabove defined); or

4) $R^{12a}Y^7C_{1-4}$alkyl- (wherein R^{12a} is as hereinabove defined and Y^7 is Y^7 is -NHC(O)- or -CONH-));

and $\mathbf{R^9}$ is hydrogen;

or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

4. A compound according to claim 1 wherein:

R^1 , R^2, and R^3 are all methoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, methoxy and methyl;

R^5 is selected from one of the following groups:

1) of the formula $-A-X^1-Y^1-B$, wherein:

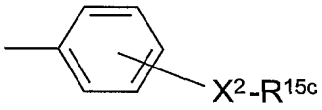
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, $-CON(R^{10})-$, $-N(R^{10})-$, $-N(R^{10})CO-$ or $-OC(O)N(R^{10})-$, wherein R^{10} is as defined in claim 1;

B is carboxy sulpho, phosphoryloxy or of the formula $-R^{12}$ (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the $-X^2-R^{15c}$ substituent is in the 3, or 4-position of the phenyl ring;

X^2 is $-(CH_2)_r-$;

r is 0, 1 and 2; and

R^{15c} is morpholinyl, piperazinyl or piperidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula $-(CH_2)_a-Y^2-(CH_2)_b-R^{15d}$, wherein:

a is 2 or 3;

b is 0 or 1; and

Y^2 is a single direct bond, -C(O)- or -NHC(O)-; and

R^{15d} is morpholinyl, piperazinyl or piperidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino, N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy; and

$\mathbf{R^8}$ is a group $-Y^3R^{17}$ (wherein Y^3 is -C(O)- or -C(O)O-; and

$\mathbf{R^{17}}$ is selected from one of the following 4 groups:

1) C_{1-4}alkyl [which alkyl, group is optionally substituted by 1 or 2 substituents selected from: fluoro, chloro and bromo];

2) R^{22b} (wherein R^{22b} is imidazolyl, pyridyl, pyrimidyl, thiazolyl or pyrazinyl, each of which is optionally substituted by C_{1-4}alkyl);

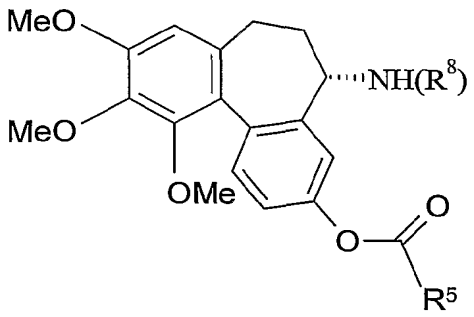
3) R^{22b} -C_{1-4}alkyl- (wherein R^{22b} is as hereinabove defined); or

4) $R^{12b}Y^7C_{1-4}$alkyl- (wherein R^{12a} is morpholinyl, piperidinyl or piperazinyl each of which is optionally substituted by methyl, ethyl, acetyl, propionyl, carbamoyl or 2-hydroxyethyl; and Y^7 is -NHC(O)- or -CONH-));

and $\mathbf{R^9}$ is hydrogen;

or a pharmaceutically-aceeptable salt, solvate or pro-drug thereof.

5. A compound of formula (II):

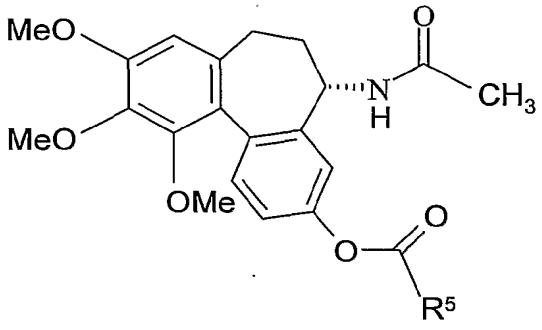


(II)

wherein R^5 and R^8 are as defined in claim 1;

or a pharmacetically-acceptable salt, solvate or pro-drug thereof.

6. A compound of the formula (III) wherein:



(III)

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

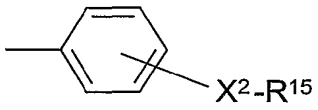
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, -CONH-, -NH-, -NHCO- or -OC(O)NH-;

B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the -X^2-R^{15} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 0, 1 and 2; and

R^{15} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted as immediately hereinabove defined for R^{12}, and substituted by at least 1 substituent selected from C_{2-4}alkanyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15}, wherein:

a is 2 or 3;

b is 0, 1, or 2; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino, N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

7. A compound according to Claim 6 wherein:

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

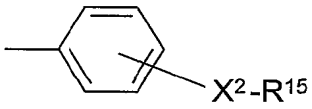
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, -NHCO-;

B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the -X^2-R^{15} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 1 and 2; and

R^{15a} is as defined in claim 3;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15b}, wherein:

a is 2 or 3;

b is 0; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; and

R^{15b} is as defined in claim 3; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, hydroxy and phosphoryloxy;

or a pharmaceutically acceptable salt, solvate or pro-drug thereof.

8. A compound according to Claim 6 wherein:

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

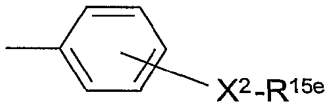
A is phenylene;

X^1 is -CO-, -NHCO-;

Y^1 is methylene or ethylene;

B is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group;

2) of the formula



wherein:

the -X^2-R^{15e} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 1; and

R^{15e} is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15f}, wherein:

a is 2 or 3;

b is 0; and

Y^2 is -C(O)-;

R^{15f} is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group; or

4) 2-[N,N-di(C_{1-4}alkyl)carbamoyl]ethyl- or 3-[N,N-di(C_{1-4}alkyl)carbamoyl]propyl-, wherein the C_{1-4}alkyl group is optionally substituted by 1 hydroxy group;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

9. A compound according to Claim 6 wherein:

R^5 is 2-[N,N-di(C_{1-4}alkyl)carbamoyl]ethyl-, or

3-[N,N-di(C_{1-4}alkyl)carbamoyl]propyl-,

wherein the C_{1-4}alkyl group is optionally substituted by 1 hydroxy group;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

10. A compound selected from:

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 5-(4-acetylpiperazin-1-yl)-5-oxopentanoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c}cyclohepten-3-yl 4-(4-acetylpiperazin-1-yl)-4-oxobutanoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-acetylpiperazin-1-ylmethyl)benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[3-(4-methylpiperazin-1-yl)propionylamino]benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-carbamoylpiperazin-1-ylmethyl)benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl N-acetylpiperidin-1-ylcarboxylate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-[N,N-di-(2-hydroxyethyl)carbamoyl]propanoate; and

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[N,N-di(2-hydroxyethyl)carbamoyl]butanoate;

and pharmaceutically-acceptable salts, solvates and pro-drugs thereof.

11. A pharmaceutical composition comprising a compound according to any one of claims 1 to 10 or pharmaceutically acceptable salt, solvate or pro-drug thereof, in association with a pharmaceutically acceptable carrier.

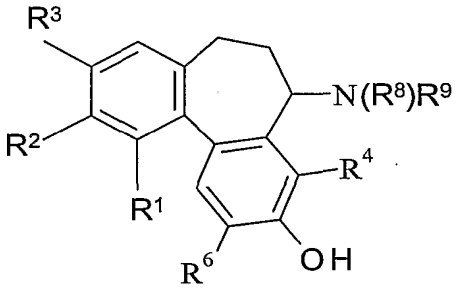
12. The use of a compound according any one of claims 1 to 10, or a pharmaceutically-acceptable salt, solvate or pro-drug thereof, in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm-blooded animal.

13. The use of a compound according to any one of claim 1 to 10 or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for

administration in divided doses for use in the production of a vascular damaging effect in a warm-blooded animal.

14. A process for preparing a compound of the formula (I), or a compound of the formula (I) wherein at least 1 functional group is protected, wherein R^1, R^2, R^3 R^4, R^5, R^6, R^7, R^8, R^9, R^{10} R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}, R^{21}, R^{22}, R^{23}, R^{24}, R^{25}, R^{26}, R^{27}, A, B, Q, X^1, X^2, Y^1, Y^2, Y^3, Y^4, Y^5, Y^7, p and r are as defined in claim 1, comprising:

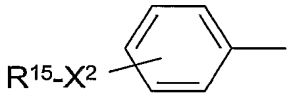
(a) reacting a compound of the formula (X):



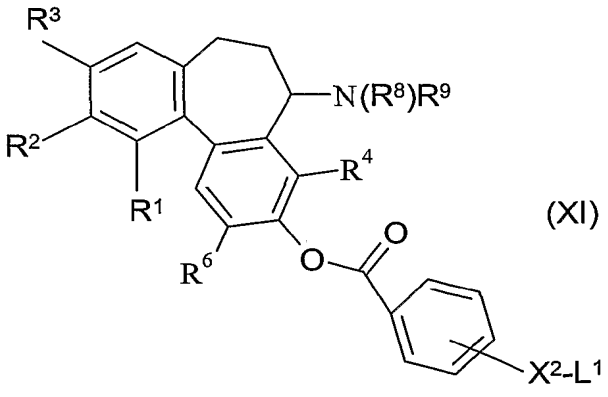
(X)

with a compound of the formula R^5-COOH or an activated derivative thereof;

(b) when R^5 is of the formula:



reacting a compound of the formula (XI):



(XI)

with R^{15} (wherein L^1 is a leaving group) ;

(c) introducing substituents onto a ring nitrogen atom in R^{12} or R^{15};

(d) converting one compound of the formula (I) into another compound of the formula (I);

(e) when a phosphoryloxy group is desired, reacting the corresponding hydroxy compound with a phosphoramidite;

wherein any functional groups are optionally protected.

and thereafter if necessary:

i) converting a compound of formula (I) into another compound of formula (I);

ii) removing any protecting groups;

iii) forming a pharmaceutically acceptable salt, solvate or pro-drug thereof.

INTERNATIONAL SEARCH REPORT

Int plication No
PCT/GB 01/02966

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D295/16 C07D295/18 C07D295/14 C07D211/62 C07C235/74
A61K31/495 A61K31/16 A61K31/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D C07C A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99 02166 A (ANGIOGENE PHARM LTD ;DOUGHERTY GRAEME (GB)) 21 January 1999 (1999-01-21) the whole document ---	1-14
A,P	WO 00 40529 A (ARNOULD JEAN CLAUDE ;ANGIOGENE PHARM LTD (GB); DAVIS PETER DAVID () 13 July 2000 (2000-07-13) the whole document ---	1-14

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 October 2001	17/10/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Frelon, D

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	BOYE O ET AL: "Synthesis of carbon-14 labeled electrophilic ligands of the colchicine binding site of tubulin: chloroacetates of demethylthiocolchicines and of N-acetylcolchinol, isothiocyanates of 9-deoxy-N-acetylcolchinol" JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS, SUSSEX, GB, vol. 33, no. 4, 1993, pages 293-299, XP002081866 ISSN: 0362-4803 example 5 ---	1-14
A	GIL-JONG KANG ET AL: "n-acetylcolchinol O-methyl ether and thiocolchicine, potent nalogs of colchicine modified in the C-ring" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 265, no. 18, 25 June 1990 (1990-06-25), pages 10255-10259, XP002081868 ISSN: 0021-9258 the whole document -----	1-14

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-14 relate to an extremely large number of possible compounds. In fact, the claims contain many options, variables, possible permutations and combinations.
"Prodrugs" are also claimed which are not defined so that it is impossible to determine which compounds are actually meant. Furthermore many brackets are opened but in some definitions they do not appear clearly closed.
Consequently a lack of clarity and conciseness within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. The search includes the compounds as defined by the general formula (I). Prodrugs were only searched as far as they are covered by the formula as such.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int lication No
PCT/GB 01/02966

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9902166	A	21-01-1999	AU	8231198 A	08-02-1999
			BR	9810681 A	15-08-2000
			CN	1262624 T	09-08-2000
			EP	1001785 A1	24-05-2000
			WO	9902166 A1	21-01-1999
			HU	0002493 A2	28-08-2001
			NO	20000077 A	07-01-2000
			PL	337926 A1	11-09-2000
			SK	132000 A3	11-12-2000
			TR	9903149 T2	21-09-2000
WO 0040529	A	13-07-2000	AU	1882300 A	24-07-2000
			WO	0040529 A1	13-07-2000

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(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
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PCT

(10) International Publication Number
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(51) International Patent Classification[7]: C07D 295/185, A61K 31/495, A61P 35/00, C07K 5/062, C07F 9/09, C07D 295/088, C07C 217/74, C07D 233/61

(21) International Application Number: PCT/GB01/02964

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(25) Filing Language: English

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(30) Priority Data:

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(71) Applicant *(for all designated States except US)*: **ANGIOGENE PHARMACEUTICALS LIMITED** [GB/GB]; 14 Plowden Park, Aston Rowant, Watlington, Oxfordshire OX9 5SX (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **ARNOULD, Jean, Claude** [FR/FR]; Z.I. La Pompelle Boîte postale 1050, 51689 Reims Cedex 2 (FR).

(74) Agents: **BRYANT, Tracey** et al.; Astrazeneca, Global Intellectual Property, Alderley Park, P.O. Box 272, Mereside, Macclesfield, Cheshire SK10 4GR (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/08213 A1

(54) Title: COLCHINOL DERIVATIVES AS ANGIOGENESIS INHIBITORS

(57) Abstract: The invention related to colchinol derivatives of the formula (I): Wherein: R^1, R^2 and R^3 are each independently hydroxy, phosphoryloxy (-OPO_3H_2), C_{1-4}alkoxy or an in vivo hydrolysable ester of hydroxy, with the proviso that at least 2 of R^1, R^2 and R^3 are C_{1-4}alkoxy; A is -CO-, -C(O)O-, -CON(R^8)- (wherein R^8 is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl or hydroxyC_{1-3}alkyl); a is an integer from 1 to 4 inclusive; R^a and R^b are independently selected from hydrogen, hydroxy and amino; B is -O-, -CO-, N(R^9)CO-, -CON(R^9)-, -N(R^9)C(O)O-, -N(R^9)CON(R^{10})-, -N(R^9)SO_2-, -SO_2N(R^9)- or a direct single bond (wherein R^9 and R^{10} are independently selected from hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl and hydroxyC_{1-3}alkyl); b is O or an integer from 1 to 4 inclusive, (provided that when b is O, B is a single direct bond); D is carboxy, sulpho, tetrazolyl, imidazolyl, phosphoryloxy, hydroxy, amino, N-(C_{1-4}alkyl)amino, N,N-di(C_{1-3}alkyl)amino, or of the formula-$Y^1(CH_2)_0R^{11}$ or -NHCH(R^{12})COOH;[wherein Y^1 is a direct single bond, -O-, -C(O)-, -N(R^{13})C(O)- or -C(O)N(R^{13})- (wherein R^{13} is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{2-3}alkyl, aminoC_{2-3}alkyl or hydroxyC_{2-3}alkyl); e is O or an integer from 1 to 4 inclusive.

COLCHINOL DERIVATIVES AS ANGIOGENESIS INHIBITORS

The present invention relates to vascular damaging agents, to the use of compounds of the invention in the manufacture of medicaments for use in the production of antiangiogenic effects in warm-blooded animals such as humans, to processes for the preparation of such compounds, to pharmaceutical compositions containing such compounds as active ingredient, to methods for the treatment of disease states associated with angiogenesis and to the use of such compounds as medicaments.

Normal angiogenesis plays an important role in a variety of processes including embryonic development, wound healing and several components of female reproductive function. Undesirable or pathological angiogenesis has been associated with disease states including diabetic retinopathy, psoriasis, cancer, rheumatoid arthritis, atheroma, Kaposi's sarcoma and haemangioma (Fan et al, 1995, Trends Pharmacol. Sci. 16: 57-66; Folkman, 1995, Nature Medicine 1: 27-31). Formation of new vasculature by angiogenesis is a key pathological feature of several diseases (J. Folkman, New England Journal of Medicine 333, 1757-1763 (1995)). For example, for a solid tumour to grow it must develop its own blood supply upon which it depends critically for the provision of oxygen and nutrients; if this blood supply is mechanically shut off the tumour undergoes necrotic death. Neovascularisation is also a clinical feature of skin lesions in psoriasis, of the invasive pannus in the joints of rheumatoid arthritis patients and of atherosclerotic plaques. Retinal neovascularisation is pathological in macular degeneration and in diabetic retinopathy.

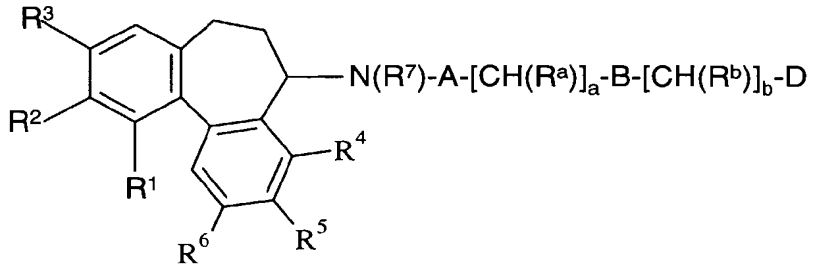
Reversal of neovascularisation by damaging the newly-formed vascular endothelium is expected to have a beneficial therapeutic effect. The present invention is based on the discovery of tricyclic compounds that surprisingly specifically damage newly formed vasculature without affecting the normal, established vascular endothelium of the host species, a property of value in the treatment of disease states associated with angiogenesis such as cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation, endometriosis, dysfunctional uterine bleeding and ocular diseases with retinal vessel proliferation.

Compounds of the present invention are colchinol derivatives. Colchinol derivatives for example *N*-acetyl-colchinol are known. Anti-tumour effects have been noted on animal

models (see for example - Jnl. Natl. Cancer Inst. 1952, 13, 379-392). However, the effect studied was that of gross damage (haemorrhage, softening and necrosis) and there is no suggestion of treatment of inappropriate angiogenesis by destruction of neovasculature.

It is believed, though this is not limiting on the invention, that the use of compounds of the invention damages newly-formed vasculature, for example the vasculature of tumours, thus effectively reversing the process of angiogenesis as compared to known anti-angiogenic agents which tend to be less effective once the vasculature has formed.

According to one aspect of the present invention there is provided a compound of the formula (I):



(I)

wherein:

R^1, R^2 and **R^3** are each independently hydroxy, phosphoryloxy ($-OPO_3H_2$), C_{1-4}alkoxy or an in vivo hydrolysable ester of hydroxy, with the proviso that at least 2 of R^1, R^2 and R^3 are C_{1-4}alkoxy;

A is - CO-, -C(O)O-, $-CON(R^8)-$, $-SO_2-$ or $-SO_2N(R^8)-$ (wherein **R^8** is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl or hydroxyC_{1-3}alkyl);

a is an integer from 1 to 4 inclusive;

R^a and **R^b** are independently selected from hydrogen, hydroxy and amino;

B is -O-, -CO-, $-N(R^9)CO-$, $-CON(R^9)-$, -C(O)O-, $-N(R^9)-$, $-N(R^9)C(O)O-$, $-N(R^9)CON(R^{10})-$, $-N(R^9)SO_2-$, $-SO_2N(R^9)-$ or a direct single bond (wherein **R^9** and **R^{10}** are independently selected from hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl and hydroxyC_{1-3}alkyl);

b is 0 or an integer from 1 to 4 inclusive, (provided that when b is 0, B is a single direct bond);

D is carboxy, sulpho, tetrazolyl, imidazolyl, phosphoryloxy, hydroxy, amino,

N-(C_{1-4}alkyl)amino, N,N-di(C_{1-3}alkyl)amino or of the formula $-Y^1-(CH_2)_cR^{11}$ or $-NHCH(R^{12})COOH$; [wherein $\mathbf{Y^1}$ is a direct single bond, -O-, -C(O)-, $-N(R^{13})-$, $-N(R^{13})C(O)-$ or $-C(O)N(R^{13})-$ (wherein $\mathbf{R^{13}}$ is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{2-3}alkyl, aminoC_{2-3}alkyl or hydroxyC_{2-3}alkyl); **c** is 0 or an integer from 1 to 4 inclusive; $\mathbf{R^{11}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, or a 5-6-membered unsaturated or partially unsaturated heteroaryl group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently form O, S and N, which heterocyclic group or heteroaryl group may bear 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-(C_{1-4}alkyl)carbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, aminoC_{1-4}alkyl, N-C_{1-4}alkylaminoC_{1-4}alkyl, di-N,N-(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein $\mathbf{R^{14}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl);

$\mathbf{R^{12}}$ is an amino acid side chain;

$\mathbf{R^5}$ is C_{1-4}alkoxy;

$\mathbf{R^4}$ and $\mathbf{R^6}$ are each independently selected from:
hydrogen, fluoro, nitro, amino, N-C_{1-4}alkylamino, N,N-di-(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy and C_{1-4}alkyl;

$\mathbf{R^7}$ is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl or hydroxyC_{1-3}alkyl;
or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

In another aspect, the invention relates to a compound of the formula (I) as hereinabove defined or to a pharmaceutically-acceptable salt thereof.

In this specification the generic term "alkyl" includes both straight-chain and branched-chain alkyl groups. However references to individual alkyl groups such as "propyl" are specific for the straight-chain version only and references to individual branched-chain

alkyl groups such as "isopropyl" are specific for the branched-chain version only. An analogous convention applies to other generic terms.

R^{12} is an amino acid side chain. This includes side chains from natural and non-natural amino acids and includes the possibility of R^{12} joining to the NH group so as to form a ring as in the amino acid proline. It includes α-amino acids β-amino acids and γ-amino acids. In addition, the amino acids may be L-isomers or D-isomers, but preferably L-isomers. Preferred amino acids include glycine, alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tryptophan, serine, threonine, cysteine, tyrosine, asparaginine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, β-alanine and ornithine. More preferred amino acids include glutamic acid, serine, threonine, arginine, glycine, alanine, β-alanine and lysine. Especially preferred amino acids include glutamic acid, serine, threonine, arginine, alanine and β-alanine. Specific values for R^{12} include hydrogen, C_{1-4}alkyl, C_{1-4}alkylthioC_{1-4}alkyl, hydroxyC_{1-4}alkyl, thioC_{1-4}alkyl, phenylC_{1-4}alkyl (optionally substituted by hydroxy), guanidinoC_{1-4}alkyl, carboxyC_{1-4}alkyl, carbamoylC_{1-4}alkyl, aminoC_{1-4}alkyl and imidazolyl C_{1-4}alkyl and R^{12} forming a pyrrolidinyl ring with the NH group. Preferred values for R^{12} include hydrogen, C_{1-4}alkyl, C_{1-4}alkylthioC_{1-4}alkyl, hydroxyC_{1-4}alkyl, thioC_{1-4}alkyl, guanidinoC_{1-4}alkyl, carboxyC_{1-4}alkyl, carbamoylC_{1-4}alkyl and aminoC_{1-4}alkyl.

It is to be understood that, insofar as certain of the compounds of Formula (I) defined above may exist in optically active or racemic forms by virtue of one or more asymmetric carbon atoms, the invention includes in its definition any such optically active or racemic form which possesses vascular damaging activity. The synthesis of optically active forms may be carried out by standard techniques of organic chemistry well known in the art, for example by synthesis from optically active starting materials or by resolution of a racemic form. Similarly, the above-mentioned activity may be evaluated using the standard laboratory techniques referred to hereinafter.

Suitable values for the generic radicals referred to above include those set out below. Within the present invention it is to be understood that a compound of the formula (I) or a salt thereof may exhibit the phenomenon of tautomerism and that the formulae drawings within this specification can represent only one of the possible tautomeric forms. It is to be understood that the invention encompasses any tautomeric form which has vascular damaging

activity and is not to be limited merely to any one tautomeric form utilised within the formulae drawings.

It is also to be understood that certain compounds of the formula (I) and salts thereof can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms which have vascular damaging activity.

The present invention relates to the compounds of formula (I) as hereinbefore defined as well as to the salts thereof. Salts for use in pharmaceutical compositions will be pharmaceutically acceptable salts, but other salts may be useful in the production of the compounds of formula (I) and their pharmaceutically acceptable salts. Pharmaceutically acceptable salts of the invention may, for example, include acid addition salts of the compounds of formula (I) as hereinbefore defined which are sufficiently basic to form such salts. Such acid addition salts include for example salts with inorganic or organic acids affording pharmaceutically acceptable anions such as with hydrogen halides (especially hydrochloric or hydrobromic acid of which hydrochloric acid is particularly preferred) or with sulphuric or phosphoric acid, or with trifluoroacetic, citric or maleic acid. Suitable salts include hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates and tartrates. In addition where the compounds of formula (I) are sufficiently acidic, pharmaceutically acceptable salts may be formed with an inorganic or organic base which affords a pharmaceutically acceptable cation. Such salts with inorganic or organic bases include for example an alkali metal salt, such as a sodium or potassium salt, an alkaline earth metal salt such as a calcium or magnesium salt, an ammonium salt or for example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

Various forms of prodrugs are known in the art. For examples of such prodrug derivatives, see:

a) Design of Prodrugs, edited by H. Bundgaard, (Elsevier, 1985) and Methods in Enzymology, Vol. 42, p. 309-396, edited by K. Widder, *et al.* (Academic Press, 1985);

b) A Textbook of Drug Design and Development, edited by Krogsgaard-Larsen and H. Bundgaard, Chapter 5 "Design and Application of Prodrugs", by H. Bundgaard p. 113-191 (1991);

c) H. Bundgaard, Advanced Drug Delivery Reviews, 8, 1-38 (1992);

d) H. Bundgaard, *et al.*, Journal of Pharmaceutical Sciences, 77, 285 (1988); and

e) N. Kakeya, *et al.*, Chem. Pharm. Bull., 32, 692 (1984).

Examples of such pro-drugs may be used to form in-vivo-cleavable esters of a compound of the Formula (I). An in-vivo-cleavable ester of a compound of the Formula (I) containing a carboxy group is, for example, a pharmaceutically-acceptable ester which is cleaved in the human or animal body to produce the parent acid. Suitable pharmaceutically-acceptable esters for carboxy include C_{1-6}alkoxymethyl esters, for example methoxymethyl; C_{1-6}alkanoyloxymethyl esters, for example pivaloyloxymethyl; phthalidyl esters; C_{3-8}cycloalkoxycarbonyloxy C_{1-6}alkyl esters, for example 1-cyclohexylcarbonyloxyethyl; 1,3-dioxolan-2-ylmethyl esters, for example 5-methyl-1,3-dioxolan-2-ylmethyl; and C_{1-6}alkoxycarbonyloxyethyl esters, for example 1-methoxycarbonyloxyethyl; and may be formed at any carboxy group in the compounds of this invention.

Suitable values for R^1, R^2, R^3 R^4, R^5, R^6, R^7, R^8, R^9, R^{10} or R^{13} or for various substituents on D or R^{14} include:

for halogeno	fluoro, chloro, bromo and iodo;
for C_{1-4}alkyl:	methyl, ethyl, propyl, isopropyl and tert-butyl;
for N-C_{1-4}alkylamino:	methylamino, ethylamino, propylamino, isopropylamino and butylamino;
for N,N-di-[C_{1-4}alkyl]amino:	dimethylamino, diethylamino, N-ethyl-N-methylamino and diisopropylamino;
for C_{2-4}alkanoyl:	acetyl and propionyl;
for C_{1-4}alkoxy:	methoxy and ethoxy;
for cyanoC_{1-4}alkyl:	cyanomethyl and 2-cyanoethyl;
for N-C_{1-4}alkylcarbamoyl:	N-methylcarbamoyl, N-ethylcarbamoyl and N-propylcarbamoyl;
for N,N-di-[(C_{1-4}))alkyl]carbamoyl:	N,N-dimethylcarbamoyl, N-ethyl-N-methylcarbamoyl and N,N-diethylcarbamoyl;
for C_{1-4}alkylsulphonylalkyl:	methylsulphonylmethyl and ethylsulphonylmethyl;
for hydroxyC_{1-4}alkyl:	hydroxymethyl, 2-hydroxyethyl, 1-hydroxyethyl and 3-hydroxypropyl as appropriate;

for C_{1-4}alkoxyC_{1-4}alkyl:	methoxymethyl, ethoxymethyl, 1-methoxyethyl, 2-methoxyethyl, 2-ethoxyethyl and 3-methoxypropyl as appropriate;
for aminoC_{1-4}alkyl	aminomethyl, 2-aminoethyl, 1-aminoethyl and 3-aminopropyl as appropriate;
for N-C_{1-4}alkylaminoC_{1-4}alkyl:	methylaminomethyl, ethylaminomethyl, 1-methylaminoethyl, 2-methylaminoethyl, 2-ethylaminoethyl and 3-methylaminopropyl as appropriate;
for N,N-di-[C_{1-4}alkyl]aminoC_{1-4}alkyl:	dimethylaminomethyl, diethylaminomethyl, 1-dimethylaminoethyl, 2-dimethylaminoethyl and 3-dimethylaminopropyl as appropriate:
for carboxyC_{1-4}alkyl:	carboxymethyl, 1-carboxyethyl, 2-carboxyethyl, 3-carboxypropyl and 4-carboxybutyl;
for carbamoylC_{1-4}alkyl:	carbamoylmethyl, 1-carbamoylethyl, 2-carbamoylethyl and 3-carbamoylpropyl;
for C_{1-4}alkoxyC_{1-4}alkyl	methoxymethyl, ethoxyethyl, methoxyethyl, and methoxypropyl.

Carbamoyl refers to —$CONH_2$.

Piperazino refers to piperazin-1-yl.

Examples of 5- or 6-membered saturated heterocyclic groups include pyrrolidinyl, imidazolidinyl, pyrazolidinyl, piperidyl, piperazinyl and morpholinyl.

Examples of 5- or 6-membered unsaturated or partially unsaturated heteroaryl groups include: imidazolyl, imidazolinyl pyridyl pyrrolyl, furanyl, triazolyl, pyrazinyl, pyrazolinyl, pyrimidinyl, pyridazinyl, isoxazolyl, oxazolyl, isothiazolyl, thiazolyl and thienyl.

Preferably at least 2 of R^1, R^2, and R^3 are methoxy.

Preferably R^1 , R^2, and R^3 are all C_{1-4}alkoxy.

Most preferably R^1, R^2, and R^3 are all methoxy.

Preferably R^8 is hydrogen, methyl, ethyl, 2-methoxyethyl, 2-aminoethyl or 2-hydroxyethyl.

More preferably R^8 is hydrogen, 2-aminoethyl or 2-hydroxyethyl and most preferably R^8 is hydrogen.

Preferably A is -CO-, -C(O)O- or -CON(R^8)-. Most preferably A is -C(O)O-.

Preferably a is 1, 2 or 3 and most preferably a is 2 or 3.

Preferably R^a, and R^b are hydrogen.

Preferably B is -N(R^9)CO-, -CON(R^9), -C(O)O-, -N(R^9)-, -N(R^9)C(O)O-, N(R^9)CON(R^{10})- or a single direct bond.

More preferably B is -CO-, -N(R^9)CO- or a single direct bond.

Yet more preferably B is -CO- or a single direct bond.

Most preferably B is -CO-. In another aspect B is a single direct bond.

Preferably R^9, and R^{10} are independently selected from hydrogen, methyl, ethyl, 2-methoxyethyl, 2-aminoethyl and 2-hydroxyethyl.

More preferably R^9 and R^{10} are independently selected from hydrogen, 2-aminoethyl and 2-hydroxyethyl.

Most preferably R^9, and R^{10} are hydrogen.

Preferably b is 0, 1 or 2, more preferably b is 0 or 1 and most preferably b is 0.

Preferably R^{11} is a 5 or 6 membered saturated heterocyclic ring containing 1 or 2 ring heteroatoms selected from N and O.

More preferably R^{11} is a 6 membered saturated heterocyclic ring containing 1 or 2 ring heteroatoms selected from N and O.

Further preferably R^{11} contains at least 1 ring nitrogen atom.

Further preferably R^{11} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents mentioned above for R^{11}.

Further preferably R^{11} is linked via a ring nitrogen atom.

Most preferably R^{11} is piperazino or morpholino, each ring being optionally substituted by 1 or 2 of the substituents mentioned hereinabove for R^{11}.

The saturated heterocyclic ring may be substituted on ring carbon or ring nitrogen atoms, providing this does not result in quaternisation.

Preferred substituents for the saturated heterocyclic ring in R^{11} include C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl.

More preferred substituents for the saturated heterocyclic ring in R^{11} include C_{1-3}alkyl, C_{2-3}alkanoyl, carbamoyl and hydroxyC_{2-3}alkyl.

Yet more preferred substituents for the saturated heterocyclic ring in R^{11} include methyl, acetyl, carbamoyl and 2-hydroxyethyl.

The most preferred substituents for the saturated heterocyclic ring include methyl, acetyl and carbamoyl.

Preferably the saturated heterocyclic ring in R^{11} is unsubstituted or substituted by 1 substituent.

When the saturated heterocyclic ring in R^{11} is morpholino, preferably it is unsubstituted. When the saturated heterocyclic ring in R^{11} is piperazino, preferably it is unsubstituted or substituted by 1 substituent on a ring nitrogen atom.

Preferably Y^1 is -CONH - or -NHCO -.

Preferably c is 0, 1 or 2.

More preferably c is 0.

Preferred values for R^{11} include morpholino, 4-methylpiperazin-1-yl and 4-acetylpiperazin-1-yl.

Preferably R^{14} is morpholino or piperazin-1-yl, each optionally substituted by 1 or 2 substituents selected from C_{1-3}alkyl, hydroxyC_{2-3}alkyl, C_{1-3}alkoxy and C_{1-3}alkoxy C_{1-3}alkyl.

More preferably R^{14} is morpholino, or piperazin-1-yl unsubstituted or substituted by methyl.

Preferably D is carboxy, phosphoryloxy, hydroxy, amino, N-C_{1-4} alkylamino, N,N-di(C_{1-4} alkyl)amino or of the formula $-Y^1(CH_2)_cR^{11}$ wherein Y^1, c and R^{11} are as hereinabove defined.

More preferably D is carboxy phosphoryloxy, hydroxy, amino or of the formula $-Y^1-(CH_2)_cR^{11}$ wherein Y^1, c and R^{11} are as hereinabove defined.

More preferably D is phosphoryloxy, amino or of the formula $-Y^1-(CH_2)_cR^{11}$ wherein Y^1, c and R^{11} are as hereinabove defined.

Yet more preferably D is phosphoyloxy, amino or of the formula $-Y^1-(CH_2)_cR^{11}$ wherein Y^1 and c are as hereinabove defined and R^{11} is morpholino, imidazolyl, or piperazinyl, which heterocyclic group may bear one or more substituents as defined above.

Yet more preferably D is phosphoyloxy, amino or of the formula $-Y^1-(CH_2)_cR^{11}$ wherein Y^1 and c are as hereinabove defined and R^{11} is morpholino, imidazolyl, 4-methylpiperazin-l-yl or 4-acetylpiperazin-1-yl.

Yet even more preferably D is phosphoyloxy, amino or of the formula $-Y^1-(CH_2)_cR^{11}$ wherein Y^1 is a direct single bond and c is 0 and R^{11} is morpholino, imidazol-1-yl, 4-methylpiperazin-1-yl or 4-acetylpiperazin-1-yl.

Preferably R^5 is methoxy.

Preferably R^4 and R^6 are independently selected from hydrogen, hydroxy, C_{1-3} alkoxy, and C_{1-3}alkyl.

More preferably at least one of R^4 and R^6 is hydrogen.

Most preferably R^4 and R^6 are both hydrogen.

Preferably R^7 is hydrogen or methyl. Most preferably R^7 is hydrogen.

A preferred class of compound is of the formula (I) wherein:

R^1 , R^2, and R^3 are all C_{1-4}alkoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, C_{1-3} alkoxy, and C_{1-3}alkyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 1, 2 or 3;

B is -CO-, -NHCO-, -CONH, -C(O)O-, -NH-, -NHC(O)O-, NHCONH- or a single direct bond;

b is 0, 1 or 2;

D is carboxy, sulpho, phosphoryloxy, hydroxy, amino, N-C_{1-4} alkylamino, N,N-di(C_{1-4} alkyl)amino or of the formula $-Y^1(CH_2)_cR^{11}$ (wherein $\mathbf{Y^1}$ is -NHC(O)- or -C(O)NH-; **c** is 1 or 2; $\mathbf{R^{11}}$ is a 5-6-membered saturated heterocyclic group (linked via nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O and N, which heterocyclic group may bear 1 or 2 substituents selected from:

C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

R^7 is hydrogen;

or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

Another preferred class of compound is of the formula (I) wherein:

R^1 , R^2, and R^3 are all methoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, methoxy and methyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

B is -CO-, -NHCO-, -CONH or a single direct bond;

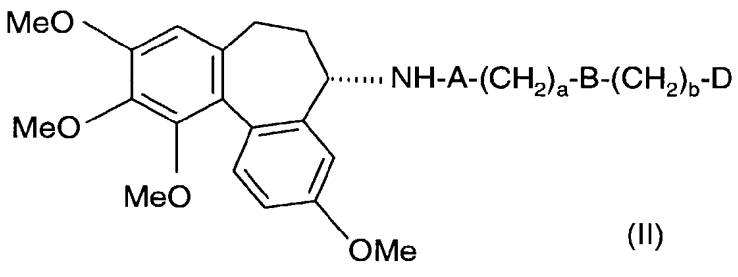
b is 0 or 1;

D is carboxy, phosphoryloxy, hydroxy, amino, N-C_{1-4} alkylamino, N,N-di(C_{1-4} alkyl)amino or of the formula $-Y^1(CH_2)_cR^{11}$ (wherein $\mathbf{Y^1}$ is -NHC(O)- or -C(O)NH-; **c** is 1 or 2; $\mathbf{R^{11}}$ is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 or 2 substituents selected from:
C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

R^7 is hydrogen;

or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

Another preferred class of compounds is that of the formula (II):



(II)

wherein a, b, A, B and D are as hereinabove defined;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Another preferred class of compounds is that of the formula (II) wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

B is -CO-, -NHCO-, -CONH or a single direct bond;

b is 0 or 1;

D is carboxy, phosphoryloxy, hydroxy, amino, N-C_{1-4} alkylamino, N,N-di(C_{1-4} alkyl)amino or of the formula $-Y^1(CH_2)_cR^{11}$ (wherein $\mathbf{Y^1}$ is -NHC(O)- or -C(O)NH-; **c** is 1 or 2; $\mathbf{R^{11}}$ is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 or 2 substituents selected from: C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Another preferred class of compounds is that of the formula (II) wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

B is -CO-, -NHCO-, -CONH or a single direct bond;

b is 0 or 1;

D is phosphoryloxy, carboxy, amino or imidazolyl;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Another preferred class of compounds is that of the formula (II) wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

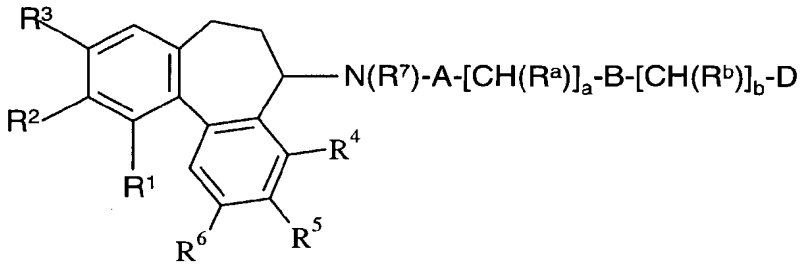
B is -CO-, -NHCO- or a single direct bond;

b is 0 or 1;

D is phosphoryloxy amino or imidazolyl;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

A further preferred class of compounds of the invention is that of a compound of formula (III)::



(III)

wherein:

R^1, R^2 and **R^3** are each independently hydroxy, phosphoryloxy ($-OPO_3H_2$), C_{1-4}alkoxy or an in vivo hydrolysable ester of hydroxy, with the proviso that at least 2 of R^1, R^2 and R^3 are C_{1-4}alkoxy;

A is - CO-, -C(O)O-, $-CON(R^8)$-, $-SO_2$- or $-SO_2N(R^8)$- (wherein **R^8** is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{2-3}alkyl, aminoC_{2-3}alkyl or hydroxyC_{2-3}alkyl);

a is an integer from 1 to 4 inclusive;

R^a and **R^b** are independently selected from hydrogen, hydroxy and amino;

B is -O-, -CO-, $-N(R^9)CO$-, $-CON(R^9)$ -, $-C(O)O$-, $-N(R^9)$ -, $-N(R^9)C(O)O$-, $-N(R^9)CON(R^{10})$-, $-N(R^9)SO_2$-, $-SO_2N(R^9)$- or a direct single bond (wherein **R^9** and **R^{10}** are independently selected from hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{2-3}alkyl, aminoC_{2-3}alkyl and hydroxyC_{2-3}alkyl);

b is 0 or an integer from 1 to 4 inclusive;

D is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O and N, which heterocyclic group may bear 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-(C_{1-4}alkyl)carbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, aminoC_{1-4}alkyl, N-C_{1-4}alkylaminoC_{1-4}alkyl, di-N,N-(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein **R^{14}** is a 5-6-membered saturated heterocyclic group (linked via carbon or

nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from: oxo, hydroxy, halogeno, C_{1-4}alkyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl);

$\mathbf{R^5}$ is C_{1-4}alkoxy;

$\mathbf{R^4}$ and $\mathbf{R^6}$ are each independently selected from: hydrogen, halogeno, nitro, amino, N-C_{1-4}alkylamino, N,N-di-(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy and C_{1-4}alkyl;

$\mathbf{R^7}$ is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl or hydroxyC_{1-3}alkyl;

or a pharmaceutically acceptable salt, solvate or pro-drug thereof.

Another further preferred class of compound is of the formula (III) wherein:

R^1 , R^2, and R^3 are all C_{1-4}alkoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, C_{1-3} alkoxy, and C_{1-3}alkyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 1, 2 or 3;

B is -CO-, -NHCO-, -CONH, -C(O)O-, -NH-, -NHC(O)O-, NHCONH- or a single direct bond;

b is 0, 1 or 2;

D is piperazinyl or morpholinyl or piperidinyl, each ring being optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl;

R^7 is hydrogen;

or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

Another further preferred class of compound is of the formula (III) wherein:

R^1 , R^2, and R^3 are all methoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, methoxy and methyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

B is -CO-, -NHCO-, -CONH or a single direct bond;

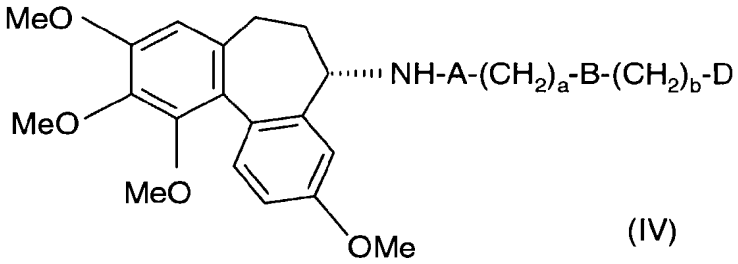
b is 0 or 1;

D is piperazino or morpholino, each ring being optionally substituted by 1 or 2 substituents selected from methyl, ethyl, acetyl, propionyl, carbamoyl and 2-hydroxyethyl;

R^7 is hydrogen;

or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

In another aspect the invention relates to a compound of the formula (IV):



(IV)

wherein a, b, A, B and D are as hereinabove defined for formula (III);

or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

Another preferred class of compounds is that of the formula (IV) wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

B is -CO-, -NHCO-, -CONH or a single direct bond;

b is 0 or 1;

D is piperazino or morpholino, each ring being optionally substituted by 1 or 2 substituents selected from methyl, ethyl, acetyl, propionyl, carbamoyl and 2-hydroxyethyl;

or a pharmaceutically acceptable salt, solvate or pro-drug thereof.

Another preferred class of compounds is that of the formula (IV) wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

B is -CO-, -NHCO-, -CONH or a single direct bond;

b is 0 or 1;

D is morpholino, 4-methylpiperazin-1-yl or 4-acetylpiperazin-1-yl;

or a pharmaceutically acceptable salt, solvate or pro-drug thereof.

Another preferred class of compounds is that of the formula (IV) wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

B is -CO- or a single direct bond;

b is 0;

D is morpholino, 4-methylpiperazin-1-yl or 4-acetylpiperazin-1-yl;

or a pharmaceutically acceptable salt, solvate or pro-drug thereof.

Particular compounds of the present invention include:

N-[(5S) -3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-2-[2-aminoacetylamino]acetamide;

4-oxo-4-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]amino]butyl disodium phosphate;

N-{N-[2-(imidazol-1-yl)ethyl]carbamoyl}-5(S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine; and

2-{N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamoyloxy}ethyl disodium phosphate;

and pharmaceutically-acceptable salts, solvates or pro-drugs thereof.

Further particular compounds of the present invention include:

2-morpholinoethyl *N*-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5*H*-dibenzo [a,c]cyclohepten-5-yl]carbamate;

3-(1-methylpiperazin-4-yl)propyl N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo [a,c]cyclohepten-5-yl] carbamate;

N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-2-[2-aminoacetylamino]acetamide;

2-(1-acetylpiperazin-4-yl)ethyl-N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl] carbamate;

N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-4-(1-methylpiperazin-4-yl)-4-oxobutan-l-amide;

4-oxo-4-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]amino]butyl disodium phosphate;

N-{N-[2-(imidazol-1-yl)ethyl]carbamoyl}-5(S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine;

3-(1-acetylpiperazin-4-yl) propyl-N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamate;

N-l-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamoyloxy]ethyl disodiumphosphate;

4-morpholino-4-oxobutyl-N-[(5S)-3,9,10, 11-tetramethoxy-6,7-dihydro-5H-dibenzo [a-c]cyclohepten-5-yl]carbamate; and

4-(1-methylpiperazin-4-yl)-4-oxobutyl-N-[(5S)-3,9,10, 11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cylcohepten-5-yl]carbamate;

and pharmaceutically-acceptable salts, , solvates or pro-drugs thereof.

Synthesis of Compounds of the Formula (I)

Compounds of Formula (I) may be prepared by a number of processes as generally described hereinbelow and more specifically in the Examples hereinafter. Processes for the preparation of novel compounds of formula (I), are provided as a further feature of the invention and are as described hereinafter. Necessary starting materials may be obtained by standard procedures of organic chemistry. The preparation of such starting materials is described within the accompanying non-limiting Examples. Alternatively necessary starting materials are obtainable by analogous procedures to those illustrated which are within the ordinary skill of an organic chemist.

Thus according to another aspect of the invention, a compound of the Formula (I) may be formed by deprotecting a compound of the formula (I) wherein at least 1 functional group is protected. For example, amino, hydroxy, carboxy or phosphoryloxy groups may be protected during the reaction sequence used to prepare a compound of the formula (I).

Protecting groups may in general be chosen from any of the groups described in the literature or known to the skilled chemist as appropriate for the protection of the group in question, and may be introduced by conventional methods.

Protecting groups may be removed by any convenient method as described in the literature or known to the skilled chemist as appropriate for the removal of the protecting

group in question, such methods being chosen so as to effect removal of the protecting group with minimum disturbance of groups elsewhere in the molecule.

A suitable protecting group for a hydroxy group is, for example, an arylmethyl group (especially benzyl), a triC_{1-4}alkysilyl group (especially trimethysilyl or tert-butyldimethylsilyl), an aryldi-C_{1-4}alkylsilyl group (especially dimethylphenylsilyl), a diarylC_{1-4}alkylsilyl group (especially tert-butyldiphenylsilyl), a C_{1-4}alkyl group (especially methyl), a C_{2-4}alkenyl group (especially allyl), a C_{1-4}alkoxymethyl group (especially methoxymethyl) or a tetrahydropyranyl group (especially tetrahydroyran-2-yl). The deprotection conditions for the above protecting groups will necessary vary with the choice of protecting group. Thus, for an example, arylmethyl group such as a benzyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-charcoal. Alternatively a trialkylsilyl or an aryldialkylsilyl group such as tert-butydimethylsilyl or a dimethylphenylsilyl group may be removed, for example, by treatment with a suitable acid such as hydrochloric, sulphuric, phosphoric or trifluoroacetic acid, or with an alkali metal or ammonium fluoride such as sodium fluoride or, preferably tetrabutylammonium fluroide. Alternatively an alkyl group may be removed, for example, by treatment with an alkali metal C_{1-4}alkylsulphide such as sodium thioethoxide or, for example, by treatment with an alkali metal diarylphosphide such as lithium diphenylphosphide or, for example, by treatment with a boron or aluminium trihalide such as boron tribromide. Alternatively a C_{1-4}alkoxymethyl group or tetrahydropyranyl group may be removed, for example, by treatment with a suitable acid such as hydrochloric or trifluoroacetic acid.

Alternatively a suitable protecting group for a hydroxy group is, for example, an acyl group, for example a C_{2-4}alkanoyl group (especially acetyl) or an aroyl group (especially benzoyl). The deprotection conditions for the above protecting groups will necessarily vary with the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl or an aroyl group may be removed, for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide.

A suitable protecting group for an amino, imino or alkylamino group is, for example, an acyl group, for example a C_{2-4}alkanoyl group (especially acetyl), a C_{1-4}alkoxycarbonyl) group (especially methoxycarbonyl), ethoxycarbonyl or tert-butoxycarbonyl), an arylmethoxycarbonyl group (especially benzyloxycarbonyl) or an aroyl group (especially benzoyl). The deprotection conditions for the above protecting groups necessarily vary with

the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl, alkoxycarbonyl or aroyl group may be removed for example, by hydrolysis with a suitable base such as alkali metal hydroxide, for example lithium or sodium hydroxide. Alternatively an acyl group such as a tert-butoxycarbonyl group may be removed, for example, by treatment with a suitable acid such as hydrochloric, sulphuric or phosphoric acid or trifluoroacetic acid, and an arylmethoxycarbonyl group such as a benzyloxycarbonyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-charcoal.

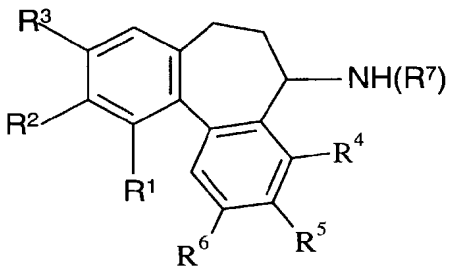
A suitable protecting group for a carboxy group is, for example, an esterifying group, for example a C_{1-4}alkyl group (especially methyl or ethyl) which may be removed, for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide; or for example, a tert-butyl group which may be removed, for example, by treatment with a suitable acid such as hydrochloric, sulphuric or phosphoric acid or trifluoroacetic acid.

The reader is referred to Advanced Organic Chemistry, 4th Edition, by J. March, published by John Wiley & Sons 1992, for general guidance on reaction conditions and reagents and to Protective Groups in Organic Synthesis, 2nd Edition, by T. Green et al., also published by John Wiley & Son, for general guidance on protecting groups.

In the following process description the symbols R^1 - R^7, A, B, D, R^a R^b , a and b are to be understood to represent those groups described above in relation to formulae (I) and (II) unless otherwise stated.

A compound of the formula (I), or a compound of the formula (I) wherein at least 1 functional group is protected, may be prepared using one of the following processes:

a) reacting a compound of the formula (X)



(X)

with a compound of the formula (XI):

L^1 - A - $[CH(R^a)]_a$-B-$[CH\ R^b)]_b$-D (XI)

wherein L^1 is a leaving group; or

b) converting one compound of the formula (I) into another compound of the formula (I); or

c) when a phosphoryloxy group is desired, reacting the corresponding hydroxy compound with a phosphoramidite;

wherein any functional groups are optionally protected.

and thereafter if necessary:

i) converting a compound of formula (I) into another compound of formula (I);

ii) removing any protecting groups;

iii) forming a pharmaceutically acceptable salt, solvate or pro-drug thereof.

The reaction between a compound of the formula (X) and a compound of the formula L^1 - A - $[CH(R^a)]_a$-B-$[CH\ R^b)]_b$-D is conveniently performed under standard acylation or sulphonylation conditions. L^1 is usually halogeno, for example chloro or bromo, hydroxy, mesyloxy, tosyloxy or an 'activated' hydroxy group. The precise conditions depending largely upon the nature of A.

For example, when -A- is -CO-, L^1 may be hydroxy and the reaction carried out in the presence of coupling agent such as dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide. Optionally, a base may be used, for example an organic base such as triethylamine. Suitable solvents are usually aprotic solvents, for example dimethylformamide, or chlorinated solvents, for example trichloromethane or dichloromethane. The temperature is usually in the range of about -30°C to about 60°C, conveniently at or near ambient temperature.

When -A- is -C(O)O-, L^1 is usually an "activated" hydroxy group. That is a group which acts as a leaving group in the same way as hydroxy, but is more labile. It can be formed in situ. An example of an activated hydroxy group is 4-nitrophenoxy, which can be formed by reacting a hydroxy group (HO-$[CH(R^a)]_a$-B-$[CH(R^b)]_b$-D) with 4-nitrophenylchloroformate. This reaction is usually carried out in an organic solvent such as dichloromethane, acetonitrile or tetrahydrofuran, in a temperature range of about -20^{0}C to the reflux temperature of the solvent. In addition, organic base such as triethylamine or N-methylmorpholine is normally present. Alternatively, a compound of the formula (X) can be reacted with 4-nitrophenylchloroformate and the resulting intermediate reacted with

$HO-[CH(R^a)]_a-B-[CH(R^b)]_b-D$ under similar conditions to those described above for the reaction of a compound of the formula (X) with a compound of the formula $L^2-[CH(R^a)]_a-B-[CH(R^b)]_b-D$ wherein L^2 is 4-nitrophenoxy.

When -A- is $-CON(R^8)-$, L^1 is preferably halogeno, particularly chloro. Alternatively when -A- is -CONH-, a compound of the formula (X) can be reacted with an isocyanate of the formula $C\equiv N-[CH(R^a)]_a-B-[CH(R^b)]_b-D$. These reactions are conveniently carried out in the presence of a base, particularly an organic base, such as triethylamine, pyridine or N-methylmorpholine, in a solvent such as an ether solvent, for example tetrahydrofuran, or in a chlorinated solvent, for example dichloromethane, at a temperature in the range from about -20°C to the reflux temperature of the solvent. Alternatively, a compound of the formula (X) can be reacted with 4-nitrophenylchloroformate and the resulting intermediate reacted with $R^{17}-NH_2$ under similar conditions to those described above for the reaction of a compound of the formula (X) with a compound of the formula $L^2-[CH(R^a)]_a-B-[CH(R^b)]_b-D$ wherein L^2 is 4-nitrophenoxy.

When -A- is of the formula $-SO_2-$ or $-SO_2N(R^8)-$, L^1 is preferably halogeno, for example chloro. The reaction is conveniently carried out in the presence of a base such as dimethylaniline, in a chlorinated solvent such as trichloromethane and at a temperature in the range of -20°C to about 60 °C, more preferably in pyridine, at a temperature in the range from -20°C to about 60 °C.

A compound of formula (I) may be prepared from another compound of formula (I) by chemical modification. Examples of chemical modifications include standard alkylation, arylation, heteroarylation, acylation, sulphonylation, phosphorylation, aromatic halogenation and coupling reactions. These reactions may be used to add new substituents or to modify existing substituents. Alternatively , existing substituents in compounds of formula (I) may be modified by, for example, oxidation, reduction, elimination, hydrolysis or other cleavage reactions to yield other compounds of formula (I).

Thus for example a compound of formula (I) containing an amino group may be acylated on the amino group by treatment with, for example, an acyl halide or anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for example, a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In another general example of an interconversion process, an amino group in a compound of formula (I) may be sulphonylated by treatment with, for example, an alkyl or aryl sulphonyl chloride or an alkyl or aryl sulphonic anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for example a solvent such as a hydrocarbon solvent e.g. dichloromethane, at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In a further general example, a compound of formula (I) containing a hydroxy group can be converted into the corresponding dihydrogenphosphate ester by treatment with for example di-tert-butyl diisopropylphosphoramidite or di-tert-butyl diethylphosphoramidite in the presence of a suitable catalyst, for example tetrazole. A solvent such as an ether solvent, for example tetrahydrofuran can be used. The reaction is usually carried out at a temperature in the range -40°C to 40°C, conveniently at or near ambient temperature, followed by treatment with an oxidising agent for example 3-chloroperoxy benzoic acid at a temperature in the range -78°C to 40°C preferably -40°C to 10°C. The resulting intermediate phosphate triester is treated with an acid, for example trifluoroacetic acid, in a solvent such as a chlorinated solvent e.g. dichloromethane at a temperature in the range -30°C to 40°C, conveniently at or near 0°C, to give the compound of formula (I) containing a dihydrogenphosphate ester.

In a further general example a compound of formula (I) containing an amide can be hydrolysed by treatment with for example an acid such as hydrochloric acid in a solvent such as an alcohol, for example methanol at an elevated temperature conveniently at the reflux temperature.

In another general example an alkoxy group may be converted to the corresponding alcohol (OH) by reaction with boron tribromide in a solvent such as a chlorinated solvent e.g. dichloromethane at a low temperature e.g. around -78°C.

In a further general example a compound of formula (I) may be alkylated by reaction with a suitable alkylating agent such as an alkyl halide, an alkyl toluenesulphonate, an alkyl methanesulphonate or an alkyl triflate. The alkylation reaction can be carried out in the presence of a base, for example an inorganic base such as a carbonate e.g. caesium or potassium carbonate, a hydride such as sodium hydride or an alkoxide such as potassium t-butoxide, in a suitable solvent such as an aprotic solvent e.g. dimethylformamide or an ether solvent such as tetrahydrofuran, at a temperature of around -10°C to 80°C.

In a further example, an unsubstituted ring nitrogen in a saturated heterocyclic ring may be acylated using similar reaction conditions to those described above for the acylation of an amino group.

Synthesis of Intermediates

A compound of the formula (X) may be known or prepared according to processes described in International Patent Application No. PCT/GB98/01977.

A compound of the formula (XI) may be known or prepared by methods known in the art. For example, when A is of the formula -C(O)O- and L^1 is 4-nitrophenyloxy, the compound of the formula (XI) may be formed by reacting a compound of the formula:

$$HO\text{-}[CH(R^7)]_a\text{ - B -}[CH(R^7)]_b\text{-D}$$

with 4-nitrophenyl chloroformate, in the presence of a base, preferably an organic base such as triethylamine, in an inert organic solvent such as dichloromethane. The reaction is usually carried out in a temperature range of -30°C to 60 °C, most commonly at around ambient temperature.

Acid addition salts of the compounds of formula (I) are prepared in a conventional manner by treating a solution or suspension of the free base of a compound of formula (I) with about one equivalent of a pharmaceutically acceptable acid. Salts of compounds of formula (I) derived from inorganic or organic bases are prepared in a conventional manner by treating a solution or suspension of the free acid of a compound of formula (I) with about one equivalent of a pharmaceutically acceptable organic or inorganic base. Alternatively both acid addition salts and salts derived from bases may be prepared by treatment of the parent compound with the appropriate ion-exchange resin in a standard fashion. Conventional concentration and recrystallistion techniques are employed in isolating the salts.

Compounds according to the invention are able to destroy vasculature that has been newly formed such as tumour vasculature while leaving unaffected normal, mature vasculature. The identification of compounds which selectively, and preferably potently, damage newly-formed vasculature is desirable and is the subject of the present invention. The ability of the compounds to act in this way may be assessed, for example, using one or more of the procedures set out below:

(a) Activity against tumour vasculature measured by radioactive tracer

This assay demonstrates the ability of compounds to damage selectively tumour vasculature.

Subcutaneous CaNT tumours were initiated by injecting 0.05ml of a crude tumour cell suspension, approximately 10^6 cells, under the skin overlying the rear dorsum of 12-16 week-old mice. The animals were selected for treatment after approximately 3-4 weeks, when their tumours reached a geometric mean diameter of 5.5-6.5 mm. Compounds were dissolved in sterile saline and injected intraperitoneally in a volume of 0.1 ml per 10g body weight. Tumour perfusion was measured 6 hours after intraperitoneal administration in tumour, kidney, liver, skin, muscle, gut and brain by the ^{86}RbCl extraction technique (Sapirstein, Amer. Jnl. Physiol., 1958, 193, 161-168). Tissue radioactivity measured 1 minute after an intravenous injection of ^{86}RbCl was used to calculate relative blood flow as a proportion of cardiac output (Hill and Denekamp, Brit. Jnl. Radiol., 1982, 55, 905-913). Five animals were used in control and treated groups. Results were expressed as a percentage of the blood flow in the corresponding tissues in vehicle treated animals.

(b) Activity against tumour vasculature measured by fluorescent dye

This assay demonstrates the ability of compounds to damage tumour vasculature.

Tumour functional vascular volume in CaNT tumour-bearing mice was measured using the fluorescent dye Hoechst 33342 according to the method of Smith et al (Brit. Jnl. Cancer 1988, 57, 247-253). Five animals were used in control and treated groups. The fluorescent dye was dissolved in saline at 6.25mg/ml and injected intravenously at 10mg/kg 24 hours after intraperitoneal drug treatment. One minute later, animals were killed and tumours excised and frozen; 10μm sections were cut at 3 different levels and observed under UV illumination using an Olympus microscope equipped with epifluorescence. Blood vessels were identified by their fluorescent outlines and vascular volume was quantified using a point scoring system based on that described by Chalkley, (Jnl. Natl. Cancer Inst., 1943, 4, 47-53). All estimates were based on counting a minimum of 100 fields from sections cut at the 3 different levels.

The ability of the compounds to bind to preparations of mammalian tubulin can be evaluated by a number of methods available in the literature, for example by following temperature initiated tubulin polymerisation by turbidity in the absence and presence of the compound (for example O.Boye *et al* Med. Chem. Res., 1991, 1, 142-150).

The activity of *N*-[3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide, (V. Fernholz Justus Liebigs Ann., 1950, 568, 63-72), against tumour vasculature was measured by the fluorescent dye method described above. This compound decreased perfused vascular volume by 88% relative to control when dosed at 50mg/kg intraperitoneally. The IC_{50} of this compound in a tubulin polymerisation assay was 58 micromolar (O.Boye *et al* Med. Chem. Res., 1991, 1, 142-150).

(c) HUVEC detachment assay

This assay examined the effects of compounds on the adherence of HUVECs to tissue culture plasticware.

HUVECs were plated in 0.2% gelatin-coated 12 well tissue culture plates at a concentration of $3x10^4$ cells per well in 1ml TCS medium. After 24 hours, when the cells were at ~30% confluency, the cells were dosed with compound for 40 minutes at 37°C, 5% CO_2. After this incubation the medium containing drug was pipetted off, and the cells were then gently washed in 2mls of HBSS (Hanks' Balanced Salt Solution purchased from Life Technologies Ltd, Paisley UK; Catalogue # 24020-083) to remove any detached cells. The washing solution was then removed, and the adherent cells remaining were trypsinised using 300µl of 1x Trypsin-EDTA solution (Life Technologies Ltd, Paisley, UK; Catalogue # 43500-019) at ambient temperature for 2 minutes. The trypsinised cells were then made up to 1ml with TCS Biologicals medium, then centrifuged at 2000rpm for 2 minutes. The cell pellet was then resuspended in a volume of 50µl of TCS Biologicals medium. Total cell counts were obtained by counting the cells on a haemocytometer. The amount of cell detachment was calculated by comparing the number of cells remaining attached following treatment with the number in undosed control wells.

(d) Hras5 necrosis model

NIH 3T3 fibroblasts transfected with Harvey ras, clone 5, (Hras5 cells) were kept in continual passage in Dulbecco's modifed Eagles medium (DMEM) containing 10% foetal bovine serum (FBS) and 1% glutamine, at 37°C in a humidified incubator gassed with 7.5% carbon dioxide and 92.5% oxygen. Cells were implanted subcutaneously into the left flank of male nude mice (8-10weeks of age) at an inoculum of 2×10^5 cells/mouse. Tumours were measured using calipers and randomised into groups of 2-4 mice between days 9-14 after implant. Mice were dosed with compounds, either intravenously or intraperitoneally, once on

day of randomisation and culled 24 hours after dosing. Compounds were dissolved in 20% hydroxypropyl beta cyclodextrin in physiological saline at pH 7 and dosed in a volume of 0.1ml per 10g body weight. Tumours were excised, weighed and placed in buffered formalin. Area of necrosis in individual tumours was assessed from a haematoxylin/eosin stained-slide by a pathologist and scored from 0, meaning no significant change, to 10, meaning 91-100% necrosis. The activity of examples 5 and 7 (described hereinafter) against tumour vasculature was measured by the fluorescent dye method described hereinabove. Example 1 scored 6.0 at 100m/kg and example 4 scored 3.2 at 50m/kg.

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises a compound of the formula (I) as defined hereinbefore or a pharmaceutically acceptable salt, solvate or pro-drug thereof, in association with a pharmaceutically acceptable excipient or carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for nasal administration or administration by inhalation, for example as a powder or solution, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) for example as a sterile solution, suspension or emulsion, for topical administration for example as an ointment or cream or for rectal administration for example as a suppository. In general the above compositions may be prepared in a conventional manner using conventional excipients.

The compositions of the present invention are advantageously presented in unit dosage form. The compound will normally be administered to a warm-blooded animal at a unit dose within the range 5-5000mg per square metre body area of the animal, i.e. approximately 0.1-100mg/kg. A unit dose in the range, for example, 1-100mg/kg, preferably 1-50mg/kg is envisaged and this normally provides a therapeutically-effective dose. A unit dose form such as a tablet or capsule will usually contain, for example 1-250mg of active ingredient.

As stated above the size of the dose required for the therapeutic or prophylactic treatment of a particular disease state will necessarily be varied depending on the host treated, the route of administration and the severity of the illness being treated. Preferably a daily dose in the range of 1-50mg/kg is employed. However the daily dose will necessarily be varied depending upon the host treated, the particular route of administration, and the severity

of the illness being treated. Accordingly the optimum dosage may be determined by the practitioner who is treating any particular patient.

According to a further aspect of the present invention there is provided a compound of the formula (I) or a pharmaceutically acceptable salt, solvate or pro-drug thereof as defined hereinbefore for use in a method of treatment of the human or animal body by therapy.

A further feature of the present invention is a compound of formula (I), or a pharmaceutically acceptable salt, solvate or pro-drug thereof, for use as a medicament, conveniently a compound of formula (I), or a pharmaceutically acceptable salt, solvate or pro-drug thereof, for use as a medicament for producing a vascular damaging effect in a warm-blooded animal such as a human being.

Thus according to a further aspect of the invention there is provided the use of a compound of the formula (I), or a pharmaceutically acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm-blooded animal such as a human being.

According to a further feature of the invention there is provided a method for producing a vascular damaging effect in a warm-blooded animal, such as a human being, in need of such treatment which comprises administering to said animal an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt, solvate or pro-drug thereof as defined hereinbefore.

According to a further aspect of the present invention there is provided a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition, when dosed in divided doses (also known as split doses) produces a greater anti-tumour effect than when a single dose is given.

Anti-tumour effects of a method of treatment of the present invention include but are not limited to, inhibition of tumour growth, tumour growth delay, regression of tumour, shrinkage of tumour, increased time to re-growth of tumour on cessation of treatment, slowing of disease progression. It is expected that when a method of treatment of the present invention is administered to a warm-blooded animal such as a human, in need of treatment for cancer involving a solid tumour, said method of treatment will produce an effect, as measured by, for example, one or more of: the extent of the anti-tumour effect, the response rate, the time to disease progression and the survival rate.

According to a further aspect of the present invention there is provided a method for the production of a vascular damaging effect in a warm-blooded animal such as a human, which comprises administering to said animal in divided doses an effective amount of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition.

According to a further aspect of the present invention there is provided a method for the treatment of a cancer involving a solid tumour in a warm-blooded animal such as a human, which comprises administering to said animal in divided doses an effective amount of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition.

According to a further aspect of the present invention there is provided a medicament comprising two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition, which together add up to a total daily dose, for administration in divided doses for use in a method of treatment of a human or animal body by therapy.

According to a further aspect of the present invention there is provided a kit comprising two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition, which together add up to a total daily dose, for administration in divided doses.

According to a further aspect of the present invention there is provided a kit comprising:

a) two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, which together add up to a total daily dose, in unit dosage forms for administration in divided doses; and

b) container means for containing said dosage forms.

According to a further aspect of the present invention there is provided a kit comprising:

a) two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, which together add up to a total daily dose, together with a pharmaceutically acceptable excipient or carrier, in unit dosage forms; and

b) container means for containing said dosage forms.

According to a further aspect of the present invention there is provided the use of compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for administration in divided doses for use in the production of a vascular damaging effect in a warm-blooded animal such as a human.

According to a further aspect of the present invention there is provided the use of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for administration in divided doses for use in the production of an anti-cancer effect in a warm-blooded animal such as a human.

According to a further aspect of the present invention there is provided the use of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for administration in divided doses for use in the production of an anti-tumour effect in a warm-blooded animal such as a human.

Divided doses, also called split doses, means that the total dose to be administered to a warm-blooded animal, such as a human, in any one day period (for example one 24 hour period from midnight to midnight) is divided up into two or more fractions of the total dose and these fractions are administered with a time period between each fraction of about greater than 0 hours to about 10 hours, preferably about 1 hour to about 6 hours, more preferably about 2 hours to about 4 hours. The fractions of total dose may be about equal or unequal.

Preferably the total dose is divided into two parts which may be about equal or unequal.

The time intervals between doses may be for example selected from:
about 1 hour, about 1.5 hours, about 2 hours, about 2.5 hours, about 3 hours, about 3.5 hours, about 4 hours, about 4.5 hours, about 5 hours, about 5.5 hours and about 6 hours.

The time intervals between doses may be any number (including non-integers) of minutes between greater than 0 minutes and 600 minutes, preferably between 45 and 375 minutes inclusive. If more than two doses are administered the time intervals between each dose may be about equal or unequal.

Preferably two doses are given with a time interval in between them of greater than or equal to 1 hour and less than 6 hours.

More preferably two doses are given with a time interval in between them of greater than or equal to two hours and less than 5 hours.

Yet more preferably two doses are given with a time interval in between them of greater than or equal to two hours and less than or equal to 4 hours.

Particularly the total dose is divided into two parts which may be about equal or unequal with a time interval between doses of greater than or equal to about two hours and less than or equal to about 4 hours.

More particularly the total dose is divided into two parts which may be about equal with a time interval between doses of greater than or equal to about two hours and less than or equal to about 4 hours.

For the avoidance of doubt the term 'about' in the description of time periods means the time given plus or minus 15 minutes, thus for example about 1 hour means 45 to 75 minutes, about 1.5 hours means 75 to 105 minutes. Elsewhere the term 'about' has its usual dictionary meaning.

The antiangiogenic treatment defined hereinbefore may be applied as a sole therapy or may involve, in addition to a compound of the invention, one or more other substances and/or treatments. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate administration of the individual components of the treatment. In the field of medical oncology it is normal practice to use a combination of different forms of treatment to treat each patient with cancer. In medical oncology the other component(s) of such conjoint treatment in addition to the antiangiogenic treatment defined hereinbefore may be: surgery, radiotherapy or chemotherapy. Such chemotherapy may include the following categories of therapeutic agent:

(i) other antiangiogenic agents that work by different mechanisms from those defined hereinbefore (for example linomide, inhibitors of integrin $\alpha v\beta 3$ function, angiostatin, endostatin, razoxin, thalidomide) and including vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitors (RTKIs) (for example those described in International Patent Applications Publication Nos. WO 97/22596, WO 97/30035, WO 97/32856 and WO 98/13354 the entire disclosure of which documents is incorporated herein by reference);

(ii) cytostatic agents such as antioestrogens (for example tamoxifen,toremifene, raloxifene, droloxifene, iodoxyfene), progestogens (for example megestrol acetate), aromatase inhibitors (for example anastrozole, letrazole, vorazole, exemestane), antiprogestogens, antiandrogens (for example flutamide, nilutamide, bicalutamide, cyproterone acetate), LHRH agonists and antagonists (for example goserelin acetate, luprolide), inhibitors of testosterone 5α-dihydroreductase (for example finasteride), anti-invasion agents (for example metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator

receptor function) and inhibitors of growth factor function, (such growth factors include for example epidermal growth factor (EGF), platelet derived growth factor and hepatocyte growth factor such inhibitors include growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors and serine/threonine kinase inhibitors);

(iii) biological response modifiers (for example interferon);

(iv) antibodies (for example edrecolomab); and

(v) antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as antimetabolites (for example antifolates like methotrexate, fluoropyrimidines like 5-fluorouracil, purine and adenosine analogues, cytosine arabinoside); antitumour antibiotics (for example anthracyclines like doxorubicin, daunomycin, epirubicin and idarubicin, mitomycin-C, dactinomycin, mithramycin); platinum derivatives (for example cisplatin, carboplatin); alkylating agents (for example nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide, nitrosoureas, thiotepa); antimitotic agents (for example vinca alkaloids like vincristine and taxoids like taxol, taxotere); enzymes (for example asparaginase); thymidylate synthase inhibitors (for example raltitrexed); topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan, irinotecan).

As stated above the compounds defined in the present invention are of interest for their vascular damaging effects. Such compounds of the invention are expected to be useful in the prophylaxis and treatment of a wide range of disease states where inappropriate angiogenesis occurs including cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation, endometriosis, dysfunctional uterine bleeding and ocular diseases with retinal vessel proliferation. In particular such compounds of the invention are expected to slow advantageously the growth of primary and recurrent solid tumours of, for example, the colon, breast, prostate, lungs and skin.

In addition to their use in therapeutic medicine, the compounds of formula (I) and their pharmaceutically acceptable salts, solvates or pro-drugs are also useful as pharmacological tools in the development and standardisation of in vitro and in vivo test systems for the evaluation of the effects of vascular damaging agents in laboratory animals such as cats, dogs, rabbits, monkeys, rats and mice, as part of the search for new therapeutic agents.

It is to be understood that where the term "ether" is used anywhere in this specification it refers to diethyl ether.

The invention will now be illustrated in the following non-limiting Examples in which, unless otherwise stated:

(i) evaporations were carried out by rotary evaporation in vacuo and work-up procedures were carried out after removal of residual solids such as drying agents by filtration;

(ii) operations were carried out at ambient temperature, that is in the range 18-25°C and under an atmosphere of an inert gas such as argon or nitrogen;

(iii) yields are given for illustration only and are not necessarily the maximum attainable;

(iv) the structures of the end-products of the formula (I) were confirmed by nuclear (generally proton) magnetic resonance (NMR) and mass spectral techniques; proton magnetic resonance chemical shift values were measured on the delta scale and peak multiplicities are shown as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; q, quartet, quin, quintet;

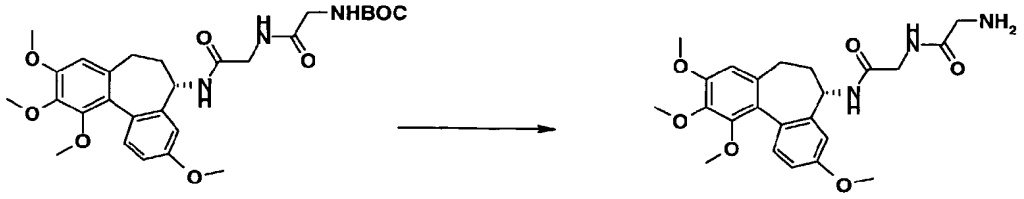
(v) intermediates were not generally fully characterised and purity was assessed by thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), infra-red (IR) or NMR analysis;

Abbreviations

4-Dimethylaminopyridine	DMAP
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride	EDCI
Dimethyl sulphoxide	DMSO
Trifluoroacetic acid	TFA

Example 1

N-[(5S) -3,9,10,11-Tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-2-[2-aminoacetylamino]acetamide



A solution of N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-2-[2-(butoxycarbonylamino)acetylamino]acetamide (0.9 g ; 0.64 mmol) in dichloromethane (6 ml) was treated with TFA (6 ml) at ambient temperature for 0.5 hour. After evaporation to dryness, the residue was neutralised to pH 6.5 with solid sodium hydrogen carbonate and purified on reverse phase silica eluting with a gradient of 30-40 % methanol / ammonium carbonate buffer (2 g / l, pH 7). The appropriate fractions were evaporated to dryness and triturated in ether to give the title compound.

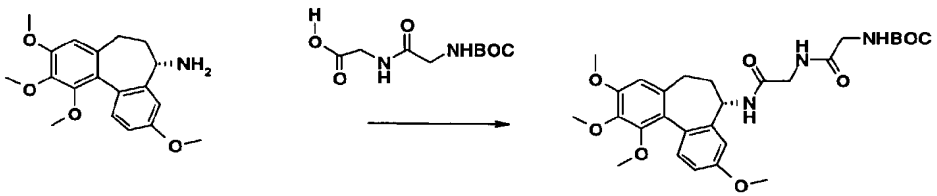
Yield : 65 %

^{1}H NMR (DMSO-d_6) : 1.88-2.21 (m, 3H) ; 2.58 partially obscured by DMSO peak (m, 1H) ; 3.10 (s, 2H) ; 3.46 (s, 3H) ; 3.79 (s, 3H) ; 3.82 (s, 3H) ; 3.83 (s, 3H) ; 3.84 (s, 3H) ; 4.47-4.58 (m, 1H) ; 6.77 (s, 1H) ; 6.87 (dd, 1H) ; 6.91 (d, 1H) ; 7.25 (d, 1H) ; 8.06 (m, 1H) ; 8.41 (d, 1H).

MS-ESI : 444 $[MH]^+$

Elemental analysis	Found	C 59.14	H 6.44	N 9.08
$C_{23}H_{29}N_3O_6$, 1.2 H_2O	Requires	C 59.39	H 6.80	N 9.03

The starting material was prepared as follows:



A solution of (5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine [Collect. Czech. Chem. Commun. 1999, 64(2), 217-228] (0.329 g ; 1.36 mmol),

EDCI (0.230 g ; 1.2 mmol); DMAP (0.025 g, 0.2 mmol) and 2[2-(tert-butoxycarbonylamino) acetylamino] acetic acid (0.189 g ; 1.2 mmol) in dichloromethane was stirred under argon atmosphere overnight. The resulting precipitate was filtered and washed with ether to give N-[(5S) -3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo [a,c] cyclohepten-5-yl]-2-[2-(butoxycarbonylamino)acetylamino]acetamide as a white solid.

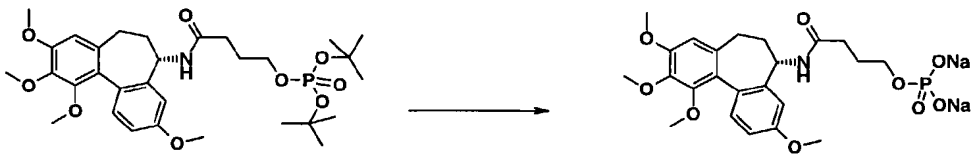
Yield : 65 %.

^{1}H NMR (DMSO-d_6) : 1.33 (s, 9H) ; 1.94-2.24 (m, 3H) ; 2.97-3.08 (m, 1H) ; 3.35 (s, 3H) ; 3.56 (t, 3H) ; 3.71-3.77 (m, 1H) ; 3.75 (s, 3H) ; 3.78 (s, 3H) ; 3.80 (s, 3H) ; 4.48-4.59 (m, 1H) ; 6.79 (s, 1H) ; 6.87 (dd, 1H) ; 6.93 (d, 1H) ; 7.14 (t, 1H) ; 7.25 (d, 1H) ; 8.17 (t, 1H) ; 8.21 (d, 1H).

MS-ESI : 544 $[MH]^+$

Example 2

4-Oxo-4-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]amino]butyl disodium phosphate

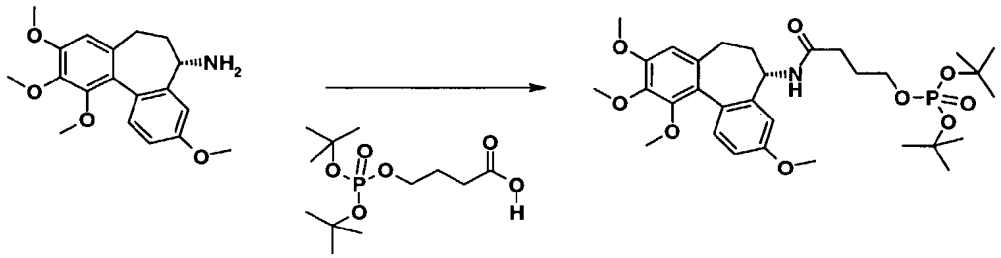


A solution of N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-4-[di(tert-butoxy)phosphoryloxy]butanamide (0.529g; 0.892 mmol) in a mixture of (12N) HCl (5 ml) and dioxan (25 ml) was stirred under argon atmosphere for 4 hours. After evaporation of the dioxan, the pH was adjusted at 7.2 with sodium hydroxide solution (2N) and the residue purified on HP20SS resin eluting with a 0-40 % gradient of methanol/water to give the title compound after freeze drying.

Yield : 75 %

^{1}H NMR (DMSO-d_6) : 1.71-2.36 (m, 7H) ; 2.58 partially obscured by DMSO peak (m, 1H) ; 3.49 (s, 3H) ; 3.78 -3.85 (m, 11H) ; 5.20 (dd, 1H) ; 5.00 (s, 1H) ; 6.77 (s, 1H) ; 6.88 (dd, 1H) ; 6.91 (d, 1H) ; 6.26 (d, 1H).

The starting material was prepared as follows:



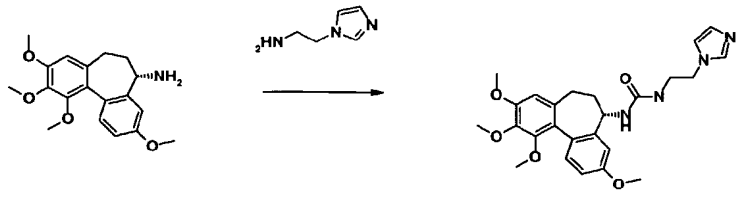
N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-4-[di-(tert-butoxy)phosphoryloxy]butanamide was prepared using a similar method to that of Example 1 by reacting (5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine with 4-[di(tert-butoxy)phosphoryloxy]butanoic acid.

Yield : 89 %

^{1}H NMR (DMSO-d_6): 1.40 (s, 18H) ; 1.80 (t, 2H) ; 1.82-1.94 (m, 1H) ; 2.00-2.20 (m, 2H ; 2.23-2.33 (m, 2H) ; 2.52-2.58 (m, 1H) ; 3.48 (s, 3H) ; 3.78 (s, 3H) ; 3.80-3.85 (m, 8H) ; 4.50-4.59 (m, 1H) ; 6.78 (s, 1H) ; 6.89 (dd, 1H) ; 6.90 (d, 1H) ; 7.26 (d, 1H) ; 8.42 (d, 1H).

Example 3

N-{N-[2-(Imidazol-1-yl)ethyl]carbamoyl}-5(S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine



A solution of (5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine (0.263 g ; 0.8 mmol), 4-nitrophenol chloroformate (0.177 g ; 0.88 mmol) and triethylamine (0.123 ml ; 0.88 mmol) in dichloromethane was stirred under argon atmosphere for 1 hour. 2-(Imidazol-1-yl)ethylamine (0.145 ml ; 1.2 mmol) was added. After stirring for 2 hours, the mixture was evaporated to dryness and the residue purified on reverse phase silica

eluting with a 40-60 % gradient of methanol / ammonium carbonate buffer (2 g/l, pH 7) to give the title compound after evaporation and trituration in ether.

Yield : 52 %.

^{1}H NMR (DMSO-d_6) : 1.66-1.77 (m, 1H) ; 1.97-2.10 (m, 1H) ; 2.13-2.25 (m, 1H) ; 2.53 partially obscured by DMSO peak (m, 1H) ; 3.12-3.32 (m, 2H) ; 3.47 (s, 3H) ; 3.77 (s, 3H) ; 3.79 (s, 3H) ; 3.83 (s, 1H) ; 3.94 (t, 3H) ; 4.32-4.42 (m, 1H) ; 5.97 (t, 1H) ; 6.63 (d, 1H) ; 6.77 (s, 1H) ; 6.83-6.92 (m, 3H) ; 7.11 (s, 1H) ; 7.24 (d, 1H) ; 7.54 (s, 1H).

MS-ESI : 481 $[MH]^+$

Elemental analysis	Found	C 64.68	H 6.89	N 11.55
$C_{26}H_{32}N_4O_5$	Requires	C 64.98	H 6.71	N 11.66

Example 4

2-{N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamoyloxy}ethyl disodium phosphate

A solution of 2-[di-(benzyloxy)phosphoryloxy]ethyl N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamate (0.576 g ; 0.85 mmol) in solution in methanol (10 ml) and ethyl acetate (5 ml) was hydrogenated in the presence of 10 % C/Pd (0.165 mg) for 4 hours. After filtration on celite and evaporation, the residue was purified on HP2O SS resin eluting with a 0-80 % gradient of methanol/distilled water. The pH of the corresponding fractions was adjusted at 8 with aqueous sodium hydroxide solution (2N), after evaporation of the methanol. After freeze drying the title compound was obtained as a white solid.

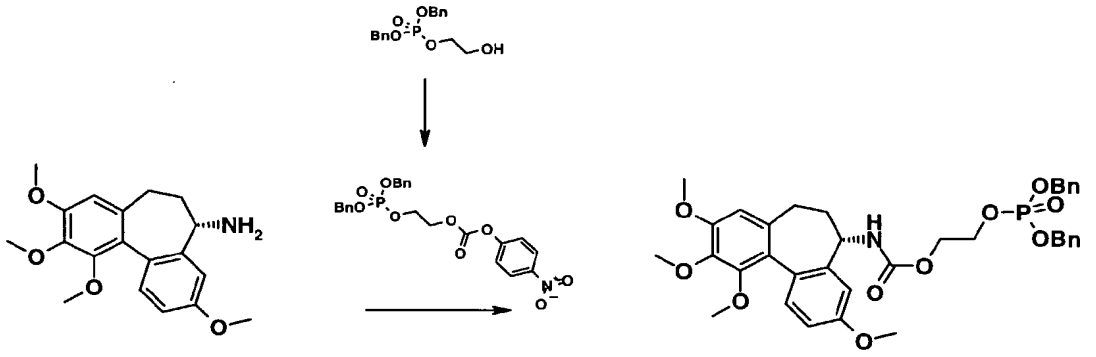
Yield : 83 %

^{1}H NMR (DMSO-d_6 + TFA-d) : 1.85-1.97 (m, 1H) ; 1.98-2.09 (m, 1H) ; 2.13-2.27 (m, 1H) ; 2.42-2.52 (m, 1H) ; 3.48 (s, 3H) ; 3.79 (s, 3H) ; 3.80 (s, 3H) ; 3.84 (s, 3H) ; 3.98 (m, 2H) ;

4.03-4.18 (m, 2H) ; 4.04-4.17 (m, 2H) ; 4.24-4.35 (m, 1H) ; 6.77 (s, 1H) ; 6.89 (dd, 1H) ; 6.96 (d, 1H) ; 7.27 (d, 1H).

MS-ESI : 498 $[MH]^+$

The starting material was prepared as follows:



4-Nitrophenyl chloroformate (1.01 g ; 5.04 mmol) was added at 0°C under argon atmosphere to a solution of 2-[di (benzyloxy)phosphoryloxy]ethanol (1.62 g ; 5.09 mmol) and triethylamine (0.7 ml ; 5 mmol) in dichloromethane (20 ml). The mixture was stirred at ambient temperature for 30 minutes, evaporated and purified by flash chromatography, eluting with petroleum ether / ethyl acetate (40/60) to give 2-[di(benzyloxy)phosphoryloxy]ethyl 4-nitrophenyl carbonate.

Yield : 45 %

^{1}HNMR ($CDCl_3$) : 4.21-4.30 (m, 2H) ; 4.41 (m, 2H) ; 5.01-5.15 (m, 4H) ; 7.29-7.42 (m, 12H) ; 8.25 (d, 2H).

A solution of (5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine (0.329 ; 1 mmol) and 2-[di-(benzyloxy)phosphoryloxy]ethyl 4-nitrophenyl carbonate (0.633 g ; 1.3 mmol) in acetonitrile (8 ml) was heated at 65°C, under argon atmosphere for 8 hours. After evaporation to dryness, the residue was purified by flash chromatography eluting with a 50-80 % gradient of ethyl acetate / petroleum ether to give 2-[di(benzyloxy) phosphoryloxy]ethyl N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamate.

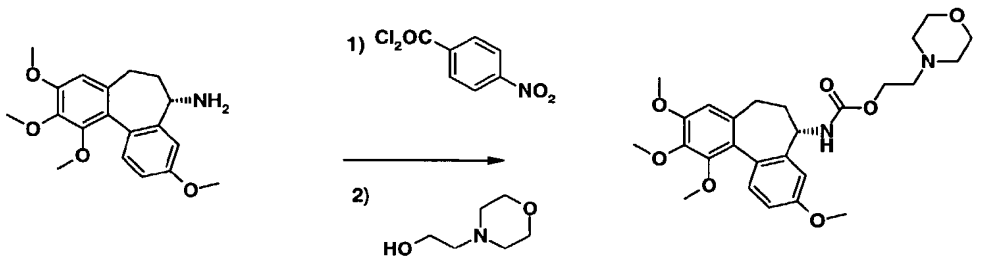
Yield : 85 %

^{1}H NMR (DMSO-d_6) : 1.81-1.93 (m, 1H) ; 1.94-2.06 (m, 1H) ; 2.06-2.20 (m, 1H) ; 2.40-2.52 (m, 1H) ; 3.43 (s, 3H) ; 3.73 (s, 3H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.11 (m, 4H) ; 4.20-4.33 (m,

4H); 5.02 (d, 4H); 6.76 (s, 1H); 6.86 -dd, 1H) ; 6.93 (d, 1H) ; 7.25 (d, 1H) . 7.35 (s, 10H) ; 7.99 (d, 1H).

Example 5

2-Morpholinoethyl *N*-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5*H*-dibenzo [a,c]cyclohepten-5-yl]carbamate



A solution of (5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine [Collect. Czech. Chem. Commun. 1999, 64(2), 217-228] (0.263 g ; 0.8 mmol), 4-nitrophenylchloroformate (0.177 g ; 0.88 mmol) and triethylamine (0.123 ml ; 0.88 mmol) in acetonitrile (5 ml) was stirred under argon atmosphere for 2 hours at ambient temperature ; 4-(2-hydroxyethyl)morpholine (0.145 ml ; 1.2 mmol) in solution in acetonitrile (2 ml) was then added to the above solution. The mixture was heated at 60°C overnight. After evaporation to dryness, the residue was purified by flash chromatography, eluted with ethanol/dichloromethane (4/96) to give the title compound.

Yield : 64 %

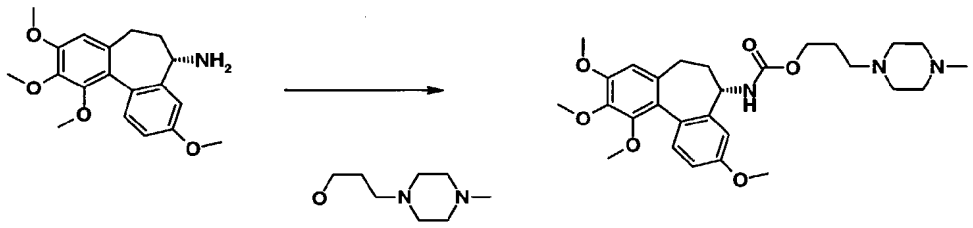
^{1}H NMR Spectrum (DMSO-d_6 + AcO-d_4) : 1.82-2.31 (m, 3H) ; 2.44 (m, 4H) ; 2.49 (m, 2H) ; 2.57 partially obscured by DMSO peak (m, 1H) ; 3.47 (s, 3H) ; 3.56 (m, 4H) ; 3.78 (s, 3H) ; 3.79 (s, 3H) ; 3.83 (s, 3H) ; 4.03 (m, 2H) ; 4.17-4.33 (m, 1H) ; 6.76 (s, 1H) ; 6.88 (dd, 1H) ; 6.93 (d, 1H) ; 7.26 (d, 1H) ; 7.86 (d, 1H).

MS-ESI : 487 $[MH]^+$

Elemental analysis :	Found	C 63.38	H 7.04	N 5.74
$C_{26}H_{34}N_2O_7$,0.3 H_2O	Requires	C 63.48	H 7.09	N 5.69

Example 6

3-(1-Methylpiperazin-4-yl)propyl N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo [a,c]cyclohepten-5-yl] carbamate



Using a similar procedure to that described for Example 5, (5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine was reacted with 4-(3-hydroxypropyl)-1-methylpiperazine to give the title compound.

Yield : 40 %.

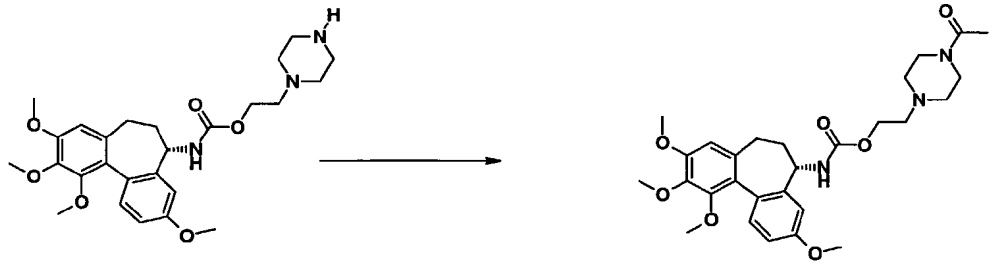
^{1}H NMR (DMSO-d_6) : 1.62-2.44 (m, 20H) ; 2.54 partially obscured by DMSO peak (m, 1H) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.78 (s, 3H) ; 3.82 (s, 3H) ; 6.78 (s, 1H) ; 6.89 (dd, 1H) ; 6.93 (d, 1H) ; 7.27 (d, 1H) ; 7.80 (d, 1H).

MS-ESI : 514 $[MH]^+$

Elemental analysis :	Found	C 65.42	H 7.54	N 8.18
$C_{28}H_{39}N_3O_6$	Requires	C 65.48	H 7.65	N 8.18

Example 7

2-(1-Acetylpiperazin-4-yl)ethyl-N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl] carbamate



A solution of 2-(piperazin-4-yl)ethyl-N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamate (0.255 g ; 0.525 mmol), acetyl chloride (0.038 ml ; 0.53 mmol) and triethylamine (0.073 ml ; 0.525 mmol) in dichloromethane (10 ml) was stirred at ambient temperature under argon atmosphere for 2 hours. After evaporation to

dryness, the residue was purified by flash chromatography, eluting with dichhloromethane/ethanol (93/7) to give the title compound.

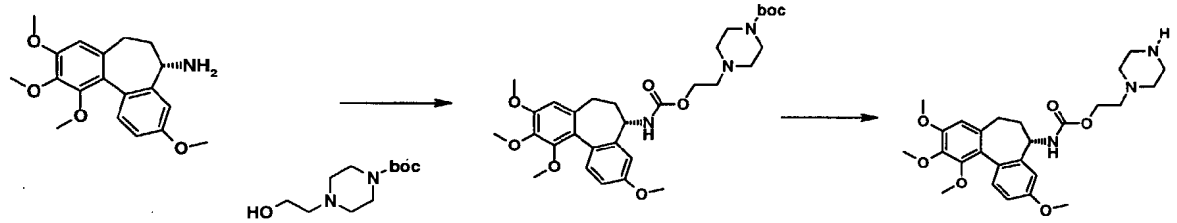
Yield : 90 %

^{1}H NMR (DMSO-d_6) : 1.81-2.32 (m, 3H) ; 1.97 (s, 3H) ; 2.33 (m, 2H) ; 2.40 (m, 2H) ; 2.47 partially obscured by DMSO peak (m, 2H) ; 2.58 partially obscured by DMSO peak (m, 1H) ; 3.38 (m, 4H) ; 3.46 (s, 3H) ; 3.77 (m, 3H) ; 3.80 (s, 3H) ; 3.82 (s, 3H) ; 3.92-4.10 (m, 2H) ; 4.20-4.30 (m, 1H) ; 6.77 (s, 1H) ; 6.88 (dd, 1H) ; 6.92 (d, 1H) ; 7.25 (d, 1H) ; 7.87 (d, 1H).

MS-ESI : 528 $[MH]^+$

Elemental analysis :	Found	C 63.21	H 7.30	N 7.86
$C_{28}H_{37}N_3O_7$	Requires	C 63.52	H 7.08	N 7.94

The starting material was prepared as follows :



2-(1-tert-Butoxycarbonylpiperazin-4-yl)ethyl-N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamate was prepared using a similar method to that described for Example 1, but using 2-[1-(tert-butoxycarbonyl)piperazin-4-yl]ethanol.

Yield : 75 %

^{1}H NMR (DMSO-d_6) : 1.78-1.94 (m, 1H) ; 1.95-2.07 (m, 1H) ; 2.10-2.23 (m, 1H) ; 2.30 (m, 4H) ; 2.38-2.53 (m, 3H) ; 2.65 (t, 2H) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.78 (s, 3H) ; 3.82 (s, 3H) ; 3.99 (t, 2H) ; 4.20-4.32 (m, 1H) ; 6.77 (s, 1H) ; 6.88 (dd, 1H) ; 6.92 (d, 1H) ; 7.25 (d, 1H) ; 7.86 (d, 1H).

2-(-1- tert-Butoxycarbonylpiperazin-4-yl)ethyl-N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo [a,c]cyclohepten-5-yl] carbamate (0.617 g ; 1.05 mmol) in dichloromethane (10 ml) was treated with TFA (5 ml) at ambient temperature for 1 hour. After evaporation to dryness, the residue was neutralised to pH 8 with sodium hydroxide solution and purified on reverse phase silica eluting with a gradient of 30 - 40 % methanol / ammonium carbonate

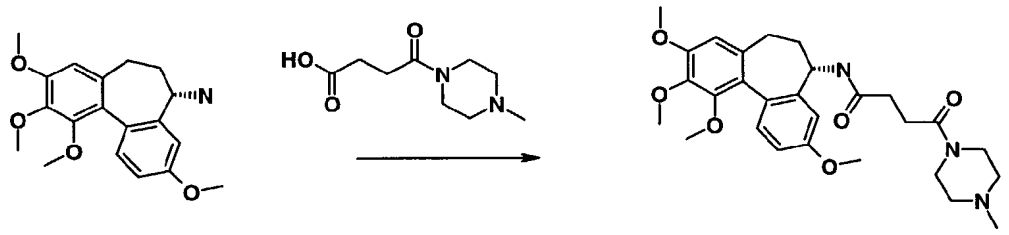
buffer (2 g/l, pH 7) to give 2-(piperazin-4-yl) ethyl- N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl] carbamate.

Yield : 60 %

^{1}H NMR (DMSO-d_6) : 1.39 (s, 9H) ; 1.81-1.94 (m, 1H) ; 1.95-2.07 (m, 1H) ; 2.09-2.27 (m, 1H) ; 2.34 (m, 4H) ; 2.52-2.64 (m, 1H) ; 3.28 (m, 2H) ; 3.36 (s, 3H) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 3.94-4.09 (m, 2H) ; 4.20-4.30 (m, 1H) ; 6.77 (s, 1H) ; 6.87 (dd, 1H) ; 6.92 (d, 1H) ; 7.25 (d, 1H) ; 7.86 (d, 1H).

Example 8

N-[(5S)-3,9,10,11-Tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl] -4-(1-methylpiperazin-4-yl)-4-oxobutan-l-amide



A solution of 4-(1-methylpiperazin-4-yl)-4-oxo butanoic acid (0.356 g ; 1.78 mmol), EDCI (0.367 g ; 1.78 mmol) and DMAP (0.05 g ; 0.41 mmol) in dichloromethane (30 ml) was stirred for 35 minutes under argon atmosphere. (5S)-3,9,10,11-Tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine 0.45 g ; 1.37 mmol) was then added and the mixture stirred at ambient temperature overnight. After evaporation of the solvent, the residue was purified by flash chromatography, eluting with dichloromethane/ethanol (95/5) to give after evaporation and trituration in pentane, the title compound as a white solid.

Yield : 60 %

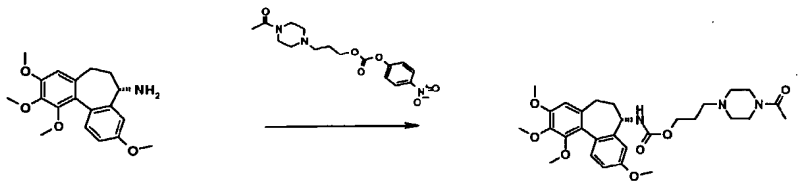
^{1}H NMR (DMSO-d_6) : 1.85-1.96 (m, 1H) ; 2.01-2.15 (m, 1H) ; 2.16 (s, 3H) ; 2.22 (t, 2H) ; 2.26 (t, 2H) ; 2.33-2.42 (m, 1H) ; 2.47-2.53 (m, 1H) ; 3.35-3.46 (m, 4H) ; 3.46 (s, 3H) ; 3.79 (s, 3H) ; 3.82 (s, 3H) ; 3.84 (s, 3H) ; 4.44-4.56 (m, 1H) . 6.79 (s, 1H) ; 6.86 (dd, 1H) ; 6.98 (d, 1H) ; 7.25 (d, 1H) ; 8.40 (d, 1H).

MS-ESI : 512 $[MH]^+$

Elemental analysis	Found	C 65.51	H 7.40	N 8.19
$C_{28}H_{37}N_3O_6$	Requires	C 65.73	H 7.29	N 8.21

Example 9

3-(1-Acetylpiperazin-4-yl) propyl-N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamate



A solution of (5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine (0.329 g ; 1 mmol) and 3-(-4-acetylpiperazino)propyl 4-nitrophenylcarbonate (0.456 g; 1.3 mmol) in acetonitrile (8 ml) was heated under argon atmosphere at 70°C for 6 hours. After evaporation to dryness, the residue was purified by flash chromatography eluting with dichloromethane/ethanol (93/7) to give the title compound.

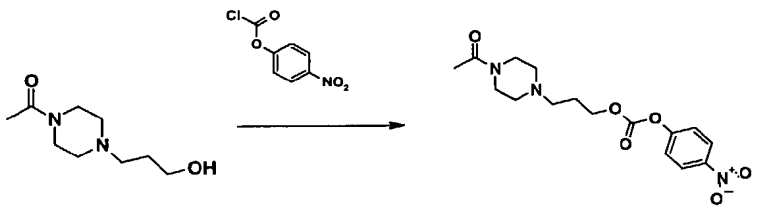
Yield : 80 %

^{1}H NMR (DMSO-d_6) : 1.62-2.51 (m, 12H) ; 1.97 (s, 3H) ; 3.32-3.44 (m, 4H) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.78 (s, 3H) ; 3.82 (s, 3H) ; 3.87-4.01 (m, 2H) ; 4.19-4.31 (m, 1H) ; 6.77 (s, 1H) ; 6.88 (dd, 1H) ; 6.92 (d, 1H) ; 7.25 (d, 1H) ; 7.80 (d, 1H).

MS-ESI : 542 $[MH]^+$

Elemental analysis	Found	C 63.48	H 7.25	N 7.72
$C_{29}H_{39}N_3O_7$, 0.4 H_2O	Requires	C 63.46	H 7.31	N 7.66

The starting material was prepared as follows :

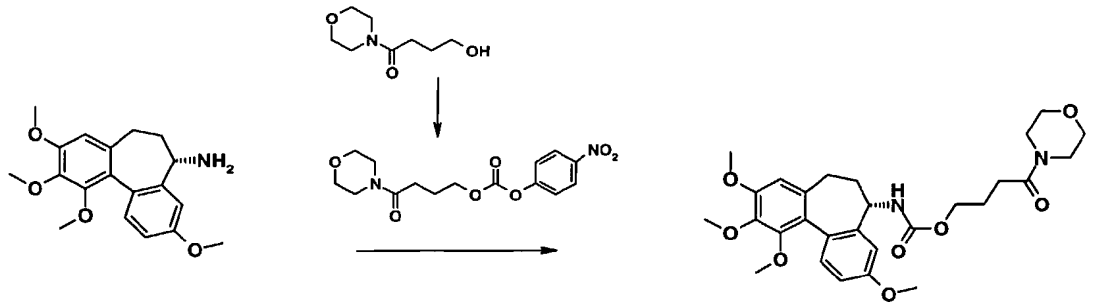


4-Nitrophenyl chloroformate (0.733 g ; 3.63 mmol) was added to a solution of 3-(4-acetylpiperazin-1-yl) propanol [Synthesis (1997), 6, 643-648] (0.645 g ; 3.46 mmol) and triethylamine (0.51 ml ; 3.36 mmol) in dichloromethane (7 ml) under argon atmosphere at 0°C. The mixture was stirred at ambient temperature for 1 hour, evaporated to dryness and purified by flash chromatography, eluting with dichloromethane/ethanol (95/5) to give 3(-4-acetylpiperazino)propyl 4-nitrophenyl carbonate.

^{1}H NMR ($CDCl_3$) : 1.96 (m, 2H) ; 2.09 (s, 3H) ; 2.39-2.48 (m, 4H) ; 2.51 (t, 2H) ; 3.47 (t, 3H); 3.63 (t, 2H) ; 3.68-3.78 (m, 2H) ; 4.38 (t, 2H) ; 7.39 (d, 2H) ; 8.29 (d, 2H).

Example 10

4-Morpholino-4-oxobutyl-N-[(5S)-3,9,10, 11-tetramethoxy-6,7-dihydro-5H-dibenzo [a-c]cyclohepten-5-yl]carbamate



The compound was prepared using a similar method to that of Example 9, but replacing 3-(4-acetylpiperazino)propyl 4-nitrophenyl carbonate by 4-morpholino-4-oxobutyl 4-nitrophenylcarbonate.

Yield : 55 %.

^{1}H NMR (DMSO-d_6) : 1.71-1.81 (m, 2H) ; 1.82-1.94 (m, 1H) ; 1.95-2.07 (s, 1H) ; 2.11-2.24 (s, 1H) ; 2.34 (t, 2H) ; 2.46 (m, 1H) ; 3.31-3.44 (m, 4H) ; 3.45 (s, 3H) ; 3.52 (m, 4H) ; 3.77 (s, 3H) ; 3.78 (s, 3H) ; 3.82 (s, 3H) ; 3.86-3.98 (m, 2H) ; 4.20-4.32 (m, 1H) ; 6.77 (s, 1H) ; 6.88 (dd, 1H) ; 6.92 (d, 1H) ; 7.25 (d, 1H) ; 7.80 (d, 1H).

MS-ESI : 529 $[MH]^+$

Elemental analysis	Found	C 62.81	H 6.95	N 5.27
$C_{28}H_{36}N_2O_8$, 0.3 H_2O	Requires	C 62.98	H 6.91	N 5.25

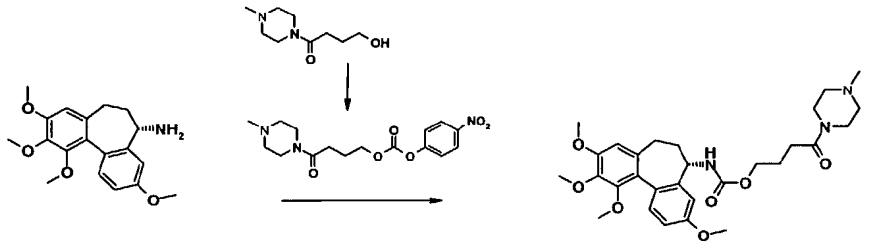
The starting material was prepared using a similar method to that of example 9, starting from 4-morpholino-4-oxobutyl 4-nitrophenyl carbonate.

Yield : 92 %

^{1}H NMR ($CDCl_3$) : 2.15 (m, 2H) ; 2.50 (t, 2H) ; 3.46-3.53 (m, 2H) ; 3.51-3.75 (m, 6H) ; 3.38 (t, 2H) ; 7.33 (d, 2H) ; 8.29 (d, 2H).

Example 11

4-(1-Methylpiperazin-4-yl)-4-oxobutyl-N-[(5S)-3,9,10, 11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cylcohepten-5-yl]carbamate



The title compound was prepared using a similar method to that of Example 9, but replacing 3-(4-acetylpiperazino)propyl 4-nitrophenyl carbonate by 4-methylpiperazin-l-yl)-4-oxobutyl 4-nitrophenyl carbonate.

Yield : 65%

^{1}H NMR (DMSO-d_6) : 1.75 (m, 2H) ; 1.81-2.07 (m, 2H) ; 2.08-2.40 (m, 7H) ; 2.15 (s, 3H) ; 2.50-2.60 (m, 1H) ; 3.22-3.56 (m, 4H) ; 3.45 (s, 3H) ; 3.77 (s, 3H) ; 3.78 (s, 3H) ; 3.82 (s, 3H) ; 3.82-3.99 (m, 2H) ; 4.12-4.32 (m, 1H) ; 6.76 (s, 1H) ; 6.87 (dd, 1H) ; 6.92 (d, 1H) ; 7.25 (d, 1H) ; 7.80 (d, 1H).

MS-ESI : 542 $[MH]^+$

Elemental analysis	Found	C 63.38	H 7.58	N 7.64
$C_{29}H_{39}N_3O_7$, 0.4 H_2O	Requires	C 63.46	H 7.31	N 7.66

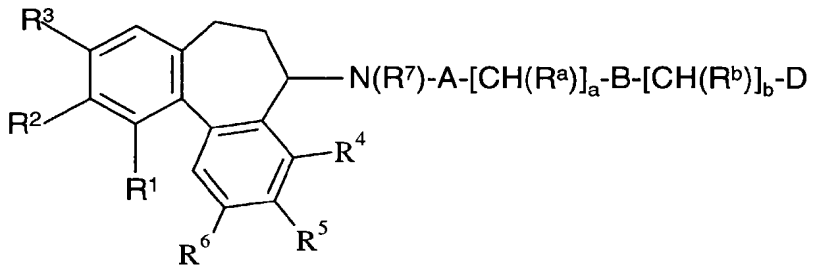
The starting material was prepared using a similar method to that of Example 9, from 4-(4-methylpiperazin-l-yl)-4-oxobutanol.

Yield : 65 %.

^{1}H NMR ($CDCl_3$) : 2.08-2.19 (m, 2H) ; 2.32 (s, 3H) ; 2.35-2.46 (m, 4H) ; 2.49 (t, 2H) ; 3.51 (t, 2H) ; 2.66 (t, 2H) ; 4.38 (t, 2H) ; 7.39 (d, 2H) ; 8.29 (d, 2H).

Claims

1. A compound of the formula (I):



(I)

wherein:

$\mathbf{R^1}$, $\mathbf{R^2}$ and $\mathbf{R^3}$ are each independently hydroxy, phosphoryloxy ($-OPO_3H_2$), C_{1-4}alkoxy or an in vivo hydrolysable ester of hydroxy, with the proviso that at least 2 of R^1, R^2 and R^3 are C_{1-4}alkoxy;

A is - CO-, -C(O)O-, $-CON(R^8)$-, $-SO_2$- or $-SO_2N(R^8)$- (wherein $\mathbf{R^8}$ is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl or hydroxyC_{1-3}alkyl);

a is an integer from 1 to 4 inclusive;

$\mathbf{R^a}$ and $\mathbf{R^b}$ are independently selected from hydrogen, hydroxy and amino;

B is -O-, -CO-, $-N(R^9)CO$-, $-CON(R^9)$ -, -C(O)O-, $-N(R^9)$ -, - $N(R^9)C(O)O$-, $-N(R^9)CON(R^{10})$-, $-N(R^9)SO_2$-, $-SO_2N(R^9)$- or a direct single bond (wherein $\mathbf{R^9}$ and $\mathbf{R^{10}}$ are independently selected from hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl and hydroxyC_{1-3}alkyl);

b is 0 or an integer from 1 to 4 inclusive, (provided that when b is 0, B is a single direct bond);

D is carboxy, sulpho, tetrazolyl, imidazolyl, phosphoryloxy, hydroxy, amino, N-(C_{1-4}alkyl)amino, N,N-di(C_{1-3}alkyl)amino or of the formula $-Y^1-(CH_2)_cR^{11}$ or $-NHCH(R^{12})COOH$; [wherein $\mathbf{Y^1}$ is a direct single bond, -O-, -C(O)-, $-N(R^{13})$-, $-N(R^{13})C(O)$- or $-C(O)N(R^{13})$- (wherein $\mathbf{R^{13}}$ is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{2-3}alkyl, aminoC_{2-3}alkyl or hydroxyC_{2-3}alkyl); **c** is 0 or an integer from 1 to 4 inclusive; $\mathbf{R^{11}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from

O, S and N, or a 5-6-membered unsaturated or partially unsaturated heteroaryl group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, which heterocyclic group or heteroaryl group may bear 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-(C_{1-4}alkyl)carbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, aminoC_{1-4}alkyl, N-C_{1-4}alkylaminoC_{1-4}alkyl, di-N,N-(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein **R^{14}** is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl);

R^{12} is an amino acid side chain;

R^5 is C_{1-4}alkoxy;

R^4 and **R^6** are each independently selected from: hydrogen, fluoro, nitro, amino, N-C_{1-4}alkylamino, N,N-di-(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy and C_{1-4}alkyl;

R^7 is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl or hydroxyC_{1-3}alkyl;

or a pharmaceutically acceptable salt, solvate or pro-drug thereof.

2. A compound according to claim 1 where R^1, R^2 and R^3 are all methoxy.
or a pharmaceutically acceptable salt, solvate or pro-drug thereof.

3 A compound according to claim 1 wherein:

R^1 , R^2, and R^3 are all C_{1-4}alkoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, C_{1-3} alkoxy, and C_{1-3}alkyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 1, 2 or 3;

B is -CO-, -NHCO-, -CONH, -C(O)O-, -NH-, -NHC(O)O-, NHCONH- or a single direct bond;

b is 0, 1 or 2;

D is carboxy, sulpho, phosphoryloxy, hydroxy, amino, N-C_{1-4} alkylamino, N,N-di(C_{1-4} alkyl)amino or of the formula $-Y^1(CH_2)_cR^{11}$ (wherein $\mathbf{Y^1}$ is -NHC(O)- or -C(O)NH-; **c** is 1 or 2; $\mathbf{R^{11}}$ is a 5-6-membered saturated heterocyclic group (linked via nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O and N, which heterocyclic group may bear 1 or 2 substituents selected from:

C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

R^7 is hydrogen;

or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

4. A compound according to claim 1 wherein:

R^1 , R^2, and R^3 are all methoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, methoxy and methyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

B is -CO-, -NHCO-, -CONH or a single direct bond;

b is 0 or 1;

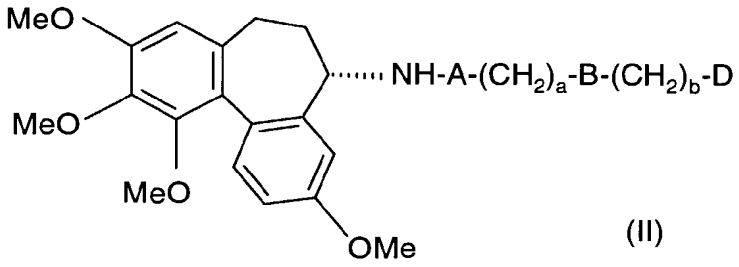
D is carboxy, phosphoryloxy, hydroxy, amino, N-C_{1-4} alkylamino, N,N-di(C_{1-4} alkyl)amino or of the formula $-Y^1(CH_2)_cR^{11}$ (wherein $\mathbf{Y^1}$ is -NHC(O)- or -C(O)NH-; **c** is 1 or 2; $\mathbf{R^{11}}$ is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 or 2 substituents selected from:

C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

R^7 is hydrogen;

or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

5. A compound of formula (II):



(II)

wherein a, b, A, B and D are as defined in claim 1;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

6. A compound according to claim 5 wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

B is -CO-, -NHCO-, -CONH or a single direct bond;

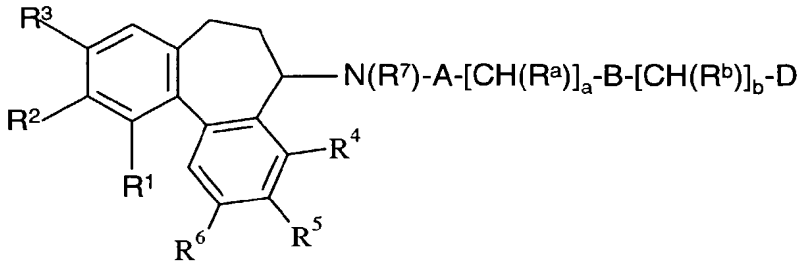
b is 0 or 1;

D is carboxy, phosphoryloxy, hydroxy, amino, N-C_{1-4} alkylamino, N,N-di(C_{1-4} alkyl)amino or of the formula $-Y^1(CH_2)_cR^{11}$ (wherein $\mathbf{Y^1}$ is -NHC(O)- or -C(O)NH-; **c** is 1 or 2; $\mathbf{R^{11}}$ is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 or 2 substituents selected from:

C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

7. A compound of formula (III):



(III)

wherein:

R^1, R^2 and **R^3** are each independently hydroxy, phosphoryloxy (-OPO_3H_2), C_{1-4}alkoxy or an in vivo hydrolysable ester of hydroxy, with the proviso that at least 2 of R^1, R^2 and R^3 are C_{1-4}alkoxy;

A is - CO-, -C(O)O-, -$CON(R^8)$-, -SO_2- or -$SO_2N(R^8)$- (wherein **R^8** is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{2-3}alkyl, aminoC_{2-3}alkyl or hydroxyC_{2-3}alkyl);

a is an integer from 1 to 4 inclusive;

R^a and **R^b** are independently selected from hydrogen, hydroxy and amino;

B is -O-, -CO-, -$N(R^9)$CO-, -$CON(R^9)$ -, -C(O)O-, -$N(R^9)$ -, - $N(R^9)$C(O)O-, -$N(R^9)CON(R^{10})$-, -$N(R^9)SO_2$-, -$SO_2N(R^9)$- or a direct single bond (wherein **R^9** and **R^{10}** are independently selected from hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{2-3}alkyl, aminoC_{2-3}alkyl and hydroxyC_{2-3}alkyl);

b is 0 or an integer from 1 to 4 inclusive;

D is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O and N, which heterocyclic group may bear 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-(C_{1-4}alkyl)carbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, aminoC_{1-4}alkyl, N-C_{1-4}alkylaminoC_{1-4}alkyl, di-N,N-(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein **R^{14}** is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl);

R^5 is C_{1-4}alkoxy;

R^4 and **R^6** are each independently selected from:

hydrogen, halogeno, nitro, amino, N-C_{1-4}alkylamino, N,N-di-(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy and C_{1-4}alkyl;

R^7 is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl or hydroxyC_{1-3}alkyl;

or a pharmaceutically acceptable salt, solvate or pro-drug thereof.

8. A compound according to claim 7 wherein:

R^1 , R^2, and R^3 are all C_{1-4}alkoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, C_{1-3} alkoxy, and C_{1-3}alkyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 1, 2 or 3;

B is -CO-, -NHCO-, -CONH, -C(O)O-, -NH-, -NHC(O)O-, NHCONH- or a single direct bond;

b is 0, 1 or 2;

D is piperazinyl or morpholinyl or piperidinyl, each ring being optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl;

R^7 is hydrogen;

or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

9. A compound according to claim 7 wherein:

R^1 , R^2, and R^3 are all methoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, methoxy and methyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

B is -CO-, -NHCO-, -CONH or a single direct bond;

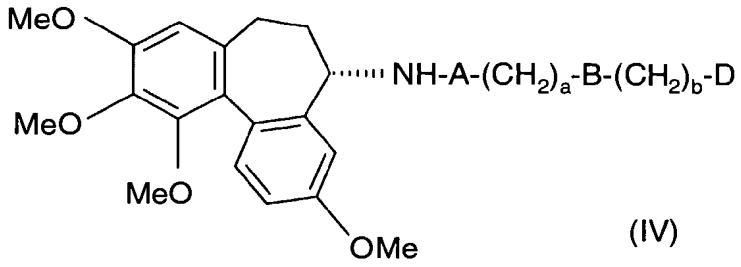
b is 0 or 1;

D is piperazino or morpholino, each ring being optionally substituted by 1 or 2 substituents selected from methyl, ethyl, acetyl, propionyl, carbamoyl and 2-hydroxyethyl;

R^7 is hydrogen;

or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

10. A compound according to claim 7 wherein:



(IV)

wherein a, b, A, B and D are as hereinabove defined in claim 7;

or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

11. A compound according to claim 10 wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

B is -CO-, -NHCO-, -CONH or a single direct bond;

b is 0 or 1;

D is piperazino or morpholino, each ring being optionally substituted by 1 or 2 substituents selected from methyl, ethyl, acetyl, propionyl, carbamoyl and 2-hydroxyethyl;

or a pharmaceutically acceptable salt, solvate or pro-drug thereof.

12. A compound according to claim 10 wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

B is -CO-, -NHCO-, -CONH or a single direct bond;

b is 0 or 1;

D is morpholino, 4-methylpiperazin-1-yl or 4-acetylpiperazin-1-yl;

or a pharmaceutically acceptable salt, solvate or pro-drug thereof.

13. A compound selected from:

N-[(5S) -3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-2-[2-aminoacetylamino]acetamide;

4-oxo-4-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]amino]butyl disodium phosphate;

N-{N-[2-(imidazol-1-yl)ethyl]carbamoyl}-5(S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine; and

2-{N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamoyloxy}ethyl disodium phosphate;

2-morpholinoethyl *N*-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5*H*-dibenzo [a,c]cyclohepten-5-yl]carbamate;

3-(1-methylpiperazin-4-yl)propyl N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo [a,c]cyclohepten-5-yl] carbamate;

N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-2-[2-aminoacetylamino]acetamide;

2-(1-acetylpiperazin-4-yl)ethyl-N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl] carbamate;

N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-4-(1-methylpiperazin-4-yl)-4-oxobutan-l-amide;

4-oxo-4-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]amino]butyl disodium phosphate;

N-{N-[2-(imidazol-1-yl)ethyl]carbamoyl}-5(S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine;

3-(1-acetylpiperazin-4-yl) propyl-N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamate;

N-l-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamoyloxy]ethyl disodiumphosphate;

4-morpholino-4-oxobutyl-N-[(5S)-3,9,10, 11-tetramethoxy-6,7-dihydro-5H-dibenzo [a-c]cyclohepten-5-yl]carbamate; and

4-(1-methylpiperazin-4-yl)-4-oxobutyl-N-[(5S)-3,9,10, 11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cylcohepten-5-yl]carbamate;

and pharmaceutically-acceptable salts, solvates or pro-drugs thereof.

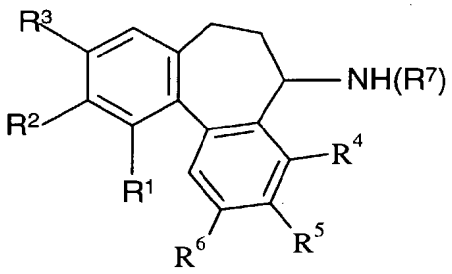
14. A pharmaceutical composition comprising a compound according to any one of claims 1 to 13 or a pharmaceutically acceptable salt, solvate or pro-drug thereof, in association with a pharmaceutically acceptable carrier.

15. The use of a compound according any one claims 1 to 13, or a pharmaceutically-acceptable salt, solvate or pro-drug thereof, in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm-blooded animal.

16. The use of a compound according to any one of claim 1 to 13 or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for administration in divided doses for use in the production of a vascular damaging effect in a warm-blooded animal.

17. A process for preparing a compound of the formula (I), or a compound of the formula (I) wherein at least 1 functional group is protected, wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, A, B, D, a, b and c are as defined in claim 1, comprising:

a) reacting a compound of the formula (X)



(X)

with a compound of the formula (XI):

$$L^1 - A - [CH(R^a)]_a\text{-}B\text{-}[CH\,R^b)]_b\text{-}D$$

(XI)

wherein L^1 is a leaving group; or

b) converting one compound of the formula (I) into another compound of the formula (I);

c) when a phosphoryloxy group is desired, reacting the corresponding hydroxy compound with a phosphoramidite;

wherein any functional groups are optionally protected.

and thereafter if necessary:

i) converting a compound of formula (I) into another compound of formula (I);

ii) removing any protecting groups;

iii) forming a pharmaceutically acceptable salt, solvate or pro-drug thereof.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 01/02964

A. CLASSIFICATION OF SUBJECT MATTER
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C07D295/088 C07C217/74 C07D233/61

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D C07K C07F C07C A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99 02166 A (ANGIOGENE PHARM LTD ;DOUGHERTY GRAEME (GB)) 21 January 1999 (1999-01-21) page 15 -page 21; claims	1,5,7, 10,13-17

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9902166 A	21-01-1999	AU 8231198 A	08-02-1999
		BR 9810681 A	15-08-2000
		CN 1262624 T	09-08-2000
		EP 1001785 A1	24-05-2000
		WO 9902166 A1	21-01-1999
		HU 0002493 A2	28-08-2001
		NO 20000077 A	07-01-2000
		PL 337926 A1	11-09-2000
		SK 132000 A3	11-12-2000
		TR 9903149 T2	21-09-2000

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(71) Applicants *(for all designated States except US)*: ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB). ZENECA PHARMA S.A. [FR/FR]; "Le Galien", 1, rue des Chauffours, Boîte postale 27, F-95022 Cergy Cédex (FR).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: THOMAS, Andrew Peter [GB/GB]; Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). JOHNSTONE, Craig [GB/GB]; Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). HENNEQUIN, Laurent, François, André [FR/FR]; Zeneca Pharma S.A., Centre de Recherches, Z.I. La Pompelle, Chemin de Vrilly, Boîte postale 1050, F-51689 Reims Cédex 2 (FR).

(74) Agent: MACK, John, Richard; Zeneca Pharmaceuticals, Intellectual Property Dept., Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).

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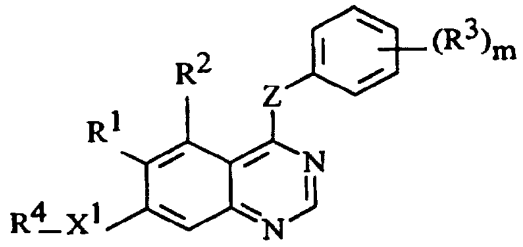
(54) Title: QUINAZOLINE DERIVATIVES AS VEGF INHIBITORS

(57) Abstract

The invention relates to quinazoline derivatives of formula (I) [wherein: Z represents -O-, -NH- or -S-; m is an integer from 1 to 5; R^1 represents hydrogen, hydroxy, halogeno, nitro, trifluoromethyl, cyano, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkylthio, or $-NR^5R^6$ (wherein R^5 and R^6, which may be the same or different, each represents hydrogen or C_{1-3}alkyl); R^2 represents hydrogen, hydroxy, halogeno, methoxy, amino or nitro; R^3 represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkanoyloxy, trifluoromethyl, cyano, amino or nitro; X^1 represents -O-, $-CH_2-$, -S-, -SO-, $-SO_2-$, $-NR^7-$, $-NR^8CO-$, $-CONR^9-$, $-SO_2NR^{10}-$ or $-NR^{11}SO_2-$, (wherein R^7, R^8, R^9, R^{10} and R^{11} each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl); R^4 represents a group which is alkenyl, alkynyl or optionally substituted alkyl, which alkyl group may contain a heteroatom linking group, which alkenyl, alkynyl or alkyl group may carry a terminal optionally substituted 5 or 6 membered saturated carbocyclic or heterocyclic group] and salts thereof; processes for their preparation, pharmaceutical compositions containing a compound of formula (I) or a pharmaceutically acceptable salt thereof as active ingredient. The compounds of formula (I) and the pharmaceutically acceptable salts thereof inhibit the effects of VEGF, a property of value in the treatment of a number of disease states including cancer and rheumatoid arthritis.



(I)

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QUINAZOLINE DERIVATIVES AS VEGF INHIBITORS

The present invention relates to quinazoline derivatives, processes for their preparation, pharmaceutical compositions containing them as active ingredient, methods for the treatment of disease states associated with angiogenesis and/or increased vascular permeability, to their use as medicaments and to their use in the manufacture of medicaments for use in the production of antiangiogenic and/or vascular permeability reducing effects in warm-blooded animals such as humans.

Normal angiogenesis plays an important role in a variety of processes including embryonic development, wound healing and several components of female reproductive function. Undesirable or pathological angiogenesis has been associated with disease states including diabetic retinopathy, psoriasis, cancer, rheumatoid arthritis, atheroma, Kaposi's sarcoma and haemangioma (Fan et al, 1995, Trends Pharmacol. Sci. 16: 57-66; Folkman, 1995, Nature Medicine 1: 27-31). Alteration of vascular permeability is thought to play a role in both normal and pathological physiological processes (Cullinan-Bove et al, 1993, Endocrinology 133: 829-837; Senger et al, 1993, Cancer and Metastasis Reviews, 12: 303-324). Several polypeptides with in vitro endothelial cell growth promoting activity have been identified including, acidic and basic fibroblast growth factors (aFGF & bFGF) and vascular endothelial growth factor (VEGF). By virtue of the restricted expression of its receptors, the growth factor activity of VEGF, in contrast to that of the FGFs, is relatively specific towards endothelial cells. Recent evidence indicates that VEGF is an important stimulator of both normal and pathological angiogenesis (Jakeman et al, 1993, Endocrinology, 133: 848-859; Kolch et al, 1995, Breast Cancer Research and Treatment, 36:139-155) and vascular permeability (Connolly et al, 1989, J. Biol. Chem. 264: 20017-20024). Antagonism of VEGF action by sequestration of VEGF with antibody can result in inhibition of tumour growth (Kim et al, 1993, Nature 362: 841-844).

Receptor tyrosine kinases (RTKs) are important in the transmission of biochemical signals across the plasma membrane of cells. These transmembrane molecules characteristically consist of an extracellular ligand-binding domain connected through a segment in the plasma membrane to an intracellular tyrosine kinase domain. Binding of ligand to the receptor results in stimulation of the receptor-associated tyrosine kinase activity

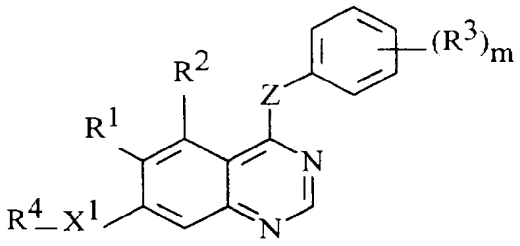
which leads to phosphorylation of tyrosine residues on both the receptor and other intracellular molecules. These changes in tyrosine phosphorylation initiate a signalling cascade leading to a variety of cellular responses. To date, at least nineteen distinct RTK subfamilies, defined by amino acid sequence homology, have been identified. One of these subfamilies is presently comprised by the fms-like tyrosine kinase receptor, Flt or Flt1, the kinase insert domain-containing receptor, KDR (also referred to as Flk-1), and another fms-like tyrosine kinase receptor, Flt4. Two of these related RTKs, Flt and KDR, have been shown to bind VEGF with high affinity (De Vries et al, 1992, Science 255: 989-991; Terman et al, 1992, Biochem. Biophys. Res. Comm. 1992, 187: 1579-1586). Binding of VEGF to these receptors expressed in heterologous cells has been associated with changes in the tyrosine phosphorylation status of cellular proteins and calcium fluxes.

Compounds which have good activity against epidermal growth factor (EGF) receptor tyrosine kinase are disclosed in the European Patent Publication No 0566226, but there is no disclosure or suggestion that the compounds inhibit the effects of VEGF. European Patent Publication No. 0326330 discloses certain quinoline, quinazoline and cinnoline plant fungicides. Certain of these plant fungicides are also stated to possess insecticidal and miticidal activity. There is however no disclosure or any suggestion that any of the compounds disclosed may be used for any purpose in animals such as humans. In particular, the European Patent Publication contains no teaching whatsoever concerning angiogenesis and/or increased vascular permeability mediated by growth factors such as VEGF.

The present invention is based on the discovery of compounds that surprisingly inhibit the effects of VEGF, a property of value in the treatment of disease states associated with angiogenesis and/or increased vascular permeability such as cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation and ocular diseases with retinal vessel proliferation. Compounds of the present invention possess higher potency against VEGF receptor tyrosine kinase whilst possessing some activity against EGF receptor tyrosine kinase. Furthermore, compounds of the present invention, possess substantially higher potency against VEGF receptor tyrosine kinase than against EGF receptor tyrosine kinase or FGF R1 receptor tyrosine kinase. Thus compounds of the invention which have

been tested possess activity against VEGF receptor tyrosine kinase such that they may be used in an amount sufficient to inhibit VEGF receptor tyrosine kinase whilst demonstrating no significant activity against EGF receptor tyrosine kinase or FGF R1 receptor tyrosine kinase.

According to one aspect of the present invention there is provided a quinazoline derivative of the formula I:



(I)

[wherein:

Z represents -O-, -NH- or -S-;

m is an integer from 1 to 5 with the proviso that where Z is -NH- m is an integer from 3 to 5;

R^1 represents hydrogen, hydroxy, halogeno, nitro, trifluoromethyl, cyano, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkylthio, or $-NR^5R^6$ (wherein R^5 and R^6, which may be the same or different, each represents hydrogen or C_{1-3}alkyl);

R^2 represents hydrogen, hydroxy, halogeno, methoxy, amino or nitro;

R^3 represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkanoyloxy, trifluoromethyl, cyano, amino or nitro;

X^1 represents -O-, $-CH_2-$, -S-, -SO-, $-SO_2-$, $-NR^7-$, $-NR^8CO-$, $-CONR^9-$, $-SO_2NR^{10}-$ or $-NR^{11}SO_2-$, (wherein R^7, R^8, R^9, R^{10} and R^{11} each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

R^4 is selected from one of the following seven groups:

1) hydrogen, C_{1-5}alkyl, C_{1-5}hydroxyalkyl, (preferably C_{2-5}hydroxyalkyl), C_{1-5}fluoroalkyl, C_{1-5}aminoalkyl;

2) C_{1-5}alkylX^2COR^{12} (wherein X^2 represents -O- or $-NR^{13}-$ (in which R^{13} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{12} represents C_{1-3}alkyl, $-NR^{14}R^{15}$ or $-OR^{16}$ (wherein R^{14}, R^{15} and R^{16} which may be the same or different each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl));

3) C_{1-5}alkylX^3R^{17} (wherein X^3 represents -O-, -S-, -SO-, $-SO_2$-, -OCO-, $-NR^{18}CO$-, $-CONR^{19}$-, $-SO_2NR^{20}$-, $-NR^{21}SO_2$- or $-NR^{22}$- (wherein R^{18}, R^{19}, R^{20}, R^{21} and R^{22} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{17} represents hydrogen, C_{1-3}alkyl, cyclopentyl, cyclohexyl or a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which C_{1-3}alkyl group may bear one or two substituents selected from oxo, hydroxy, halogeno and C_{1-4}alkoxy and which cyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy);

4) C_{1-5}alkylR^{23} (wherein R^{23} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy);

5) C_{2-5}alkenylR^{23} (wherein R^{23} is as defined hereinbefore);

6) C_{2-5}alkynylR^{23} (wherein R^{23} is as defined hereinbefore); and

7) C_{1-5}alkylX^4C_{1-5}alkylX^5R^{24} (wherein X^4 and X^5 which may be the same or different are each -O-, -S-, -SO-, $-SO_2$-, $-NR^{25}CO$-, $-CONR^{26}$-, $-SO_2NR^{27}$-, $-NR^{28}SO_2$- or $-NR^{29}$- (wherein R^{25}, R^{26}, R^{27}, R^{28} and R^{29} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{24} represents hydrogen or C_{1-3}alkyl)];

and salts thereof.

Z is advantageously -S-, preferably -O-, but especially -NH-.

Where Z is -S- or -O- m is advantageously an integer from 2 to 5, preferably 2 or 3.

Where Z is -NH- m is preferably 3.

R^1 is advantageously hydrogen, hydroxy, cyano, nitro, trifluoromethyl, C_{1-3} alkyl, C_{1-3} alkoxy or amino.

R^1 is preferably hydrogen, hydroxy, cyano, nitro, trifluoromethyl, methyl, ethyl, methoxy, or ethoxy, more preferably hydrogen, cyano, nitro, trifluoromethyl, hydroxy, methyl or methoxy, but especially methoxy.

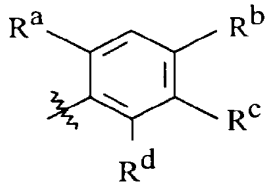
Where X^1 is $-NR^8CO$-, R^1 is preferably hydrogen.

R^2 is preferably hydrogen, fluoro, amino or nitro, but especially hydrogen.

In one embodiment of the present invention R^3 represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, trifluoromethyl, cyano, amino or nitro, preferably hydroxy, halogeno or C_{1-2} alkyl, especially hydroxy or halogeno.

Advantageously in another embodiment of the present invention one R^3 substituent is advantageously hydroxy, preferably meta-hydroxy, and the other one or more are each selected from halogeno, methyl and methoxy.

In another embodiment of the invention the phenyl group bearing $(R^3)_m$ is preferably of the formula II:



(II)

wherein:

R^a represents hydrogen, methyl, fluoro or chloro, preferably hydrogen, fluoro or chloro, especially fluoro;

R^b represents hydrogen, methyl, methoxy, bromo, fluoro or chloro;

R^c represents hydrogen or hydroxy, especially hydroxy;

R^d represents hydrogen, fluoro or chloro, especially hydrogen or fluoro.

Preferably in another embodiment of the invention two R^3 substituents are halogeno, especially ortho,ortho'- difluoro, and the other one or more are each selected from halogeno, hydroxy and methyl, especially from halogeno and methyl.

In a particular aspect of the present invention, the phenyl group bearing $(R^3)_m$ is the 2-fluoro-5-hydroxy-4-methylphenyl group, the 4-bromo-2,6-difluorophenyl group, the 4-chloro-2-fluoro-5-hydroxyphenyl group, the 4-chloro-2,6-difluorophenyl group or the 2,4-difluoro-5-hydroxyphenyl group or, where Z is O or S, the 4-chloro-2-fluorophenyl group.

Preferably the phenyl group bearing $(R^3)_m$ is the 4-chloro-2-fluoro-5-hydroxyphenyl group or the 2-fluoro-5-hydroxy-4-methylphenyl group or, where Z is O or S, the 4-chloro-2-

fluorophenyl group. The 4-chloro-2-fluoro-5-hydroxyphenyl group is an especially preferred value for the phenyl group bearing $(R^3)_m$.

Conveniently X^1 represents -O-, -S-, $-CH_2-$, $-NR^8CO-$, $-CONR^9-$, $-NR^{11}SO_2-$ or $-NR^7-$ (wherein R^7, R^8, R^9 and R^{11} each independently represents hydrogen, C_{1-3}alkyl (especially C_{1-2}alkyl) or C_{1-2}alkoxyethyl).

Advantageously X^1 represents -O-, -S-, $-NR^8CO-$, $-NR^{11}SO_2-$ or $-NR^7-$ (wherein R^7, R^8 and R^{11} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Preferably X^1 represents -O-, -S-, $-NR^8CO-$, $-NR^{11}SO_2-$ (wherein R^8 and R^{11} each independently represents hydrogen or C_{1-2}alkyl) or NH.

More preferably X^1 represents -O-, -S-, $-NR^8CO-$ (wherein R^8 represents hydrogen or methyl) or NH.

Particularly X^1 represents -O- or -NHCO-, especially -O-.

Advantageously X^2 represents -O- or $-NR^{13}-$ (wherein R^{13} represents hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl).

Advantageously X^3 represents -O-, -S-, -SO-, $-SO_2-$, $-NR^{18}CO-$, $-NR^{21}SO_2-$ or $-NR^{22}-$ (wherein R^{18}, R^{21} and R^{22} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Preferably X^3 represents -O-, -S-, -SO-, $-SO_2-$ or $-NR^{22}-$ (wherein R^{22} represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

More preferably X^3 represents -O- or $-NR^{22}-$ (wherein R^{22} represents hydrogen or C_{1-2}alkyl).

Advantageously X^4 and X^5 which may be the same or different each represents -O-, -S-, -SO-, $-SO_2-$ or $-NR^{29}-$ (wherein R^{29} represents hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl).

Preferably X^4 and X^5 which may be the same or different each represents -O-, -S- or $-NR^{29}-$ (wherein R^{29} represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

More preferably X^4 and X^5 which may be the same or different each represents -O- or -NH-.

Conveniently R^4 is selected from one of the following nine groups:

1) C_{1-5}alkyl, C_{2-5}hydroxyalkyl, C_{1-5}fluoroalkyl, C_{1-5} aminoalkyl;

2) C_{1-5}alkylX^2COR^{12} (wherein X^2 is as hereinbefore defined and R^{12} represents C_{1-3}alkyl, $-NR^{14}R^{15}$ or $-OR^{16}$ (wherein R^{14}, R^{15} and R^{16} which may be the same or different each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl));

3) C_{1-5}alkylX^3R^{17} (wherein X^3 is as hereinbefore defined and R^{17} represents hydrogen, C_{1-3}alkyl, cyclopentyl, cyclohexyl or a 5 or 6 membered saturated heterocyclic group with one or

two heteroatoms, selected independently from O, S and N, which C_{1-3}alkyl group may bear one or two substituents selected from oxo, hydroxy, halogeno and C_{1-3}alkoxy and which cyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy);

4) C_{1-5}alkylR^{30} (wherein R^{30} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to C_{1-5}alkyl through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy) or C_{2-5}alkylR^{31} (wherein R^{31} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to C_{2-5}alkyl through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy);

5) C_{3-4}alkenylR^{30} (wherein R^{30} is as defined hereinbefore);

6) C_{3-4}alkynylR^{30} (wherein R^{30} is as defined hereinbefore);

7) C_{3-4}alkenylR^{31} (wherein R^{31} is as defined hereinbefore);

8) C_{3-4}alkynylR^{31} (wherein R^{31} is as defined hereinbefore); and

9) C_{1-5}alkylX^4C_{1-5}alkylX^5R^{24} (wherein X^4 and X^5 are as hereinbefore defined and R^{24} represents hydrogen or C_{1-3}alkyl).

Advantageously R^4 is selected from one of the following nine groups:

1) C_{1-5}alkyl, C_{2-5}hydroxyalkyl, C_{1-5}fluoroalkyl, C_{2-4}aminoalkyl;

2) C_{2-3}alkylX^2COR^{12} (wherein X^2 is as hereinbefore defined and R^{12} represents C_{1-3}alkyl, -$NR^{14}R^{15}$ or -OR^{16} (wherein R^{14}, R^{15} and R^{16} which may be the same or different are each C_{1-2}alkyl or C_{1-2}alkoxyethyl));

3) C_{2-4}alkylX^3R^{17} (wherein X^3 is as hereinbefore defined and R^{17} is a group selected from C_{1-3}alkyl, cyclopentyl, cyclohexyl, pyrrolidinyl and piperidinyl which group is linked to X^3 through a carbon atom and which C_{1-3}alkyl group may bear one or two substituents selected from oxo, hydroxy, halogeno and C_{1-2}alkoxy and which cyclopentyl, cyclohexyl, pyrrolidinyl or piperidinyl group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy);

4) C_{1-4}alkylR^{30} (wherein R^{30} is a group selected from pyrrolidinyl, piperazinyl, piperidinyl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl and 1,3-dithian-2-yl, which group is linked to C_{1-4}alkyl through a carbon atom and which group may carry one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy) or C_{2-4}alkylR^{31} (wherein R^{31} is a group selected from morpholino, thiomorpholino, pyrrolidin-1-yl, piperazin-1-yl and piperidino which group may carry one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy);

5) C_{3-4}alkenylR^{30} (wherein R^{30} is as defined hereinbefore);

6) C_{3-4}alkynylR^{30} (wherein R^{30} is as defined hereinbefore);

7) C_{3-4}alkenylR^{31} (wherein R^{31} is as defined hereinbefore);

8) C_{3-4}alkynylR^{31} (wherein R^{31} is as defined hereinbefore); and

9) C_{2-3}alkylX^4C_{2-3}alkylX^5R^{24} (wherein X^4 and X^5 are as hereinbefore defined and R^{24} represents hydrogen or C_{1-3}alkyl).

Preferably R^4 is selected from one of the following five groups:

1) C_{1-3}alkyl, C_{2-3}hydroxyalkyl, C_{1-3}fluoroalkyl, C_{2-3}aminoalkyl;

2) 2-(3,3-dimethylureido)ethyl, 3-(3,3-dimethylureido)propyl, 2-(3-methylureido)ethyl, 3-(3-methylureido)propyl, 2-ureidoethyl, 3-ureidopropyl, 2-(N,N-dimethylcarbamoyloxy)ethyl, 3-(N,N-dimethylcarbamoyloxy)propyl, 2-(N-methylcarbamoyloxy)ethyl, 3-(N-methylcarbamoyloxy)propyl, 2-(carbamoyloxy)ethyl, 3-(carbamoyloxy)propyl;

3) C_{2-3}alkylX^3R^{17} (wherein X^3 is as hereinbefore defined and R^{17} is a group selected from C_{1-2}alkyl, cyclopentyl, cyclohexyl, pyrrolidinyl and piperidinyl which group is linked to X^3 through a carbon atom and which C_{1-2}alkyl group may bear one or two substituents selected from hydroxy, halogeno and C_{1-2}alkoxy and which cyclopentyl, cyclohexyl, pyrrolidinyl or piperidinyl group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy);

4) C_{1-2}alkylR^{30} (wherein R^{30} is a group selected from pyrrolidinyl, piperazinyl, piperidinyl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl and 1,3-dithian-2-yl, which group is linked to C_{1-2}alkyl through a carbon atom and which group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy) or C_{2-3}alkylR^{31} (wherein R^{31} is a group selected from morpholino, thiomorpholino, piperidino, piperazin-1-yl

and pyrrolidin-1-yl which group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy); and

5) C_{2-3}alkylX^4C_{2-3}alkylX^5R^{24} (wherein X^4 and X^5 are as hereinbefore defined and R^{24} represents hydrogen or C_{1-2}alkyl).

More preferably R^4 represents methyl, ethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 2-hydroxyethyl, 3-hydroxypropyl, 2-methoxyethyl, 3-methoxypropyl, 2-(methylsulphinyl)ethyl, 2-(methylsulphonyl)ethyl, 2-(N,N-dimethylsulphamoyl)ethyl, 2-(N-methylsulphamoyl)ethyl, 2-sulphamoylethyl, 2-(N,N-dimethylamino)ethyl, 3-(N,N-dimethylamino)propyl, 2-morpholinoethyl, 3-morpholinopropyl, 2-piperidinoethyl, 3-piperidinopropyl, 2-(piperazin-1-yl)ethyl, 3-(piperazin-1-yl)propyl, 2-(pyrrolidin-1-yl)ethyl, 3-(pyrrolidin-1-yl)propyl, (1,3-dioxolan-2-yl)methyl, 2-(1,3-dioxolan-2-yl)ethyl, 2-(2-methoxyethylamino)ethyl, 2-(2-hydroxyethylamino)ethyl, 3-(2-methoxyethylamino)propyl, 3-(2-hydroxyethylamino)propyl, 2-thiomorpholinoethyl, 3-thiomorpholinopropyl, 2-(4-methylpiperazin-1-yl)ethyl, 3-(4-methylpiperazin-1-yl)propyl or 2-(2-methoxyethoxy)ethyl.

Particularly R^4 represents 2-hydroxyethyl, 3-hydroxypropyl, 2-methoxyethyl, 3-methoxypropyl, 2-(methylsulphinyl)ethyl, 2-(methylsulphonyl)ethyl, 2-(N,N-dimethylamino)ethyl, 3-(N,N-dimethylamino)propyl, 2-morpholinoethyl, 3-morpholinopropyl, 2-piperidinoethyl, 3-piperidinopropyl, 2-(piperazin-1-yl)ethyl, 3-(piperazin-1-yl)propyl, 2-(pyrrolidin-1-yl)ethyl, 3-(pyrrolidin-1-yl)propyl, (1,3-dioxolan-2-yl)methyl, 2-(1,3-dioxolan-2-yl)ethyl, 2-(2-methoxyethylamino)ethyl, 2-(2-hydroxyethylamino)ethyl, 3-(2-methoxyethylamino)propyl, 3-(2-hydroxyethylamino)propyl, 2-thiomorpholinoethyl, 3-thiomorpholinopropyl, 2-(4-methylpiperazin-1-yl)ethyl, 3-(4-methylpiperazin-1-yl)propyl or 2-(2-methoxyethoxy)ethyl.

Preferred compounds are:

4-(4-bromo-2,6-difluoroanilino)-6,7-dimethoxyquinazoline,

4-(4-bromo-2-fluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-thiomorpholinoethoxy)quinazoline,

6,7-dimethoxy-4-(3-hydroxy-4-methylphenoxy)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-hydroxyethoxy)-6-methoxyquinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-methoxyethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-(methylsulphinyl)ethoxy)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6,7-dimethoxyquinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,

7-(2-acetoxyethoxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-piperidinoethoxy)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-methoxyethylamino)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-cyclopentyloxyethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methylthioethoxy)quinazoline,

4-(2,4-difluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline,

4-(2,4-difluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-methoxyacetamidoquinazoline,

4-(4-bromo-2,6-difluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline

and salts thereof especially the hydrochloride salts thereof.

More preferred compounds are:

4-(4-bromo-2-fluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-thiomorpholinoethoxy)quinazoline,

6,7-dimethoxy-4-(3-hydroxy-4-methylphenoxy)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-hydroxyethoxy)-6-methoxyquinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-methoxyethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-(methylsulphinyl)ethoxy)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6,7-dimethoxyquinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,

7-(2-acetoxyethoxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-piperidinoethoxy)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-methoxyethylamino)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-cyclopentyloxyethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methylthioethoxy)quinazoline,

4-(2,4-difluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline,

4-(2,4-difluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-methoxyacetamidoquinazoline,

4-(4-bromo-2,6-difluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline

and salts thereof especially the hydrochloride salts thereof.

Particularly preferred compounds are:

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6,7-dimethoxyquinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,

7-(2-acetoxyethoxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-piperidinoethoxy)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-methoxyethylamino)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-cyclopentyloxyethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methylthioethoxy)quinazoline,

4-(2,4-difluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline,

4-(2,4-difluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-methoxyacetamidoquinazoline,

4-(4-bromo-2,6-difluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline

and salts thereof especially the hydrochloride salts thereof.

More particularly preferred compounds are:

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methylthioethoxy)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6,7-dimethoxyquinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-methoxyacetamidoquinazoline,

4-(4-bromo-2,6-difluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline

and salts thereof especially the hydrochloride salts thereof.

Especially preferred compounds are:

4-(2-fluoro-5-hydroxy-4-methylanilino)-6,7-dimethoxyquinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-methoxyacetamidoquinazoline,

4-(4-bromo-2,6-difluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline

and salts thereof especially the hydrochloride salts thereof.

For the avoidance of doubt it is to be understood that where in this specification a group is qualified by 'hereinbefore defined' or 'defined hereinbefore' the said group encompasses the first occurring and broadest definition as well as each and all of the preferred definitions for that group.

In this specification the term "alkyl" includes both straight and branched chain alkyl groups but references to individual alkyl groups such as "propyl" are specific for the straight chain version only. An analogous convention applies to other generic terms. Unless otherwise stated the term "alkyl" advantageously refers to chains with 1-6 carbon atoms, preferably 1-4 carbon atoms. In this specification the term "alkoxy" means an alkyl group as

defined hereinbefore linked to an oxygen atom. In this specification the term "aryl" includes C_{6-10}aromatic groups which may, if desired, carry one or more substituents selected from halogeno, alkyl, alkoxy, cyano, nitro or trifluoromethyl (wherein alkyl and alkoxy are as hereinbefore defined). The term "aryloxy" means an aryl group as defined hereinbefore linked to an oxygen atom. In this specification the term "sulphonyloxy" includes alkylsulphonyloxy and arylsulphonyloxy wherein "alkyl" and "aryl" are as defined hereinbefore. The term "alkanoyl" as used herein unless otherwise stated includes alkylC=O groups in which "alkyl" is as defined hereinbefore, for example ethanoyl refers to $CH_3C=O$. In this specification unless stated otherwise the term "alkenyl" includes both straight and branched chain alkenyl groups but references to individual alkenyl groups such as 2-butenyl are specific for the straight chain version only. Unless otherwise stated the term "alkenyl" advantageously refers to chains with 2-5 carbon atoms, preferably 3-4 carbon atoms. In this specification unless stated otherwise the term "alkynyl" includes both straight and branched chain alkynyl groups but references to individual alkynyl groups such as 2-butynyl are specific for the straight chain version only. Unless otherwise stated the term "alkynyl" advantageously refers to chains with 2-5 carbon atoms, preferably 3-4 carbon atoms.

In formula I, as hereinbefore defined, hydrogen will be present at positions 2 and 8 of the quinazoline group.

Within the present invention it is to be understood that a quinazoline of the formula I or a salt thereof may exhibit the phenomenon of tautomerism and that the formulae drawings within this specification can represent only one of the possible tautomeric forms. It is to be understood that the invention encompasses any tautomeric form which inhibits VEGF receptor tyrosine kinase activity and is not to be limited merely to any one tautomeric form utilised within the formulae drawings.

It is also to be understood that certain quinazolines of the formula I and salts thereof can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms which inhibit VEGF receptor tyrosine kinase activity.

For the avoidance of any doubt, it is to be understood that when X^1 is, for example, a group of formula $-NR^8CO-$, it is the nitrogen atom bearing the R^8 group which is attached to the quinazoline ring and the carbonyl (CO) group is attached to R^4, whereas when X^1 is, for

example, a group of formula -$CONR^9$-, it is the carbonyl group which is attached to the quinazoline ring and the nitrogen atom bearing the R^9 group is attached to R^4. A similar convention applies to the other two atom X^1 linking groups such as -$NR^{11}SO_2$- and -SO_2NR^{10}-. When X^1 is -NR^7- it is the nitrogen atom bearing the R^7 group which is linked to the quinazoline ring and to R^4. An analogous convention applies to other groups. It is further to be understood that when X^1 represents -NR^7- and R^7 is C_{1-3}alkoxyC_{2-3}alkyl it is the C_{2-3}alkyl moiety which is linked to the nitrogen atom of X^1 and an analogous convention applies to other groups.

For the avoidance of any doubt, it is to be understood that in a compound of the formula I when R^4 is, for example, a group of formula C_{1-5}alkylR^{23}, it is the terminal C_{1-5}alkyl moiety which is bound to X^1, similarly when R^4 is, for example, a group of formula C_{2-5}alkenylR^{23} it is the C_{2-5}alkenyl moiety which is bound to X^1 and an analogous convention applies to other groups. When R^4 is a group 1-R^{23}prop-1-en-3-yl it is the first carbon to which the group R^{23} is attached and it is the third carbon which is linked to X^1 and an analogous convention applies to other groups.

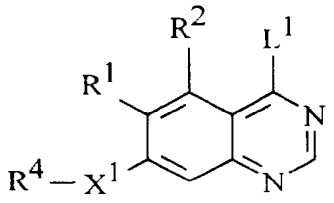
The present invention relates to the compounds of formula I as hereinbefore defined as well as to the salts thereof. Salts for use in pharmaceutical compositions will be pharmaceutically acceptable salts, but other salts may be useful in the production of the compounds of formula I and their pharmaceutically acceptable salts. Pharmaceutically acceptable salts of the invention may, for example, include acid addition salts of the compounds of formula I as hereinbefore defined which are sufficiently basic to form such salts. Such acid addition salts include for example salts with inorganic or organic acids affording pharmaceutically acceptable anions such as with hydrogen halides (especially hydrochloric or hydrobromic acid of which hydrochloric acid is particularly preferred) or with sulphuric or phosphoric acid, or with trifluoroacetic, citric or maleic acid. In addition where the compounds of formula I are sufficiently acidic, pharmaceutically acceptable salts may be formed with an inorganic or organic base which affords a pharmaceutically acceptable cation. Such salts with inorganic or organic bases include for example an alkali metal salt, such as a sodium or potassium salt, an alkaline earth metal salt such as a calcium or magnesium salt, an ammonium salt or for example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

A compound of the formula I, or salt thereof, and other compounds of the invention (as hereinafter defined) may be prepared by any process known to be applicable to the preparation of chemically-related compounds. Such processes include, for example, those illustrated in European Patent Applications, Publication Nos. 0520722, 0566226, 0602851 and 0635498. Such processes, are provided as a further feature of the invention and are as described hereinafter. Necessary starting materials may be obtained by standard procedures of organic chemistry. The preparation of such starting materials is described within the accompanying non-limiting Examples. Alternatively necessary starting materials are obtainable by analogous procedures to those illustrated which are within the ordinary skill of an organic chemist.

Thus the following processes (a) to (g) and (i) to (v) constitute further features of the present invention.

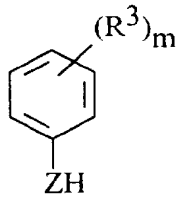
Synthesis of Compounds of Formula I

(a) Compounds of the formula I and salts thereof may be prepared by the reaction of a compound of the formula III:



(III)

(wherein R^1, R^2, X^1 and R^4 are as defined hereinbefore and L^1 is a displaceable moiety), with a compound of the formula IV:



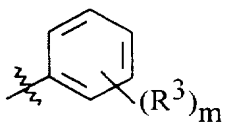
(IV)

(wherein Z, R^3 and m are as defined hereinbefore) whereby to obtain compounds of the formula I and salts thereof. A convenient displaceable moiety L^1 is, for example, a halogeno, alkoxy (preferably C_{1-4}alkoxy), aryloxy or sulphonyloxy group, for example a chloro, bromo, methoxy, phenoxy, methanesulphonyloxy or toluene-4-sulphonyloxy group.

The reaction is advantageously effected in the presence of either an acid or a base. Such an acid is, for example, an anhydrous inorganic acid such as hydrogen chloride. Such a base is, for example, an organic amine base such as, for example, pyridine, 2,6-lutidine, collidine, 4-dimethylaminopyridine, triethylamine, morpholine, N-methylmorpholine or diazabicyclo[5.4.0]undec-7-ene, or for example, an alkali metal or alkaline earth metal carbonate or hydroxide, for example sodium carbonate, potassium carbonate, calcium carbonate, sodium hydroxide or potassium hydroxide. Alternatively such a base is, for example, an alkali metal hydride, for example sodium hydride, or an alkali metal or alkaline earth metal amide, for example sodium amide or sodium bis(trimethylsilyl)amide. The reaction is preferably effected in the presence of an inert solvent or diluent, for example an alkanol or ester such as methanol, ethanol, isopropanol or ethyl acetate, a halogenated solvent such as methylene chloride, trichloromethane or carbon tetrachloride, an ether such as tetrahydrofuran or 1,4-dioxan, an aromatic hydrocarbon solvent such as toluene, or a dipolar aprotic solvent such as N,N-dimethylformamide, N,N-dimethylacetamide, N-methylpyrrolidin-2-one or dimethylsulphoxide. The reaction is conveniently effected at a temperature in the range, for example, 10 to 150°C, preferably in the range 20 to 80°C.

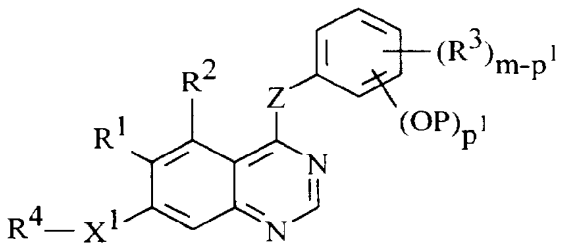
The compound of the invention may be obtained from this process in the form of the free base or alternatively it may be obtained in the form of a salt with the acid of the formula H-L^1 wherein L^1 has the meaning defined hereinbefore. When it is desired to obtain the free base from the salt, the salt may be treated with a base as defined hereinbefore using a conventional procedure.

(b) Where the group of formula IIa:



(IIa)

(wherein R^3 and m are as hereinbefore defined) represents a phenyl group carrying one or more hydroxy groups, a compound of the formula I and salts thereof can be prepared by the deprotection of a compound of formula V:

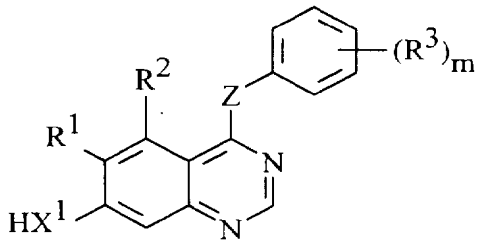


(V)

(wherein X^1, m, R^1, R^2, R^3, R^4 and Z are as hereinbefore defined, P represents a phenolic hydroxy protecting group and p^1 is an integer from 1 to 5 equal to the number of protected hydroxy groups and such that $m-p^1$ is equal to the number of R^3 substituents which are not protected hydroxy). The choice of phenolic hydroxy protecting group P is within the standard knowledge of an organic chemist, for example those included in standard texts such as "Protective Groups in Organic Synthesis" T.W. Greene and R.G.M.Wuts, 2nd Ed. Wiley 1991, including ethers (for example, methyl, methoxymethyl, allyl and benzyl), silyl ethers (for example, t-butyldiphenylsilyl and t-butyldimethylsilyl), esters (for example, acetate and benzoate) and carbonates (for example, methyl and benzyl). The removal of such a phenolic hydroxy protecting group may be effected by any of the procedures known for such a transformation, including those reaction conditions indicated in standard texts such as that indicated hereinbefore, or by a related procedure. The reaction conditions preferably being such that the hydroxy derivative is produced without unwanted reactions at other sites within the starting or product compounds. For example, where the protecting group P is acetate, the transformation may conveniently be effected by treatment of the quinazoline derivative with a base as defined hereinbefore and including ammonia, and its mono and di-alkylated derivatives, preferably in the presence of a protic solvent or co-solvent such as water or an alcohol, for example methanol or ethanol. Such a reaction can be effected in the presence of

an additional inert solvent or diluent as defined hereinbefore and at a temperature in the range 0 to 50°C, conveniently at about 20°C.

(c) Production of those compounds of formula I and salts thereof wherein the substituent X^1 is -O-, -S- or -NR^7- can be achieved by the reaction, conveniently in the presence of a base as defined hereinbefore, of a compound of the formula VI:



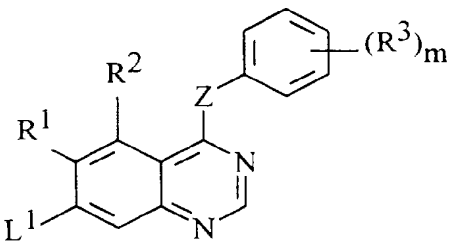
(VI)

(wherein m, X^1, R^1, R^2, R^3, and Z are as hereinbefore defined) with a compound of formula VII:

$$R^4\text{-}L^1 \qquad \text{(VII)}$$

(wherein R^4 and L^1 are as hereinbefore defined); L^1 is a displaceable moiety for example a halogeno or sulphonyloxy group such as a bromo or methanesulphonyloxy group. The reaction is preferably effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), advantageously at a temperature in the range, for example 10 to 150°C, conveniently at about 50°C.

(d) Compounds of the formula I and salts thereof may be prepared by the reaction of a compound of the formula VIII:



(VIII)

with a compound of the formula IX:

$$R^4\text{-}X^1\text{-}H \qquad \text{(IX)}$$

(wherein L^1, R^1, R^2, R^3, R^4, Z, m and X^1 are all as hereinbefore defined). The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), advantageously at a temperature in the range, for example 10 to 150°C, conveniently at about 100°C.

(e) Compounds of the formula I and salts thereof wherein R^4 is $C_{1-5}alkylR^{32}$, [wherein R^{32} is selected from one of the following four groups:

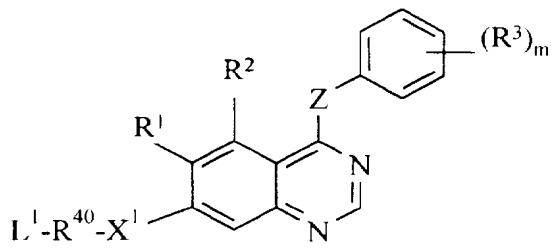
1) $X^6C_{1-3}alkyl$ (wherein X^6 represents -O-, -S-, $-SO_2-$, $-NR^{33}CO-$ or $-NR^{34}SO_2-$ (wherein R^{33} and R^{34} are each independently hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$);

2) $NR^{35}R^{36}$ (wherein R^{35} and R^{36} which may be the same or different are each hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$);

3) $X^7C_{1-5}alkylX^5R^{24}$ (wherein X^7 represents -O-, -S-, $-SO_2-$, $-NR^{37}CO-$, $-NR^{38}SO_2-$ or $-NR^{39}-$ (wherein R^{37}, R^{38} and R^{39} are each independently hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$) and X^5 and R^{24} are as defined hereinbefore); and

4) R^{31} (wherein R^{31} is as defined hereinbefore);]

may be prepared by reacting a compound of the formula X:



(X)

(wherein L^1, X^1, R^1, R^2, R^3, Z and m are as hereinbefore defined and R^{40} is C_{1-5}alkyl) with a compound of the formula XI:

$$R^{32}\text{-}H \qquad \text{(XI)}$$

(wherein R^{32} is as defined hereinbefore) to give a compound of the formula I. The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), and at a temperature in the range, for example 0 to 150°C, conveniently at about 50°C.

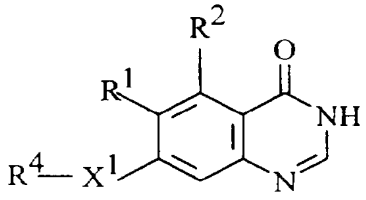
(f) The production of those compounds of the formula I and salts thereof wherein the substituent R^1 is represented by NR^5R^6, where one or both of R^5 and R^6 are C_{1-3}alkyl, may be effected by the reaction of compounds of formula I wherein the substituent R^1 is an amino group and an alkylating agent, preferably in the presence of a base as defined hereinbefore. Such alkylating agents are C_{1-3}alkyl moieties bearing a displaceable moiety as defined hereinbefore such as C_{1-3}alkyl halides for example C_{1-3}alkyl chloride, bromide or iodide. The reaction is preferably effected in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)) and at a temperature in the range, for example, 10 to 100°C, conveniently at about ambient temperature. This process can also be used for preparing compounds in which R^4-X^1 is an alkylamino or dialkylamino group.

(g) The production of compounds of formula I and salts thereof wherein one or more of the substituents R^1, R^2 or R^3 is an amino group or where R^4-X^1 is amino may be effected by the reduction of a corresponding compound of formula I wherein the substituent(s) at the corresponding position(s) of the quinazoline and/or phenyl ring is/are a nitro group(s). The

reduction may conveniently be effected as described in process (i) hereinafter. The production of a compound of formula I and salts thereof wherein the substituent(s) at the corresponding position(s) of the quinazoline and/or phenyl ring is/are a nitro group(s) may be effected by the processes described hereinbefore and hereinafter in processes (a-e) and (i-v) using a quinazoline compound selected from the compounds of the formulae (I-XXVII) in which the substituent(s) at the corresponding position(s) of the quinazoline and/or phenyl ring is/are a nitro group(s).

Synthesis of Intermediates

(i) The compounds of formula III and salts thereof, constitute a further feature of the present invention. Such compounds in which L^1 is halogeno may for example be prepared by halogenating a compound of the formula XII:

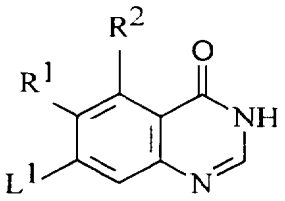


(XII)

(wherein R^1, R^2, R^4 and X^1 are as hereinbefore defined).

Convenient halogenating agents include inorganic acid halides, for example thionyl chloride, phosphorus(III)chloride, phosphorus(V)oxychloride and phosphorus(V)chloride. The halogenation reaction is conveniently effected in the presence of an inert solvent or diluent such as for example a halogenated solvent such as methylene chloride, trichloromethane or carbon tetrachloride, or an aromatic hydrocarbon solvent such as benzene or toluene. The reaction is conveniently effected at a temperature in the range, for example 10 to 150°C, preferably in the range 40 to 100°C.

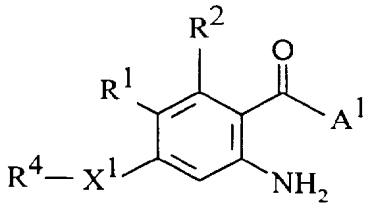
The compounds of formula XII and salts thereof which constitute a further feature of the present invention may for example be prepared by reacting a compound of the formula XIII:



(XIII)

(wherein R^1, R^2 and L^1 are as hereinbefore defined) with a compound of the formula IX as hereinbefore defined. The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), advantageously at a temperature in the range, for example 10 to 150°C, conveniently at about 100°C.

The compounds of formula XII and salts thereof may also be prepared by cyclising a compound of the formula XIV:

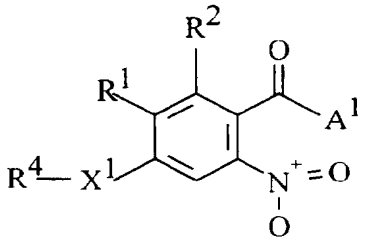


(XIV)

(wherein R^1, R^2, R^4 and X^1, are as hereinbefore defined, and A^1 is an hydroxy, alkoxy (preferably C_{1-4}alkoxy) or amino group) whereby to form a compound of formula XII or salt thereof. The cyclisation may be effected by reacting a compound of the formula XIV, where A^1 is an hydroxy or alkoxy group, with formamide or an equivalent thereof effective to cause cyclisation whereby a compound of formula XII or salt thereof is obtained, such as [3-(dimethylamino)-2-azaprop-2-enylidene]dimethylammonium chloride. The cyclisation is conveniently effected in the presence of formamide as solvent or in the presence of an inert solvent or diluent such as an ether for example 1,4-dioxan. The cyclisation is conveniently effected at an elevated temperature, preferably in the range 80 to 200°C. The compounds of formula XII may also be prepared by cyclising a compound of the formula XIV, where A^1 is an amino group, with formic acid or an equivalent thereof effective to cause cyclisation whereby a compound of formula XII or salt thereof is obtained. Equivalents of formic acid

effective to cause cyclisation include for example a tri-C_{1-4}alkoxymethane, for example triethoxymethane and trimethoxymethane. The cyclisation is conveniently effected in the presence of a catalytic amount of an anhydrous acid, such as a sulphonic acid for example p-toluenesulphonic acid, and in the presence of an inert solvent or diluent such as for example a halogenated solvent such as methylene chloride, trichloromethane or carbon tetrachloride, an ether such as diethylether or tetrahydrofuran, or an aromatic hydrocarbon solvent such as toluene. The cyclisation is conveniently effected at a temperature in the range, for example 10 to 100°C, preferably in the range 20 to 50°C.

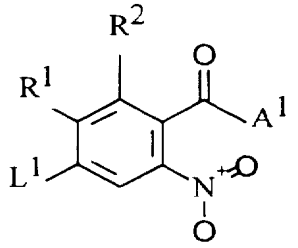
Compounds of formula XIV and salts thereof, which constitute a further feature of the present invention, may for example be prepared by the reduction of the nitro group in a compound of the formula XV:



(XV)

(wherein R^1, R^2, R^4, X^1 and A^1 are as hereinbefore defined) to yield a compound of formula XIV as hereinbefore defined. The reduction of the nitro group may conveniently be effected by any of the procedures known for such a transformation. The reduction may be carried out, for example, by the hydrogenation of a solution of the nitro compound in the presence of an inert solvent or diluent as defined hereinbefore in the presence of a metal effective to catalyse hydrogenation reactions such as palladium or platinum. A further reducing agent is, for example, an activated metal such as activated iron (produced for example by washing iron powder with a dilute solution of an acid such as hydrochloric acid). Thus, for example, the reduction may be effected by heating the nitro compound and the activated metal in the presence of a solvent or diluent such as a mixture of water and alcohol, for example methanol or ethanol, to a temperature in the range, for example 50 to 150°C, conveniently at about 70°C.

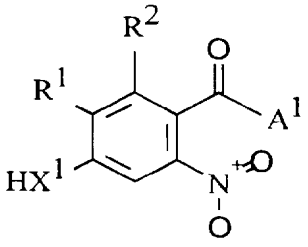
Compounds of the formula XV and salts thereof which constitute a further feature of the present invention, may for example be prepared by the reaction of a compound of the formula XVI:



(XVI)

(wherein R^1, R^2, L^1 and A^1 are as hereinbefore defined) with a compound of the formula IX as hereinbefore defined to give a compound of the formual XV. The reaction of the compounds of formulae XVI and IX is conveniently effected under conditions as described for process (d) hereinbefore.

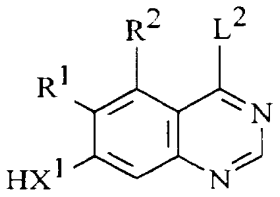
Compounds of formula XV and salts thereof, may for example also be prepared by the reaction of a compound of the formula XVII:



(XVII)

(wherein R^1, R^2, X^1 and A^1 are as hereinbefore defined with the proviso that X^1 is not $-CH_2-$) with a compound of the formula VII as hereinbefore defined to yield a compound of formula XV as hereinbefore defined. The reaction of the compounds of formulae XVII and VII is conveniently effected under conditions as described for process (c) hereinbefore.

The compounds of formula III and salts thereof may also be prepared for example by reacting a compound of the formula XVIII:

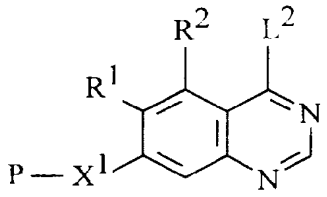


(XVIII)

(wherein R^1, R^2 and X^1 are as hereinbefore defined with the proviso that X^1 is not $-CH_2-$ and L^2 represents a displaceable protecting moiety) with a compound of the formula VII as hereinbefore defined, whereby to obtain a compound of formula III in which L^1 is represented by L^2.

A compound of formula XVIII is conveniently used in which L^2 represents a phenoxy group which may if desired carry up to 5 substituents, preferably up to 2 substituents, selected from halogeno, nitro and cyano. The reaction may be conveniently effected under conditions as described for process (c) hereinbefore.

The compounds of formula XVIII and salts thereof as hereinbefore defined may for example be prepared by deprotecting a compound of the formula XIX:



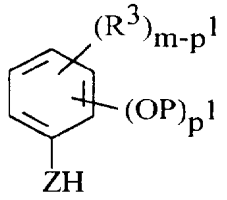
(XIX)

(wherein R^1, R^2, P, X^1 and L^2 are as hereinbefore defined with the proviso that X^1 is not $-CH_2-$). Deprotection may be effected by techniques well known in the literature, for example where P represents a benzyl group deprotection may be effected by hydrogenolysis or by treatment with trifluoroacetic acid.

One compound of formula III may if desired be converted into another compound of formula III in which the moiety L^1 is different. Thus for example a compound of formula III in which L^1 is other than halogeno, for example optionally substituted phenoxy, may be converted to a compound of formula III in which L^1 is halogeno by hydrolysis of a compound of formula III (in which L^1 is other than halogeno) to yield a compound of formula XII as

hereinbefore defined, followed by introduction of halide to the compound of formula XII, thus obtained as hereinbefore defined, to yield a compound of formula III in which L^1 represents halogen.

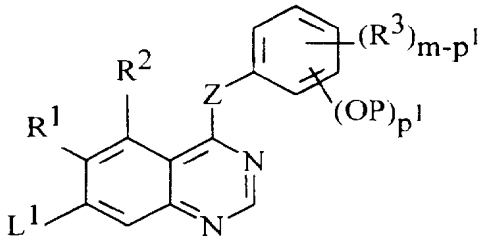
(ii) The compounds of formula V and salts thereof, constitute a further feature of the present invention, and may for example be prepared by the reaction of a compound of formula III as hereinbefore defined with a compound of the formula XX:



(XX)

(wherein R^3, m, p^1, P and Z are as hereinbefore defined). The reaction may for example be effected as described for process (a) hereinbefore.

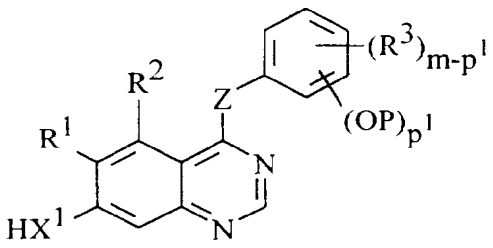
The compounds of formula V and salts thereof may also be prepared by reacting a compound of formula XXI:



(XXI)

(wherein R^1, R^2, L^1, Z, R^3, m, p^1 and P are as hereinbefore defined) with a compound of formula IX as hereinbefore defined. The reaction may for example be effected as described for process (d) above.

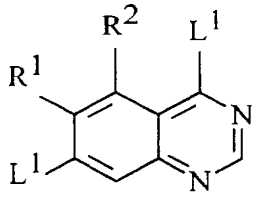
The compounds of formula V and salts thereof may also be prepared by reacting a compound of formula XXII:



(XXII)

(wherein R^1, R^2, R^3, X^1, Z, P, p^1 and m are as hereinbefore defined with the proviso that X^1 is not $-CH_2-$) with a compound of the formula VII as hereinbefore defined. The reaction may for example be effected as described for process (c) hereinbefore.

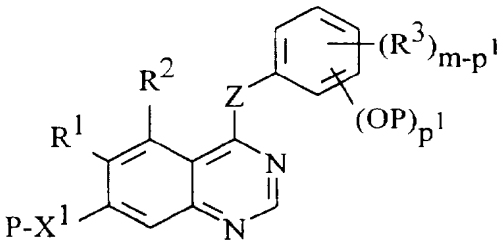
The compounds of formula XXI and salts thereof may for example be prepared by reaction of a compound of formula XXIII:



(XXIII)

(wherein R^1, R^2, and L^1 are as hereinbefore defined, and L^1 in the 4- and 7- positions may be the same or different) with a compound of the formula XX as hereinbefore defined. The reaction may be effected for example by a process as described in (a) above.

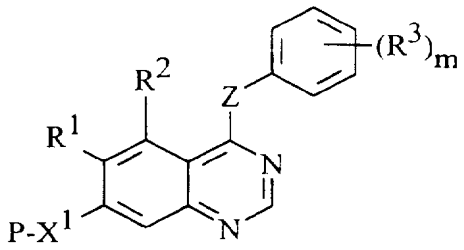
Compounds of the formula XXII and salts thereof may be made by reacting compounds of the formulae XIX and XX as hereinbefore defined, under conditions described in (a) hereinbefore, to give a compound of formula XXIV:



(XXIV)

(wherein R^1, R^2, R^3, P, Z, X^1, p^1 and m are as hereinbefore defined with the proviso that X^1 is not $-CH_2-$) and then deprotecting the compound of formula XXIV for example as described in (i) above.

(iii) Compounds of the formula VI as hereinbefore defined and salts thereof may be made by deprotecting the compound of formula XXV:



(XXV)

(wherein R^1, R^2, R^3, P, Z, X^1 and m are as hereinbefore defined) by a process for example as described in (i) above.

Compounds of the formula XXV and salts thereof may be made by reacting compounds of the formulae XIX and IV as hereinbefore defined, under the conditions described in (a) hereinbefore, to give a compound of the formula XXV or salt thereof.

(iv) Compounds of the formula VIII and salts thereof as hereinbefore defined may be made by reacting compounds of the formulae XXIII and IV as hereinbefore defined, the reaction for example being effected by a process as described in (a) above.

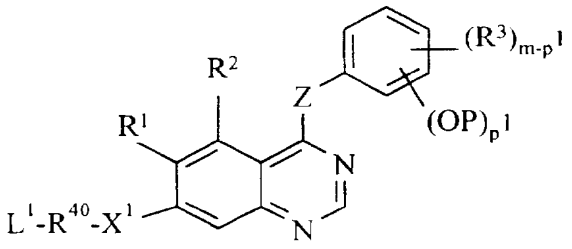
(v) Compounds of the formula X as defined hereinbefore and salts thereof may for example be made by the reaction of a compound of formula VI as defined hereinbefore with a compound of the formula XXVI:

$$L^1\text{-}R^{40}\text{-}L^1$$

(XXVI)

(wherein L^1 and R^{40} are as hereinbefore defined) to give a compound of the formula X. The reaction may be effected for example by a process as described in (c) above.

Compounds of the formula X and salts thereof may also be made for example by deprotecting a compound of the formula XXVII:



(XXVII)

(wherein L^1, R^{40}, X^1, R^1, R^2, R^3, Z, P, m and p^1 are as defined hereinbefore) by a process for example as described in (b) above.

Compounds of the formula XXVII and salts thereof may be made for example by reacting compounds of the formulae XXII and XXVI as defined hereinbefore, under the conditions described in (c) above.

When a pharmaceutically acceptable salt of a compound of the formula I is required, it may be obtained, for example, by reaction of said compound with, for example, an acid using a conventional procedure, the acid having a pharmaceutically acceptable anion.

Many of the intermediates defined herein are novel, for example, those of the formulae III, V, XII, XIV and XV, and these are provided as a further feature of the invention.

Intermediates of the formulae VIII, X, XXI, XXII, XXIV, XXV and XXVII are also provided as a further feature of the invention.

The identification of compounds which potently inhibit the tyrosine kinase activity associated with the VEGF receptors such as Flt and/or KDR and which inhibit angiogenesis and/or increased vascular permeability is desirable and is the subject of the present invention. These properties may be assessed, for example, using one or more of the procedures set out below:

(a) In Vitro Receptor Tyrosine Kinase Inhibition Test

This assay determines the ability of a test compound to inhibit tyrosine kinase activity. DNA encoding VEGF or epidermal growth factor (EGF) receptor cytoplasmic domains may be obtained by total gene synthesis (Edwards M, International Biotechnology

Lab 5(3), 19-25, 1987) or by cloning. These may then be expressed in a suitable expression system to obtain polypeptide with tyrosine kinase activity. For example VEGF and EGF receptor cytoplasmic domains, which were obtained by expression of recombinant protein in insect cells, were found to display intrinsic tyrosine kinase activity. In the case of the VEGF receptor Flt (Genbank accession number X51602), a 1.7kb DNA fragment encoding most of the cytoplasmic domain, commencing with methionine 783 and including the termination codon, described by Shibuya et al (Oncogene, 1990, 5: 519-524), was isolated from cDNA and cloned into a baculovirus transplacement vector (for example pAcYM1 (see The Baculovirus Expression System: A Laboratory Guide, L.A. King and R. D. Possee, Chapman and Hall, 1992) or pAc360 or pBlueBacHis (available from Invitrogen Corporation)). This recombinant construct was co-transfected into insect cells (for example Spodoptera frugiperda 21(Sf21)) with viral DNA (eg Pharmingen BaculoGold) to prepare recombinant baculovirus. (Details of the methods for the assembly of recombinant DNA molecules and the preparation and use of recombinant baculovirus can be found in standard texts for example Sambrook et al, 1989, Molecular cloning - A Laboratory Manual, 2nd edition, Cold Spring Harbour Laboratory Press and O'Reilly et al, 1992, Baculovirus Expression Vectors - A Laboratory Manual, W. H. Freeman and Co, New York). For other tyrosine kinases for use in assays, cytoplasmic fragments starting from methionine 806 (KDR, Genbank accession number L04947) and methionine 668 (EGF receptor, Genbank accession number X00588) may be cloned and expressed in a similar manner.

For expression of cFlt tyrosine kinase activity, Sf21 cells were infected with plaque-pure cFlt recombinant virus at a multiplicity of infection of 3 and harvested 48 hours later. Harvested cells were washed with ice cold phosphate buffered saline solution (PBS) (10mM sodium phosphate pH7.4, 138mM sodium chloride, 2.7mM potassium chloride) then resuspended in ice cold HNTG/PMSF (20mM Hepes pH7.5, 150mM sodium chloride, 10% v/v glycerol, 1% v/v Triton X100, 1.5mM magnesium chloride, 1mM ethylene glycol-bis(βaminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), 1mM PMSF (phenylmethylsulphonyl fluoride); the PMSF is added just before use from a freshly-prepared 100mM solution in methanol) using 1ml HNTG/PMSF per 10 million cells. The suspension was centrifuged for 10 minutes at 13,000 rpm at 4°C, the supernatant (enzyme stock) was removed and stored in aliquots at -70°C. Each new batch of stock enzyme was titrated in the

assay by dilution with enzyme diluent (100mM Hepes pH 7.4, 0.2mM sodium orthovanadate, 0.1% v/v Triton X100, 0.2mM dithiothreitol). For a typical batch, stock enzyme is diluted 1 in 2000 with enzyme diluent and 50µl of dilute enzyme is used for each assay well.

A stock of substrate solution was prepared from a random copolymer containing tyrosine, for example Poly (Glu, Ala, Tyr) 6:3:1 (Sigma P3899), stored as 1 mg/ml stock in PBS at -20°C and diluted 1 in 500 with PBS for plate coating.

On the day before the assay 100µl of diluted substrate solution was dispensed into all wells of assay plates (Nunc maxisorp 96-well immunoplates) which were sealed and left overnight at 4°C.

On the day of the assay the substrate solution was discarded and the assay plate wells were washed once with PBST (PBS containing 0.05% v/v Tween 20) and once with 50mM Hepes pH7.4.

Test compounds were diluted with 10% dimethylsulphoxide (DMSO) and 25µl of diluted compound was transferred to wells in the washed assay plates. "Total" control wells contained 10% DMSO instead of compound. Twenty five microlitres of 40mM manganese(II)chloride containing 8µM adenosine-5'-triphosphate (ATP) was added to all test wells except "blank" control wells which contained manganese(II)chloride without ATP. To start the reactions 50µl of freshly diluted enzyme was added to each well and the plates were incubated at room temperature for 20 minutes. The liquid was then discarded and the wells were washed twice with PBST. One hundred microlitres of mouse IgG anti-phosphotyrosine antibody (Upstate Biotechnology Inc. product 05-321), diluted 1 in 6000 with PBST containing 0.5% w/v bovine serum albumin (BSA), was added to each well and the plates were incubated for 1 hour at room temperature before discarding the liquid and washing the wells twice with PBST. One hundred microlitres of horse radish peroxidase (HRP)-linked sheep anti-mouse Ig antibody (Amersham product NXA 931), diluted 1 in 500 with PBST containing 0.5% w/v BSA, was added and the plates were incubated for 1 hour at room temperature before discarding the liquid and washing the wells twice with PBST. One hundred microlitres of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) solution, freshly prepared using one 50mg ABTS tablet (Boehringer 1204 521) in 50ml freshly prepared 50mM phosphate-citrate buffer pH5.0 + 0.03% sodium perborate (made with 1 phosphate citrate buffer with sodium perborate (PCSB) capsule (Sigma P4922) per 100ml

distilled water), was added to each well. Plates were then incubated for 20-60 minutes at room temperature until the optical density value of the "total" control wells, measured at 405nm using a plate reading spectrophotometer, was approximately 1.0. "Blank" (no ATP) and "total" (no compound) control values were used to determine the dilution range of test compound which gave 50% inhibtion of enzyme activity.

(b) In Vitro HUVEC Proliferation Assay

This assay determines the ability of a test compound to inhibit the growth factor-stimulated proliferation of human umbilical vein endothelial cells (HUVEC).

HUVEC cells were isolated in MCDB 131 (Gibco BRL) + 7.5% v/v foetal calf serum (FCS) and were plated out (at passage 2 to 8), in MCDB 131 + 2% v/v FCS + 3μg/ml heparin + 1μg/ml hydrocortisone, at a concentration of 1000 cells/well in 96 well plates. After a minimum of 4 hours they were dosed with the appropriate growth factor (i.e. VEGF 3ng/ml, EGF 3ng/ml or b-FGF 0.3ng/ml) and compound. The cultures were then incubated for 4 days at 37°C with 7.5% carbon dioxide. On day 4 the cultures were pulsed with 1μCi/well of tritiated-thymidine (Amersham product TRA 61) and incubated for 4 hours. The cells were harvested using a 96-well plate harvester (Tomtek) and then assayed for incorporation of tritium with a Beta plate counter. Incorporation of radioactivity into cells, expressed as cpm, was used to measure inhibition of growth factor-stimulated cell proliferation by compounds.

(c) In Vivo Rat Uterine Oedema Assay

This test measures the capacity of compounds to reduce the acute increase in uterine weight in rats which occurs in the first 4-6 hours following oestrogen stimulation. This early increase in uterine weight has long been known to be due to oedema caused by increased permeability of the uterine vasculature and recently Cullinan-Bove and Koos (Endocrinology, 1993,133:829-837) demonstrated a close temporal relationship with increased expression of VEGF mRNA in the uterus. We have found that prior treatment of the rats with a neutralising monoclonal antibody to VEGF significantly reduces the acute increase in uterine weight, confirming that the increase in weight is substantially mediated by VEGF.

Groups of 20 to 22-day old rats were treated with a single subcutaneous dose of oestradiol benzoate (2.5μg/rat) in a solvent, or solvent only. The latter served as unstimulated controls. Test compounds were orally administered at various times prior to the

administration of oestradiol benzoate. Five hours after the administration of oestradiol benzoate the rats were humanely sacrificed and their uteri were dissected, blotted and weighed. The increase in uterine weight in groups treated with test compound and oestradiol benzoate and with oestradiol benzoate alone was compared using a Student T test. Inhibition of the effect of oestradiol benzoate was considered significant when $p<0.05$.

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises a compound of the formula I as defined hereinbefore or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable excipient or carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) for example as a sterile solution, suspension or emulsion, for topical administration for example as an ointment or cream or for rectal administration for example as a suppository. In general the above compositions may be prepared in a conventional manner using conventional excipients.

The compositions of the present invention are advantageously presented in unit dosage form. The compound will normally be administered to a warm-blooded animal at a unit dose within the range 5-5000mg per square metre body area of the animal, i.e. approximately 0.1-100mg/kg. A unit dose in the range, for example, 1-100mg/kg, preferably 1-50mg/kg is envisaged and this normally provides a therapeutically-effective dose. A unit dose form such as a tablet or capsule will usually contain, for example 1-250mg of active ingredient.

According to a further aspect of the present invention there is provided a compound of the formula I or a pharmaceutically acceptable salt thereof as defined hereinbefore for use in a method of treatment of the human or animal body by therapy.

We have found that compounds of the present invention inhibit VEGF receptor tyrosine kinase activity and are therefore of interest for their antiangiogenic effects and/or their ability to cause a reduction in vascular permeability.

A further feature of the present invention is a compound of formula I, or a pharmaceutically acceptable salt thereof, for use as a medicament, conveniently a compound of formula I, or a pharmaceutically acceptable salt thereof, for use as a medicament for

producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal such as a human being.

Thus according to a further aspect of the invention there is provided the use of a compound of the formula I, or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for use in the production of an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal such as a human being.

According to a further feature of the invention there is provided a method for producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal, such as a human being, in need of such treatment which comprises administering to said animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof as defined hereinbefore.

As stated above the size of the dose required for the therapeutic or prophylactic treatment of a particular disease state will necessarily be varied depending on the host treated, the route of administration and the severity of the illness being treated. Preferably a daily dose in the range of 1-50mg/kg is employed. However the daily dose will necessarily be varied depending upon the host treated, the particular route of administration, and the severity of the illness being treated. Accordingly the optimum dosage may be determined by the practitioner who is treating any particular patient.

The antiangiogenic and/or vascular permeability reducing treatment defined hereinbefore may be applied as a sole therapy or may involve, in addition to a compound of the invention, one or more other substances and/or treatments. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate administration of the individual components of the treatment. In the field of medical oncology it is normal practice to use a combination of different forms of treatment to treat each patient with cancer. In medical oncology the other component(s) of such conjoint treatment in addition to the antiangiogenic and/or vascular permeability reducing treatment defined hereinbefore may be: surgery, radiotherapy or chemotherapy. Such chemotherapy may cover three main categories of therapeutic agent:

(i) other antiangiogenic agents that work by different mechanisms from those defined hereinbefore (for example linomide, inhibitors of integrin $\alpha v\beta 3$ function, angiostatin, razoxin, thalidomide);

(ii) cytostatic agents such as antioestrogens (for example tamoxifen,toremifene, raloxifene, droloxifene, iodoxyfene), progestogens (for example megestrol acetate), aromatase inhibitors (for example anastrozole, letrazole, vorazole, exemestane), antiprogestogens, antiandrogens (for example flutamide, nilutamide, bicalutamide, cyproterone acetate), LHRH agonists and antagonists (for example goserelin acetate, luprolide), inhibitors of testosterone 5α-dihydroreductase (for example finasteride), anti-invasion agents (for example metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator receptor function) and inhibitors of growth factor function, (such growth factors include for example EGF, FGFs, platelet derived growth factor and hepatocyte growth factor such inhibitors include growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors and serine/threonine kinase inhibitors); and

(iii) antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as antimetabolites (for example antifolates like methotrexate, fluoropyrimidines like 5-fluorouracil, purine and adenosine analogues, cytosine arabinoside); antitumour antibiotics (for example anthracyclines like doxorubicin, daunomycin, epirubicin and idarubicin, mitomycin-C, dactinomycin, mithramycin); platinum derivatives (for example cisplatin, carboplatin); alkylating agents (for example nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide, nitrosoureas, thiotepa); antimitotic agents (for example vinca alkaloids like vincristine and taxoids like taxol, taxotere); topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan).

As stated above the compounds defined in the present invention are of interest for their antiangiogenic and/or vascular permeability reducing effects. Such compounds of the invention are expected to be useful in a wide range of disease states including cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation and ocular diseases with retinal vessel proliferation. In particular such compounds of the invention are expected to slow advantageously the growth of primary and recurrent solid tumours of, for example, the colon, breast, prostate, lungs and skin. More particularly such compounds of the invention are expected to inhibit the growth of those primary and recurrent solid tumours which are associated with VEGF, especially those tumours which are

significantly dependent on VEGF for their growth and spread, including for example, certain tumours of the colon, breast, prostate, lung, vulva and skin.

In addition to their use in therapeutic medicine, the compounds of formula I and their pharmaceutically acceptable salts are also useful as pharmacological tools in the development and standardisation of in vitro and in vivo test systems for the evaluation of the effects of inhibitors of VEGF receptor tyrosine kinase activity in laboratory animals such as cats, dogs, rabbits, monkeys, rats and mice, as part of the search for new therapeutic agents.

It is to be understood that where the term "ether" is used anywhere in this specification it refers to diethyl ether.

The invention will now be illustrated in the following non-limiting Examples in which, unless otherwise stated:-

[(i) evaporations were carried out by rotary evaporation in vacuo and work-up procedures were carried out after removal of residual solids such as drying agents by filtration;

(ii) operations were carried out at ambient temperature, that is in the range 18-25°C and under an atmosphere of an inert gas such as argon;

(iii) column chromatography (by the flash procedure) and medium pressure liquid chromatography (MPLC) were performed on Merck Kieselgel silica (Art. 9385) or Merck Lichroprep RP-18 (Art. 9303) reversed-phase silica obtained from E. Merck, Darmstadt, Germany;

(iv) yields are given for illustration only and are not necessarily the maximum attainable;

(v) melting points are uncorrected and were determined using a Mettler SP62 automatic melting point apparatus, an oil-bath apparatus or a Koffler hot plate apparatus.

(vi) the structures of the end-products of the formula I were confirmed by nuclear (generally proton) magnetic resonance (NMR) and mass spectral techniques; proton magnetic resonance chemical shift values were measured on the delta scale and peak multiplicities are shown as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; q, quartet;

(vii) intermediates were not generally fully characterised and purity was assessed by thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), infra-red (IR) or NMR analysis;

(viii) the following abbreviations have been used:-

DMF N,N-dimethylformamide

DMSO dimethylsulphoxide

DMA N,N-dimethylacetamide

TFA trifluoroacetic acid.]

Example 1

Isopropanolic hydrogen chloride (0.1ml of a 5M solution) was added to a solution of 4-chloro-6,7-dimethoxyquinazoline (202mg, 0.9mmol) and 4-bromo-2-fluoro-5-hydroxyaniline (as described in EP 61741 A2) (206mg, 1mmol) in 2-butanol (8ml). The mixture was heated at reflux for 45 minutes, then allowed to cool. The precipitated product was collected by filtration, washed with 2-butanol, and then with ether, and dried under vacuum to give **4-(4-bromo-2-fluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline** hydrochloride hydrate (340mg, 87%) as a white solid.

m.p. 265-270°C

^{1}H NMR Spectrum: ($DMSOd_6$) 4.0(2s, 6H); 7.13(d, 1H); 7.32(s, 1H); 7.64(d, 1H); 8.17(s, 1H); 8.8(s, 1H); 10.6(s, 1H); 11.3(s, 1H)

MS - ESI: 394-396 $[MH]^+$

Elemental analysis:	Found	C 43.42	H 3.68	N 9.33
$C_{16}H_{13}BrFN_3O_3$ 1HCl 1.05H_2O	Requires	C 42.75	H 3.61	N 9.35%

The starting material was prepared as follows:

A mixture of 4,5-dimethoxyanthranilic acid (19.7g) and formamide (10ml) was stirred and heated to 190°C for 5 hours. The mixture was allowed to cool to approximately 80°C and water (50ml) was added. The mixture was stored at ambient temperature for 3 hours. The precipitate was isolated, washed with water and dried to give 6,7-dimethoxy-3,4-dihydroquinazolin-4-one (3.65g).

A mixture of a portion (2.06g) of the material so obtained, thionyl chloride (20ml) and DMF (1 drop) was stirred and heated to reflux for 2 hours. The mixture was evaporated and the residue was partitioned between ethyl acetate and a saturated aqueous sodium

hydrogen carbonate solution. The organic phase was washed with water, dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography using increasingly polar mixtures of methylene chloride and ethyl acetate as eluant to give 4-chloro-6,7-dimethoxyquinazoline (0.6g, 27%).

Example 2

Solid potassium hydroxide (71mg, 1.2mmol) and then 4-chloro-6,7-dimethoxyquinazoline (0.25g, 1.1mmol), (prepared as described for the starting material in Example 1), were added to a melt of 2,4-dihydroxytoluene (0.6g, 4.8mmol) at 140°C. The mixture was stirred at 140°C for 15 minutes, then allowed to cool. The mixture was diluted with water, and acidified to pH4 then extracted with ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The crude product was first purified by flash chromatography eluting with petroleum ether/ethyl acetate (1/9) and then by absorption HPLC eluting with trichloromethane/acetonitrile (85/15) to give **6,7-dimethoxy-4-(3-hydroxy-4-methylphenoxy)quinazoline** (116mg, 34%).

m.p. 213-216°C

^{1}H NMR Spectrum: ($CDCl_3$) 2.22(s, 3H); 4.05(s, 6H); 6.6(s, 1H); 6.69(dd, 1H); 7.2(d, 1H); 7.3(s, 1H); 7.52(s, 1H); 8.35(br s, 1H); 8.65(s, 1H)

MS - ESI: 313 $[MH]^+$

Elemental analysis:	Found	C 65.36	H 5.53	N 8.92
$C_{17}H_{16}N_2O_4$	Requires	C 65.38	H 5.16	N 8.97%

The starting material was prepared as follows:

Boron tribromide (3.1ml, 3.2mmol) was added to a solution of 2,4-dimethoxytoluene (1g, 6.5mmol) in pentane (10ml) at -70°C. The reaction mixture was allowed to warm to ambient temperature and the mixture stirred for a further 2 hours. Ice water and ethyl acetate were then added and the aqueous layer basified to pH9.5 with 2M aqueous sodium hydroxide. After stirring for 10 minutes, the organic layer was separated and the aqueous layer extracted with ethyl acetate. The combined organic extract was washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by flash chromatography

eluting with methylene chloride/ethyl acetate (9/1) to give 2,4-dihydroxytoluene (759mg, 94%) as a white solid.

Example 3

As part of the procedure described in Example 2 a second compound was extracted during the absorption HPLC by eluting with trichloromethane/acetonitrile (75/25) to give **6,7-dimethoxy-4-(5-hydroxy-2-methylphenoxy)quinazoline** (123mg, 36%).

m.p. 231-239°C

^{1}H NMR Spectrum: ($CDCl_3$) 2.1(s, 3H); 4.05(s, 6H); 6.6(s, 1H); 6.72(dd, 1H); 7.15(d, 1H); 7.32(s, 1H); 7.58(s, 1H); 8.65(s, 1H)

MS - ESI: 313 $[MH]^+$

Elemental analysis:	Found	C 65.05	H 5.68	N 8.6
$C_{17}H_{16}N_2O_4$ $0.1H_2O$	Requires	C 65.00	H 5.20	N 8.92%

Example 4

A mixture of 4-(4-chloro-2-fluorophenoxy)-7-hydroxy-6-methoxyquinazoline (160mg, 0.5mmol), 2-bromoethyl methyl ether (83mg, 0.6mmol) and potassium carbonate (207mg, 1.5mmol) in DMF (3ml) was heated at 180°C for 45 minutes. The reaction mixture was allowed to cool, diluted with water and acidified to pH3.5. This aqueous mixture was extracted with ethyl acetate and the organic extract was washed with water and brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by flash chromatography eluting with methylene chloride/ether (7/3) to give **4-(4-chloro-2-fluorophenoxy)-7-(2-methoxyethoxy)-6-methoxyquinazoline** (130mg, 68%).

m.p. 167-168°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.76(t, 2H); 3.99(s, 3H); 4.34(t, 2H); 7.4(d, 1H); 7.44(s, 1H); 7.56(t, 1H); 7.57(s, 1H); 7.70(dd, 1H); 8.56(s, 1H)

MS - ESI: 379 $[MH]^+$

Elemental analysis :	Found	C 57.03	H 4.53	N 7.41
$C_{18}H_{16}FClN_2O_4$ $0.1H_2O$	Requires	C 56.81	H 4.29	N 7.36%

The starting material was prepared as follows:

A mixture of 2-amino-4-benzyloxy-5-methoxybenzamide (J. Med. Chem. 1977, vol 20, 146-149, 10g, 0.04mol) and Gold's reagent (7.4g, 0.05mol) in dioxane (100ml) was stirred and heated at reflux for 24 hours. Sodium acetate (3.02g, 0.037mol) and acetic acid (1.65ml, 0.029mol) were added to the reaction mixture and it was heated for a further 3 hours. The mixture was evaporated, water was added to the residue, the solid was filtered off, washed with water and dried. Recrystallisation from acetic acid gave 7-benzyloxy-6-methoxy-3,4-dihydroquinazolin-4-one (8.7g, 84%).

A mixture of 7-benzyloxy-6-methoxy-3,4-dihydroquinazolin-4-one (2.82g, 0.01mol), thionyl chloride (40ml) and DMF (0.28ml) was stirred and heated at reflux for 1 hour. The mixture was evaporated and azeotroped with toluene to give 7-benzyloxy-4-chloro-6-methoxyquinazoline hydrochloride (3.45g).

4-Chloro-2-fluoro-phenol (264mg, 1.8mmol) was added to a solution of 7-benzyloxy-4-chloro-6-methoxyquinazoline hydrochloride (506mg, 1.5mmol) in pyridine (8ml) and the mixture heated at reflux for 45 minutes. The solvent was removed by evaporation and the residue partitioned between ethyl acetate and water. The organic layer was washed with 0.1M HCl, water and brine, dried ($MgSO_4$) and the solvent removed by evaporation. The solid residue was triturated with petroleum ether and the crude product collected by filtration and purified by flash chromatography eluting with methylene chloride/ether (9/1) to give 7-benzyloxy-4-(4-chloro-2-fluorophenoxy)-6-methoxyquinazoline (474mg, 77%) as a cream solid.

m.p. 179-180°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.99(s, 3H); 5.36(s, 2H); 7.35-7.5(m, 4H); 7.55-7.65(m, 5H); 7.72(d, 1H); 8.6(s, 1H)

MS - ESI: 411 $[MH]^+$

Elemental analysis: Found C 63.38 H 4.07 N 6.78

$C_{22}H_{16}ClFN_2O_3$ $0.06H_2O$ $0.05CH_2Cl_2$ Requires C 63.64 H 3.93 N 6.73%

A solution of 7-benzyloxy-4-(4-chloro-2-fluorophenoxy)-6-methoxyquinazoline (451mg, 1.1mmol) in TFA (4.5ml) was heated at reflux for 3 hours. The mixture was diluted with toluene and the volatiles removed by evaporation. The residue was triturated with methylene chloride, collected by filtration, washed with ether and dried under vacuum to give 4-(4-chloro-2-fluorophenoxy)-7-hydroxy-6-methoxyquinazoline (320mg, 90%).

^{1}H NMR Spectrum: ($DMSOd_6$) 4.0(s, 3H); 7.27(s, 1H); 7.43(dd, 1H); 7.56(t, 1H); 7.57(s, 1H); 7.72(dd, 1H); 8.5(s, 1H)

MS - ESI: 321 $[MH]^+$

Example 5

4-Chloro-6,7-dimethoxyquinazoline (200mg, 0.89mmol), (prepared as described for the starting material in Example 1), was added to a solution of 3-hydroxybenzenethiol (168mg, 1.3mmol) and N,N-diisopropylethylamine (233µl, 1.3mmol) in DMF (5ml). After heating at 40°C for 10 minutes, the reaction mixture was allowed to cool, diluted with water, acidified to pH3 and the mixture extracted with ethyl acetate. The organic extract was washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was recrystallised from a mixture of ethanol and ether to give **6,7-dimethoxy-4-(3-hydroxyphenylthio)quinazoline** (259mg, 93%) as a white solid.

m.p. 221-230°C

^{1}H NMR Spectrum: ($DMSOd_6$) 4.0(2s, 6H); 6.9(dd, 1H); 7.05(s, 1H); 7.07(d, 1H); 7.34(t, 1H); 7.35(s, 1H); 7.38(s, 1H); 8.7(s, 1H); 9.8(br s, 1H)

MS - ESI: 315 $[MH]^+$

Elemental analysis:	Found	C 61.06	H 4.61	N 8.95
$C_{16}H_{14}N_2O_3S$	Requires	C 61.13	H 4.49	N 8.91%

The starting material was prepared as follows:

Boron tribromide (1.4ml, 14mmol) was added to a solution of 3-methoxybenzenethiol (1g, 7.1mmol) in methylene chloride (10ml) at 0°C. The mixture was allowed to warm to ambient temperature and stirred for a further 60 minutes. The reaction mixture was diluted with ethyl acetate and water and basified with aqueous 2M sodium hydroxide solution to pH9. The mixture was then extracted with ethyl acetate, the combined extract washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by flash chromatography eluting with petroleum ether/ethyl acetate (8/2) to give 3-hydroxybenzenethiol (819mg, 91%).

^{1}H NMR Spectrum: ($CDCl_3$) 3.42(s, 1H); 4.85(br s, 1H); 6.6(d, 1H); 6.75(s, 1H); 6.85(d, 1H); 7.1(t, 1H)

Example 6

Concentrated aqueous ammonia (5ml) was added to a solution of 4-(5-acetoxy-4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline (180mg, 0.4mmol) in methanol (50ml). The mixture was stirred at ambient temperature for 3 hours, and then diluted with water. Most of the methanol was removed by evaporation and the resulting precipitate collected by filtration, washed with water and dried to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline** (73mg, 45%).

m.p. >250°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.29(s, 3H); 3.74(t, 2H); 3.94(s, 3H); 4.28(t, 2H); 7.15(d, 1H); 7.19(s, 1H); 7.38(d, 1H); 7.77(s, 1H); 8.36(s, 1H); 9.40(s, 1H)

MS - ESI: 394 $[MH]^-$

Elemental analysis :	Found	C 51.1	H 4.6	N 9.8
$C_{18}H_{17}N_3ClFO_4$ $1.6H_2O$	Requires	C 51.2	H 4.8	N 9.9%

The starting material was prepared as follows:

A mixture of 4-chloro-2-fluoro-5-hydroxyaniline (2.5g, 15mmol), (as described in EP 61741 A2), and 7-benzyloxy-4-chloro-6-methoxyquinazoline (4.2g, 14mmol), (prepared as described for the starting material in Example 4 but with an aqueous work up), in isopropanol was heated at reflux for 2 hours. The mixture was then allowed to cool and the solid product collected by filtration, washed with isopropanol and dried to give 7-benzyloxy-4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxyquinazoline hydrochloride (4.8g, 81%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.98(s, 3H); 5.18(s, 2H); 7.05(d, 1H); 7.18-7.27(m, 7H); 8.06(s, 1H); 8.38(s, 1H)

Triethylamine (216ml, 1.5mmol) and then acetic anhydride (133ml, 1.4mmol) were added to a stirred suspension of 7-benzyloxy-4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy quinazoline hydrochloride (600mg, 1.4mmol) in methylene chloride (7ml). The mixture was stirred at ambient temperature for 3 hours and insoluble material removed by filtration. Volatiles were removed from the filtrate by evaporation and the residue purified by flash chromatography eluting with methylene chloride/methanol (100/0 increasing in polarity

to 97/3) to give 4-(5-acetoxy-4-chloro-2-fluoroanilino)-7-benzyloxy-6-methoxyquinazoline (340mg, 52%) as a solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 2.34(s, 3H); 3.94(s, 3H); 5.28(s, 2H); 7.28(s, 1H); 7.35-7.44(m, 2H); 7.50(d, 2H); 7.58(d, 1H); 7.70(d, 1H); 7.80(s, 1H); 8.37(s, 1H); 9.30(s,1H)

MS - ESI: 468 $[MH]^+$

A solution of 4-(5-acetoxy-4-chloro-2-fluoroanilino)-7-benzyloxy-6-methoxyquinazoline (250mg, 0.54mmol) in methanol (5ml), trichloromethane (5ml) and DMF (1ml) was stirred under hydrogen at 1 atmosphere with 5% palladium-on-charcoal catalyst (100mg) for 4 hours. The catalyst was removed by filtration through diatomaceous earth and the solvent removed by evaporation. The residue was dissolved in ethyl acetate, washed with water and brine, and dried ($MgSO_4$). Most of the solvent was removed by evaporation, the mixture was cooled and hexane added to obtain solid product which was collected by filtration, washed with hexane/ethyl acetate and dried to give 4-(5-acetoxy-4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (170mg, 45%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.37(s, 3H); 3.95(s, 3H); 7.08(s, 1H); 7.59(d, 1H); 7.68(d, 1H); 7.78(s, 1H); 8.34(s, 1H); 9.48(s,1H)

1-1'-(Azodicarbonyl)dipiperidine (413mg, 1.6mmol) was added portionwise to a stirred mixture of 4-(5-acetoxy-4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (250mg, 0.66mmol), 2-methoxyethanol (63ml, 0.8mmol) and tributylphosphine (405ml, 1.6mmol) in methylene chloride at 0°C. The resulting solution was allowed to warm to ambient temperature and stirred for 2 hours. The precipitated solid was removed by filtration, the solvent removed from the filtrate by evaporation and the residue purified by flash chromatography eluting with acetonitrile/methylene chloride (1/9 increasing in polarity to 4/6) to give 4-(5-acetoxy-4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline (180mg, 62%) as a solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 2.35(s, 3H); 3.33(s, 3H); 3.75(t, 2H); 3.95(s, 3H); 4.28(t, 2H); 7.22(s, 1H); 7.60(d, 1H); 7.72(d, 1H); 7.80(s, 1H); 8.39(s, 1H); 9.60(s,1H)

MS - ESI: 436 $[MH]^+$

Example 7

A mixture of 4-chloro-6,7-dimethoxyquinazoline hydrochloride (2.1g, 8mmol), (prepared as described for the starting material in Example 1 but without the aqueous work up), and 4-chloro-2-fluoro-5-hydroxyaniline (1.43g, 8.9mmol), (as described in EP 61741 A2), in isopropanol (150ml) was heated at reflux for 2 hours. The mixture was allowed to cool, the solid product collected by filtration, washed with isopropanol and dried to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline** hydrochloride (1.45g, 47%).

m.p. >250°C

^{1}H NMR Spectrum: ($DMSOd_6$) 4.0(s, 6H); 7.17(d, 1H); 7.34(s, 1H); 7.50(d, 1H); 8.22(s, 1H); 8.80(s, 1H)

MS - ESI: 350 $[MH]^+$

Elemental analysis :	Found	C 49.2	H 3.7	N 10.9	
$C_{16}H_{13}N_3ClFO_3$ 1HCl	Requires	C 49.7	H 3.6	N 10.9%	

Example 8

A mixture of 4-chloro-6,7-dimethoxyquinazoline hydrochloride (2.5g, 9.6mmol), (prepared as described for the starting material in Example 1 but without the aqueous work up), and 2-fluoro-5-hydroxy-4-methylaniline (1.48g, 10.5mmol) in isopropanol (150ml) was heated at reflux for 2 hours. The mixture was allowed to cool, the solid product collected by filtration, washed with isopropanol and dried to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6,7-dimethoxyquinazoline** hydrochloride (2.2g, 71%).

m.p. >250°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.15(s, 3H); 3.99(s, 6H); 6.88(d, 1H); 7.10(d, 1H); 7.32(s, 1H); 8.20(s, 1H); 8.78(s, 1H) 9.66(s, 1H)

Elemental analysis:	Found	C 56.3	H 5.4	N 10.4	
$C_{17}H_{16}N_3FO_3$ 1HCl 0.65C_3H_8O	Requires	C 56.3	H 5.5	N 10.4%	

The starting material was prepared as follows:

Methyl chloroformate (6.8ml, 88mmol) was added over 30 minutes to a solution of 4-fluoro-2-methylphenol (10g, 79mmol) in 6% aqueous sodium hydroxide solution at 0°C. The mixture was stirred for 2 hours, then extracted with ethyl acetate (100ml). The ethyl

acetate extract was washed with water (100ml) and dried ($MgSO_4$) and the solvent removed by evaporation to give 4-fluoro-2-methylphenyl methyl carbonate (11.4g, 78%) as an oil.

^{1}H NMR Spectrum: ($DMSOd_6$) 2.14(s, 3H); 3.81(s, 3H); 7.05(m, 1H); 7.1-7.25(m, 2H)

A mixture of concentrated nitric acid (6ml) and concentrated sulphuric acid (6ml) was added slowly to a solution of 4-fluoro-2-methylphenyl methyl carbonate (11.34g, 62mmol) in concentrated sulphuric acid (6ml) such that the temperature of the reaction mixture was kept below 50°C. The mixture was stirred for 2 hours, then ice/water was added and the precipitated product collected by filtration. The crude product was purified by chromatography on silica eluting with methylene chloride/hexane progressing through increasingly polar mixtures to methanol/methylene chloride (1:19) to give 4-fluoro-2-methyl-5-nitrophenol (2.5g, 22%) as a solid.

^{1}H NMR Spectrum: ($DMSOd_6$, CD_3CO_2D) 2.31(s, 3H); 7.38(d, 1H); 7.58(d, 1H)

MS - ESI: 171 $[MH]^+$

A mixture of 4-fluoro-2-methyl-5-nitrophenol (2.1g, 13mmol), iron powder (1g, 18mmol) and iron(II)sulphate (1.5g, 10mmol) in water (40ml) was refluxed for 4 hours. The reaction mixture was allowed to cool, neutralised with 2M aqueous sodium hydroxide and extracted with ethyl acetate (100ml). The ethyl acetate extract was dried ($MgSO_4$) and the solvent removed by evaporation to give 2-fluoro-5-hydroxy-4-methylaniline (0.8g, 47%) as a solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 1.94(s, 3H); 4.67(s, 2H); 6.22(d, 1H); 6.65(d, 1H); 8.68(s, 1H)

MS - ESI: 142 $[MH]^+$

Example 9

A mixture of 4-chloro-6-methoxy-7-(2-methoxyethoxy)quinazoline (76mg, 0.28mmol) and 2-fluoro-5-hydroxy-4-methylaniline (40mg, 0.28mmol), (prepared as described for the starting material in Example 8), in isopropanol (2.5ml) was stirred and heated at reflux for 7 hours. The reaction mixture was allowed to cool and the precipitated product collected by filtration, washed with isopropanol and dried to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline** hydrochloride (79mg 66%) as a white solid.

m.p. >275°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.19(s, 3H); 3.36(s, 3H); 3.80(m, 2H); 4.00(s, 3H); 4.33(m, 2H); 6.90(d, 1H); 7.10(d, 1H); 7.37(s, 1H); 8.20(s, 1H); 8.75(s, 1H) 9.65(br s, 1H); 11.25(br s, 1H)

MS - ESI: 374 $[MH]^+$

Elemental analysis :	Found	C 55.7	H 4.8	N 10.1
$C_{19}H_{20}N_3FO_4$ 1HCl	Requires	C 55.7	H 5.2	N 10.3%

The starting material was prepared as follows:

A mixture of ethyl 4-hydroxy-3-methoxybenzoate (9.8g, 50mmol), 2-bromoethyl methyl ether (8.46ml, 90mmol) and potassium carbonate (12.42g, 90mmol) in acetone (60ml) was heated at reflux for 30 hours. The mixture was allowed to cool and the solids removed by filtration. The volatiles were removed from the filtrate by evaporation and the residue triturated with hexane to give ethyl 3-methoxy-4-(2-methoxyethoxy)benzoate (11.3g, 89%) as a white solid.

m.p. 57-60°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.31(t, 3H); 3.29(s, 3H); 3.32(s, 3H); 3.68(m, 2H); 4.16(m, 2H); 4.28(q, 2H); 7.06(d, 1H); 7.45(d, 1H); 7.56(dd, 1H)

MS - FAB: 255 $[MH]^+$

Ethyl 3-methoxy-4-(2-methoxyethoxy)benzoate (9.5g, 37mmol) was added portionwise to stirred concentrated nitric acid (75ml) at 0°C. The mixture was allowed to warm to ambient temperature and stirred for a further 90 minutes. The mixture was diluted with water and extracted with methylene chloride, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was triturated with hexane to give ethyl 5-methoxy-4-(2-methoxyethoxy)-2-nitrobenzoate (10.6g, 95%) as an orange solid.

m.p. 68-69°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.27(t, 3H); 3.30(s, 3H); 3.69(m, 2H); 3.92(s, 3H); 4.25(m, 2H); 4.29(q, 2H); 7.30(s, 1H); 7.65(s, 1H)

MS - CI: 300 $[MH]^+$

A mixture of ethyl 5-methoxy-4-(2-methoxyethoxy)-2-nitrobenzoate (10.24g, 34mmol), cyclohexene (30ml) and 10% palladium-on-charcoal catalyst (2.0g) in methanol

(150ml) was heated at reflux for 5 hours. The reaction mixture was allowed to cool and diluted with methylene chloride. The catalyst was removed by filtration and the volatiles removed from the filtrate by evaporation. The residue was recrystallised from ethyl acetate/hexane to give ethyl 2-amino-5-methoxy-4-(2-methoxyethoxy) benzoate (8.0g) as a buff solid. Formamide (80ml) was added to this product and the mixture heated at 170°C for 18 hours. About half the solvent was removed by evaporation under high vacuum and the residue was left to stand overnight. The solid product was collected by filtration, washed with ether and dried to give 6-methoxy-7-(2-methoxyethoxy)-3,4-dihydroquinazolin-4-one (5.3g, 62% over two steps) as a grey solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.35(s, 3H); 3.74(m, 2H); 3.89(s, 3H); 4.26(m, 2H); 7.15(s, 1H); 7.47(s, 1H); 7.98(s, 1H); 12.03(br s, 1 H)

MS - CI: 251 $[MH]^+$

DMF (0.5ml) was added to a mixture of 6-methoxy-7-(2-methoxyethoxy)-3,4-dihydroquinazolin-4-one (5.1g, 20mmol) in thionyl chloride (50ml). The mixture was stirred and heated at reflux for 3 hours, allowed to cool and the excess thionyl chloride removed by evaporation. The residue was suspended in methylene chloride and washed with aqueous sodium hydrogen carbonate solution. The aqueous phase was extracted with methylene chloride and the combined extracts dried ($MgSO_4$). The crude product was recrystallised from methylene chloride/hexane to give 4-chloro-6-methoxy-7-(2-methoxyethoxy)quinazoline (2.8g, 51%) as a fine white solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.37(s, 3H); 3.77(m, 2H); 4.01(s, 3H); 4.37(m, 2H); 7.40(s, 1H); 7.49(s, 1H); 8.88(s, 1H)

MS - CI: 269 $[MH]^+$

Example 10

A mixture of 4-chloro-6,7-dimethoxyquinazoline hydrochloride, (152mg, 0.6mmol), (prepared as described for the starting material in Example 1 but without the aqueous work up), and 4-bromo-2,6-difluoroaniline (121mg, 0.6mmol) in isopropanol (7ml) was heated at reflux for 2 hours. The mixture was allowed to cool, the solid product collected by filtration, washed with isopropanol and dried to give **4-(4-bromo-2,6-difluoroanilino)-6,7-dimethoxyquinazoline** hydrochloride (81mg, 35%).

^{1}H NMR Spectrum: ($DMSOd_6$) 4.0(s x 2, 3H each); 7.2(s, 1H); 7.35(d, 2H); 8.2(s, 1H); 8.9(s, 1H); 11.8(br s, 1H)

MS - ESI: 396 $[MH]^-$

Example 11

4-Chloro-6,7-dimethoxyquinazoline hydrochloride (300mg, 1.15mmol), (prepared as described for the starting material in Example 1 but without the aqueous work up), and 2,4-difluoro-5-hydroxyaniline (184mg, 0.90mmol) in isopropanol (10ml) were heated at reflux for 2 hours. The reaction mixture was then allowed to cool, the precipitated product collected by filtration, washed with isopropanol and dried to give **4-(2,4-difluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline** hydrochloride (250mg, 65%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.99(s, 6H); 7.05(dd, 1H); 7.17(s, 1H); 7.40(dd, 1H); 8.10(s, 1H); 8.68(s, 1H)

MS - ESI: 334 $[MH]^+$

Elemental analysis:	Found	C 51.8	H 3.9	N 11.3
$C_{16}H_{13}N_3O_3F_2$ 1HCl	Requires	C 52.0	H 3.8	N 11.4%

The starting material was prepared as follows:

Methyl chloroformate (16.35ml, 0.173mol) was added to a solution of 2,4-difluorophenol (25g, 0.192mol) and sodium hydroxide (8.1g, 0.203mol) in water (140ml). The mixture was stirred at ambient temperature for 2 hours and then extracted with ethyl acetate. The extract was washed with water, dried ($MgSO_4$) and the volatiles removed by evaporation to give 2,4-difluoro-1-methoxycarbonyloxybenzene (32g, 89%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.85(s, 3H); 7.64(d, 2H); 7.72(d,1H)

A mixture of concentrated nitric acid (4ml) and concentrated sulphuric acid (4ml) was added slowly to a cooled mixture of 2,4-difluoro-1-methoxycarbonyloxybenzene (5.0g, 0.027mol) in concentrated sulphuric acid (4ml) such that the reaction temperature was maintained below 30°C. The mixture was stirred for a further 3 hours, diluted with ice/water and the precipitated product collected by filtration washed with water and dried to give 2,4-difluoro-5-methoxycarbonyloxy-1-nitrobenzene (2.8g, 45%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.85(s, 3H); 7.97(dd, 1H); 8.44(dd, 1H)

A mixture of 2,4-difluoro-5-methoxycarbonyloxy-1-nitrobenzene (2.7g, 0.012mol) and 10% palladium-on-charcoal catalyst (500mg) in ethanol (20ml) and ethyl acetate (10ml) was stirred under 1 atmosphere of hydrogen for 4 hours. The catalyst was removed by filtration through diatomaceous earth and the solvent removed by evaporation to give 2,4-difluoro-5-methoxycarbonyloxyaniline (2.3g, 97%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.82(s, 3H); 5.20(s, 2H); 6.65(dd,1H); 7.20(dd, 1H)

MS - ESI: 204 $[MH]^+$

Concentrated aqueous ammonia (20ml) was added to a solution of 2,4-difluoro-5-methoxycarbonyloxyaniline (2.0g, 9.85mmol) in ethanol (100ml) and the mixture stirred at ambient temperature for 3 hours. The reaction mixture was diluted with water and most of the organic volatiles were removed by evaporation. The aqueous residue was neutralised to pH7 and extracted with ethyl acetate. The extracts were washed with water, dried (MgSO4) and the solvent removed by evaporation to give 2,4-difluoro-5-hydroxyaniline (1.2g, 85%).

^{1}H NMR Spectrum: ($DMSOd_6$) 4.78(s, 2H); 6.34(t,1H); 6.87(t, 1H); 9.23(s, 1H)

MS - ESI: 145 $[MH]^+$

Example 12

6-Methoxy-7-(2-methoxyethoxy)-3,4-dihydroquinazolin-4-one (200mg, 0.8mmol), (prepared as described for the starting material in Example 9), and DMF (0.1ml) in thionyl chloride (20ml) were heated at reflux for 2 hours. Excess thionyl chloride was removed by evaporation and the residue azeotroped with toluene. The residue was dissolved in isopropranol (15ml), 2,4-difluoro-5-hydroxyaniline (128mg, 0.88mmol), (prepared as described for the starting material in Example 11), added, and the mixture heated at reflux for 2 hours. The reaction mixture was then allowed to cool, the precipitated product collected by filtration, washed with isopropanol and dried to give **4-(2,4-difluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline** hydrochloride (83mg, 28%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.35(s, 3H); 3.77(t, 2H); 4.00(s, 3H); 4.30(t, 2H); 7.10)(dd, 1H); 7.36(s, 1H); 7.40(t, 2H); 8.20(s, 1H); 8.78(d, 2H)

MS - ESI: 378 $[MH]^+$

Elemental analysis:	Found	C 51.8	H 4.2	N 10.1
$C_{18}H_{17}N_3O_4F_2$ 1HCl	Requires	C 52.2	H 4.4	N 10.2%

Example 13

A mixture of 7-(2-acetoxyethoxy)-4-(5-benzyloxy-2-fluoro-4-methylanilino)-6-methoxyquinazoline (133mg, 0.27mmol) and 10% palladium-on-charcoal catalyst (50mg) in ethyl acetate (8ml) was stirred under 1 atmosphere of hydrogen at ambient temperature for 18 hours. The catalyst was removed by filtration through diatomaceous earth and most of the solvent removed by evaporation and hexane added to the residue. The resulting precipitated product was collected by filtration and dried to give **7-(2-acetoxyethoxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline** (16mg, 15%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.05(s, 3H); 2.13(s, 3H); 3.91 (s, 3H); 4.3-4.4(m, 4H); 6.90(d, 1H); 6.98(d, 1H); 7.18(s, 1H); 7.79(s, 1H); 8.30(s, 1H); 9.15(s, 2H)

MS - ESI: 402 $[MH]^-$

The starting material was prepared as follows:

A mixture of 4-fluoro-2-methyl-5-nitrophenol (4.69g, 27mmol), (prepared as described for the starting material in Example 8), benzyl bromide (3.59ml, 30mmol) and potassium carbonate (7.58g, 55mmol) in DMF (100ml) was heated at 80°C for 4 hours. The reaction mixture was allowed to cool and diluted with water and stirred for 15 minutes. The precipitated product was collected by filtration, washed with water and dried to give 5-benzyloxy-2-fluoro-4-methyl-1-nitrobenzene (6.4g, 89%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.28(s, 3H); 5.22(s, 2H); 7.3-7.5(m, 6H); 7.70(s, 1H)

5-Benzyloxy-2-fluoro-4-methyl-1-nitrobenzene (500mg, 1.9mmol) in methanol (10ml) was added to a suspension of Raney nickel (75mg) and hydrazine hydrate (465ml, 9.5mmol) in methanol (10ml) and heated at reflux. The mixture was maintained under reflux for 15 minutes and then the insoluble materials removed by filtration through diatomaceous earth. The filter pad was washed with methanol and the solvent removed from the filtrate by evaporation to give 5-benzyloxy-2-fluoro-4-methylaniline (440mg, 99%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.02(s, 3H); 4.88(s, 2H); 4.98(s, 2H); 6.44(d, 1H); 6.76(d, 1H); 7.3-7.5(m, 5H)

A mixture of 7-benzyloxy-6-methoxy-3,4-dihydroquinazolin-4-one (5.0g, mmol), (prepared as described for the starting material in Example 4), acetic anhydride (200ml),

sodium acetate (12g), 10% palladium-on-charcoal catalyst (1.5g) in toluene (100ml) was stirred under an atmosphere of hydrogen for 3 hours. The mixture was filtered and the filtrate was evaporated. The residue was partitioned between a mixture of ethyl acetate (500ml), methanol (20ml) and water (300ml). The organic phase was separated, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was triturated with hexane to give 7-acetoxy-6-methoxy-3,4-dihydroquinazolin-4-one (1.1 g, 27%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.29(s, 3H); 3.84(s, 3H); 7.42(s, 1H); 7.62(s, 1H); 8.1(br s, 1H)

MS - ESI: 235 $[MH]^+$

A mixture of 7-acetoxy-6-methoxy-3,4-dihydroquinazolin-4-one (1.69g, 7.2mmol), thionyl chloride (50ml) and DMF (3 drops) was heated at reflux for 2 hours. The excess thionyl chloride was removed by evaporation and the residue azeotroped with toluene. The residue was partitioned between methylene chloride and saturated aqueous sodium hydrogen carbonate solution. The organic phase was separated, dried ($MgSO_4$) and the solvent removed by evaporation. 5-Benzyloxy-2-fluoro-4-methylaniline (1.8g, 7.8mmol) in isopropanol (50ml) was added to the residue and the mixture heated at reflux for 2 hours. The mixture was allowed to cool, hexane added and the precipitated product collected by filtration to give 7-acetoxy-4-(5-benzyloxy-2-fluoro-4-methylanilino)-6-methoxyquinazoline (1.34g, 43%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.24(s, 3H); 2.38(s, 3H); 4.00(s, 3H); 5.10(s, 2H); 7.1-7.5(m, 7H); 7.75(s, 1H); 8.39(s, 1H); 8.77(s, 1H)

Concentrated aqueous ammonia (25ml) was added to a solution of 7-acetoxy-4-(5-benzyloxy-2-fluoro-4-methylanilino)-6-methoxyquinazoline (1.5g, 3.4mmol) in methanol (100ml). The mixture was stirred at ambient temperature for 30 minutes, and most of the organic volatiles were then removed by evaporation. Further water was added and the precipitate was collected by filtration, washed with water and dried to give 4-(5-benzyloxy-2-fluoro-4-methylanilino)-7-hydroxy-6-methoxyquinazoline (1.2g, 89%) which was used without further characterisation.

A mixture of 4-(5-benzyloxy-2-fluoro-4-methylanilino)-7-hydroxy-6-methoxyquinazoline (440mg, 1mmol), 2-bromoethanol (77ml, 1mmol) and potassium carbonate (150mg, 1.1mmol) in DMF (5ml) was heated at 50 °C for 1 hour, further 2-bromoethanol (42ml, 0.6mmol) and potassium carbonate (150mg, 1.1mmol) was added and

the mixture was maintained at 50°C for 2 hours. The reaction mixture was diluted with water, neutralised with 2M hydrochloric acid and extracted with ethyl acetate. The extracts were dried ($MgSO_4$), the solvent removed by evaporation and the residue triturated with ether and hexane to give 4-(5-benzyloxy-2-fluoro-4-methylanilino)-7-(2-hydroxyethoxy)-6-methoxyquinazoline (200mg, 41%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.21(s, 3H); 3.80(t, 2H); 3.94(s, 3H); 4.14(t, 2H); 4.90(s, 1H); 5.10(s, 2H); 7.05-7.2(m, 2H); 7.25-7.45(m, 5H); 7.79(s, 1H); 8.30(s, 1H); 9.20(s, 1H)

Acetic anhydride (55ml, 0.58mmol) was added to a mixture of 4-(5-benzyloxy-2-fluoro-4-methylanilino)-7-(2-hydroxyethoxy)-6-methoxyquinazoline (233mg, 0.52mmol), triethylamine (80ml, 0.57mmol) and 4-(N,N-dimethylamino)pyridine (5mg) in ethyl acetate (50ml). The mixture was stirred for 2 hours at ambient temperature, water was added, the organic layer separated, washed with water and brine and dried ($MgSO_4$). Most of the solvent was removed by evaporation and hexane added. The precipitated product was collected by filtration to give 7-(2-acetoxyethoxy)-4-(5-benzyloxy-2-fluoro-4-methylanilino)-6-methoxyquinazoline (110mg, 43%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.03(s, 3H); 2.22(s, 3H); 3.92(s, 3H); 4.3-4.4(m, 4H); 5.08(s, 2H); 7.13(d, 1H); 7.18(d, 1H); 7.3-7.45(m, 5H); 7.80(s, 1H); 8.30(s, 1H); 9.42(s, 1H)

Example 14

A mixture of 4-(5-benzyloxy-2-fluoro-4-methylanilino)-7-(2-hydroxyethoxy)-6-methoxyquinazoline (150mg, 0.33mmol), (prepared as described for the starting material in Example 13), and 10% palladium-on-charcoal catalyst (20mg) in ethyl acetate (8ml) was stirred under 1 atmosphere of hydrogen at ambient temperature for 18 hours. The catalyst was removed by filtration through diatomaceous earth and most of the solvent removed by evaporation and hexane added to the residue. The resulting precipitate was collected by filtration and dried to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-hydroxyethoxy)-6-methoxyquinazoline** (50mg, 41%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.14(s, 3H); 3.80(q, 2H); 3.94(s, 3H); 4.15(t, 2H); 4.90(t, 1H); 6.90(d, 1H); 7.00(d, 1H); 7.17(s, 1H); 7.80(s, 1H); 8.33(s, 1H); 9.32(s, 1H); 9.37(s, 1H)

MS - ESI: 360 $[MH]^+$

Example 15

4-Chloro-6,7-dimethoxyquinazoline hydrochloride (210mg, 0.8mmol), (prepared as described for the starting material in Example 1 but without the aqueous work up), and 4-chloro-2,6-difluoroaniline hydrochloride (177mg, 0.89mmol) in isopropanol (8ml) were heated at reflux for 2 hours. The reaction mixture was then allowed to cool, hexane added and the precipitated product collected by filtration, washed with isopropanol and dried to give **4-(4-chloro-2,6-difluoroanilino)-6,7-dimethoxyquinazoline** hydrochloride (45mg, 16%).

m.p. >250°C

^{1}H NMR Spectrum: ($DMSOd_6$) 4.00(s, 3H); 4.01(s, 3H); 7.35(s, 1H); 7.63(d, 2H); 8.22(s, 1H); 8.81(s, 1H)

MS - ESI: 352 $[MH]^+$

The starting material was prepared as follows:

A solution of 3,5-difluoronitrobenzene (20g,126mmol) and ethyl dichloroacetate (15.8ml, 129mmol) in DMF (60ml) was added to potassium t-butoxide (31.8g, 283mmol) in DMF (500ml) at -25°C over 30 minutes. The mixture was stirred for 15 minutes at -25°C then poured on to a mixture of ice (600g) and 2M hydrochloric acid (500ml). The aqueous mixture was extracted with ethyl acetate, the combined extracts were washed with water and sodium hydrogen carbonate solution and dried ($MgSO_4$) and the solvent removed by evaporation to give ethyl 2-chloro-2-(2,6-difluoro-4-nitrophenyl)ethanoate (34g, 97%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.15(t, 3H); 4.1-4.3(m, 2H); 6.44(s, 1H); 8.17(d, 2H)

2.5M Aqueous sodium hydroxide solution (300ml) was added over 5 minutes to a solution of ethyl 2-chloro-2-(2,6-difluoro-4-nitrophenyl)ethanoate (34.86g 125mmol) in ethanol (300ml) at 5°C such that the reaction temperature was kept below 25°C. The mixture was cooled to 18°C and 30% hydrogen peroxide (40ml) was added. The mixture was stirred at 20°C for 2.5 hours. Sodium sulphite was added until the peroxide test was negative, the mixture was acidified to pH1 with 6M hydrochloric acid and extracted with ethyl acetate. The organic extracts were back extracted with saturated aqueous sodium hydrogen carbonate solution, the aqueous extracts were acidified with concentrated hydrochloric acid and extracted with ethyl acetate. The extracts were dried ($MgSO_4$) and the solvent removed by evaporation to give 2,6-difluoro-4-nitrobenzoic acid (4.89g, 19%).

^{1}H NMR Spectrum: ($DMSOd_6$) 8.14(d, 2H)

A mixture of 2,6-difluoro-4-nitrobenzoic acid (2.5g, 12mmol) and 10% palladium-on-charcoal catalyst (500mg) in ethanol (150ml) was stirred under 1 atmosphere of hydrogen at ambient temperature for 3 hours. The catalyst was removed by filtration through diatomaceous earth, the filter pad washed with ethanol and the solvent removed by evaporation to give 4-amino-2,6-difluorobenzoic acid (3.8g, 91%).

^{1}H NMR Spectrum: ($DMSOd_6$) 6.12(d, 2H); 6.28(s, 2H)

MS - ESI: 174 $[MH]^+$

A solution of sodium nitrite (220mg, 3.18mmol) in concentrated sulphuric acid (2ml) was added over 15 minutes to a suspension of 4-amino-2,6-difluorobenzoic acid (550mg, 3.18mmol) in acetic acid (6ml) at 15°C. The mixture was stirred at 15°C for 1 hour then heated to 90°C and poured into a solution of copper(I)chloride (800mg) in concentrated hydrochloric acid (11ml) at 95°C. The mixture was heated at 95°C for 45 minutes and then allowed to cool. The mixture was diluted with water, extracted with ethyl acetate, the organic extracts dried ($MgSO_4$) and the solvent removed by evaporation to give 4-chloro-2,6-difluorobenzoic acid (600mg, 98%)

^{1}H NMR Spectrum: ($DMSOd_6$) 7.50(d, 2H)

MS - ESI: 192 $[MH]^-$

4-Chloro-2,6-difluorobenzoic acid (500mg, 2.6mmol) was added to a solution of diphenylphosphoryl azide (737mg, 3mmol) in t-butanol (8ml) followed by triethylamine (477ml, 6mmol) and the mixture heated at reflux for 2 hours. The reaction mixture was allowed to cool and the solvent removed by evaporation. The residue was dissolved in ethyl acetate, washed with water, dried ($MgSO_4$) and purified by column chromatography eluting with increasingly polar mixtures of methylene chloride, hexane and methanol (1/1/0 to 95/0/5) to give N-t-butoxycarbonyl-4-chloro-2,6-difluoroaniline (170mg, 25%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.41(s, 9H); 7.39(d, 2H); 8.86(s, 1H)

A saturated solution of hydrogen chloride in ethyl acetate (4ml) was added to N-t-butoxycarbonyl-4-chloro-2,6-difluoroaniline (330mg, 1.3mmol) and the mixture stirred at ambient temperature for 2 hours. The precipitate was collected by filtration to give 4-chloro-2,6-difluoroaniline hydrochloride (140mg, 56%).

^{1}H NMR Spectrum: ($DMSOd_6$) 6.12(s, 2H); 7.08(d, 2H)

Example 16

A mixture of 6-methoxy-7-(3-morpholinopropoxy)-3,4-dihydroquinazolin-4-one (370mg, 1.16mmol), thionyl chloride (5ml) and DMF (3 drops) was heated at reflux for 2 hours and allowed to cool. The excess thionyl chloride was removed by evaporation and the residue was azeotroped with toluene. A solution of 2-fluoro-5-hydroxy-4-methylaniline (220mg, 1.56mmol) in isopropanol (10ml) was added to the solid residue and the mixture was heated at reflux for 2 hours and then allowed to cool. The resulting precipitate was collected by filtration, washed with methylene chloride and dried. The impure solid product was treated with aqueous sodium hydrogen carbonate, to give a suspension and the product was recollected by filtration and purified by column chromatography eluting with methylene chloride/methanol (9/1) to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline** (140mg, 27%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.0(m, 2H); 2.15(s, 3H); 2.35-2.55(m, 6H); 3.55(br t, 4H); 3.90(s, 3H); 4.20(t, 2H); 6.85-6.95(m, 2H); 7.10(s, 1H); 7.75(s, 1H); 8.25(s, 1H); 9.20(s, 2H)

Elemental analysis:	Found	C 62.2	H 6.1	N 12.4
$C_{23}H_{27}N_4O_4F$	Requires	C 62.4	H 6.2	N 12.7%

The starting material was prepared as follows:

Sodium hydride (400mg of an 80% suspension in paraffin oil, 13.3mmol) was added to a solution of phenol (1.26g, 13.3mmol) in dry 1-methyl-2-pyrrolidinone (20ml) and the mixture stirred for 10 minutes. 7-Benzyloxy-4-chloro-6-methoxyquinazoline (1.6g, 5.3mmol), (prepared as described for the starting material in Example 4 but with an aqueous work up), was then added and the reaction mixture heated at 110°C for 2 hours. The mixture was allowed to cool, water was added and the mixture extracted with ethyl acetate (3 x 100ml). The combined extracts were then washed with 2M sodium hydroxide solution, water and brine. Removal of the solvent under reduced pressure gave 7-benzyloxy-6-methoxy-4-phenoxyquinazoline (1.6g, 84%) as a yellowish solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.98(s, 3H); 5.37(s, 2H); 7.25-7.6(m, 11H); 7.60(s, 1H); 8.54(s, 1H)

MS - ESI: 300 $[MH]^+$

7-Benzyloxy-6-methoxy-4-phenoxyquinazoline (160mg, 0.44mmol) in TFA (3ml) was heated at reflux for 30 minutes. The solvent was removed by evaporation and the residue treated with aqueous sodium hydrogen carbonate solution. The precipitated product was collected by filtration, washed with water and dried to give 7-hydroxy-6-methoxy-4-phenoxyquinazoline (105mg, 88%).

^{1}H NMR Spectrum: ($DMSOd_6$) 4.00(s, 3H); 7.20(s, 1H); 7.25-7.35(m, 3H); 7.4-7.55(m, 2H); 7.58(s, 1H); 10.73(s, 1 H)

MS - ESI: 269 $[MH]^+$

4-(3-Chloropropyl)morpholine (0.9g, 4.5mmol), (J. Am. Chem. Soc. 1945, 67, 736, 174mg, 1.06mmol), was added to 7-hydroxy-6-methoxy-4-phenoxyquinazoline (1.0g, 3.7mmol), potassium carbonate (2.6g, 18.8mmol) in DMF (30ml). The mixture was heated at 110°C for 4 hours and then allowed to cool. The solids were removed by filtration, and the volatiles were removed from the filtrate by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol, (96/4) to give 6-methoxy-7-(3-morpholinopropoxy)-4-phenoxyquinazoline (1.0g, 68%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.0 (m, 2H); 2.35-2.55(m, 6H); 3.6(br s, 4H); 3.95(s, 3H); 4.25(t, 2H); 7.25-7.35(m, 3H); 7.40(s, 1H); 7.45-7.55(m, 2H); 7.55(s, 1H); 8.50(s, 1H)

MS - ESI: 396 $[MH]^+$

A mixture of 6-methoxy-7-(3-morpholinopropoxy)-4-phenoxyquinazoline (980mg, 2.48mmol) and 2M hydrochloric acid (25ml) was heated at 100°C for 2 hours and allowed to cool. The solution was basified with solid sodium hydrogen carbonate, and the product was extracted with methylene chloride. The organic phase was passed through phase separating paper and the solvent removed by evaporation to give 6-methoxy-7-(3-morpholinopropoxy)-3,4-dihydroquinazolin-4-one (750mg, 95%) as a pale brown solid which was used without further purification.

MS - ESI: 320 $[MH]^+$

Example 17

A mixture of 6-methoxy-7-(3-morpholinopropoxy)-3,4-dihydroquinazolin-4-one (370mg, 1.16mmol), (prepared as described for the starting material in Example 16), thionyl chloride (5ml) and DMF (3 drops) was heated at reflux for 2 hours and allowed to cool. The

excess thionyl chloride was removed by evaporation and the residue was azeotroped with toluene. A solution of 4-chloro-2-fluoro-5-hydroxyaniline (210mg, 1.30mmol), (as described in EP 61741 A2), in isopropanol (10ml) was added to the solid residue and the mixture was heated at reflux for 2 hours and then allowed to cool. The mixture was diluted with acetone and the precipitate collected by filtration. The crude solid product was suspended in aqueous sodium hydrogen carbonate, collected again by filtration and purified by column chromatography eluting with methylene chloride/methanol/ammonia (100/10/1) to give **4-(4-chloro-2-fluoro-5-hydroxyaniline)-6-methoxy-7-(3-morpholinopropoxy)quinazoline** (160mg, 30%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.0(m, 2H); 2.35-2.55(m, 6H); 3.6(t, 4H); 3.95(s, 3H); 4.15(t, 2H); 7.15(m, 2H); 7.35(d, 1H); 7.75(s, 1H); 8.35(s, 1H); 9.35(s, 1H); 10.15(s, 1H)

MS - ESI: 463 $[MH]^+$

Elemental analysis:	Found	C 57.1	H 5.3	N 12.0
$C_{22}H_{24}N_4O_4FCl$	Requires	C 57.1	H 5.2	N 12.1%

Example 18

1M Ethereal hydrogen chloride (3.1ml, 3.1mmol) was added to 4-chloro-6-methoxy-7-(2-methylthioethoxy)quinazoline (0.8g, 2.8mmol) and 2-fluoro-5-hydroxy-4-methylaniline (0.44g, 3.12mmol), (prepared as described for the starting material in Example 8), in isopropanol (25ml). The mixture was heated at reflux for 2 hours, then allowed to cool. The resulting suspension was diluted with acetone and the precipitate collected by filtration and purified by column chromatography eluting with methylene chloride/methanol/ammonia (100/8/1) to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methylthioethoxy)quinazoline** (580mg, 52%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.15 (s, 3H); 2.23(s, 3H); 2.94 (t, 2H); 3.95(s, 3H); 4.33(t, 2H); 6.92(d, 1H); 7.00(d, 1H); 7.20(s, 1H); 7.83(s, 1H); 8.38(s, 1H); 9.30(s, 1H); 9.33(s, 1H)

MS - ESI: 390 $[MH]^+$

Elemental analysis:	Found	C 57.4	H 5.1	N 10.5
$C_{19}H_{20}N_3O_3FS\ 0.5H_2O$	Requires	C 57.3	H 5.3	N 10.5%

The starting material was prepared as follows:

2-Chloroethyl methyl sulphide (1.2g, 10.9mmol) was added to 7-hydroxy-6-methoxy-4-phenoxyquinazoline (2.25g, 8.4mmol), (prepared as described for the starting material in Example 16), and potassium carbonate (6.0g, 43.4mmol) in DMF (70ml). The mixture was heated at 110°C for 4 hours and allowed to cool. The mixture was filtered, and the volatiles were removed from the filtrate by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (96/4) to give 6-methoxy-7-(2-methylthioethoxy)-4-phenoxyquinazoline (1.55g, 54%).

A mixture of 6-methoxy-7-(2-methylthioethoxy)-4-phenoxyquinazoline (1.5g, 4.4mmol) and 2M hydrochloric acid (25ml) was heated at 100°C for 2 hours. The mixture was allowed to cool, and methylene chloride was added with stirring to give a white precipitate. The precipitate was collected by filtration, washed with water and methylene chloride and dried to give 6-methoxy-7-(2-methylthioethoxy)-3,4-dihydroquinazolin-4-one hydrochloride (1.1g, 83%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.22(s, 3H); 2.94(t, 2H); 3.92(s, 3H); 4.30(t, 2H); 7.36(s, 1H); 7.49(s, 1H); 8.80(s, 1H)

MS - ESI: 267 $[MH]^+$

Elemental analysis:	Found	C 46.4	H 5.2	N 8.8
$C_{12}H_{14}N_2O_3S$ 1HCl	Requires	C 47.6	H 5.0	N 9.3%

A mixture of 6-methoxy-7-(2-methylthioethoxy)-3,4-dihydroquinazolin-4-one (1.07g, 4.0mmol), thionyl chloride (20ml) and DMF (4 drops) was heated at reflux for 2 hours and then allowed to cool. The excess thionyl chloride was removed by evaporation and the residue was azeotroped with toluene. The solid residue was partitioned between aqueous sodium hydrogen carbonate and methylene chloride, the organic phase was separated and washed with brine. The organic phase was passed through phase separating paper, and the solvent removed by evaporation to give 4-chloro-6-methoxy-7-(2-methylthioethoxy)quinazoline (810mg, 71%).

MS - ESI: 285 $[MH]^+$

Examples 19 and 20

3-Chloroperoxybenzoic acid (wet, 50-60%, 500mg), (3-CPBA), was added to a solution of 4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-

methylthioethoxy)quinazoline (485mg, 1.2mmol), (prepared as described for Example 18), in methylene chloride (90ml) and DMA (6ml). After 2 hours, 2 further portions of 3-CPBA were added (total 160mg). The mixture was checked for remaining oxidant, and the volatiles were removed by evaporation. The 2 products were separated by column chromatography eluting with methylene chloride/methanol (9/1) to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-(methylsulphonyl)ethoxy)quinazoline** (94mg, 19%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.15(s, 3H); 3.18(s, 3H); 3.70(t, 2H); 3.95(s, 3H); 4.50(t, 2H); 6.92(d, 1H); 6.97(d, 1H); 7.25(s, 1H); 7.83(s, 1H); 8.33(s, 1H); 9.27(s, 1H); 9.30(s, 1H)

MS - ESI: 422 $[MH]^+$

Elemental analysis:	Found	C 53.0	H 4.9	N 9.7
$C_{19}H_{20}N_3O_5SF$ $0.5H_2O$ Requires		C 53.0	H 4.9	N 9.8%

and **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-(methylsulphinyl)ethoxy)quinazoline** (120mg, 25%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.16(s, 3H); 2.69(s, 3H); 3.15(m, 1H); 3.37(m, 1H); 3.94(s, 3H); 4.53(m, 2H); 6.92(d, 1H); 6.97(d, 1H); 7.83(s, 1H); 8.32(s, 1H); 9.27(s, 1H); 9.30(s, 1H)

MS - ESI: 406 $[MH]^+$

Elemental analysis:	Found	C 55.5	H 5.0	N 10.0
$C_{19}H_{20}N_3O_4SF$	Requires	C 56.0	H 5.4	N 10.3%

Example 21

A mixture of 6-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)-3,4-dihydroquinazolin-4-one (260mg, 0.90mmol), thionyl chloride (5ml) and DMF (2 drops) was heated at reflux for 45 minutes and allowed to cool. The excess thionyl chloride was removed by evaporation, and the residue azeotroped with toluene. A solution of 4-chloro-2-fluoro-5-hydroxyaniline (160mg, 1.0mmol), (as described in EP 61741 A2), in isopropanol (5ml) was added to the residue and the mixture was heated at reflux for 1 hour and then allowed to cool. The mixture was diluted with acetone, and the solid product collected by filtration, washed with acetone and dried to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)quinazoline** hydrochloride(381mg, 83%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.85-2.15(br m, 4H); 3.20(br s, 2H); 3.5-3.7(br m, 4H); 4.05(s, 3H); 4.65(t, 2H); 7.20(d, 1H); 7.5(m, 2H); 8.45(s, 1H); 8.80(s, 1H); 10.5(br s, 1H); 11.35(br s, 1H); 11.75(br s, 1H)

MS - ESI: 433 $[MH]^+$

Elemental analysis:	Found	C 49.7	H 5.0	N 10.6
$C_{21}H_{22}N_4O_3ClF$ 2HCl 0.17isopropanol	Requires	C 50.1	H 5.0	N 10.9%

The starting material was prepared as follows:

1-(2-Chloroethyl)pyrrolidine hydrochloride (1.27g, 7.5mmol) was added to 7-hydroxy-6-methoxy-4-phenoxyquinazoline (1.0g, 3.7mmol), (prepared as described for the starting material in Example 16), and potassium carbonate (3.9g, 28.3mmol) in DMF (30ml). The mixture was heated at 110°C for 4 hours and allowed to cool. The mixture was filtered, and the volatiles were removed from the filtrate by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol/ammonia, (100/8/1) to give an oil which was triturated with ethyl acetate to give 6-methoxy-4-phenoxy-7-(2-(pyrrolidin-1-yl)ethoxy)quinazoline (200mg, 15%) as a white solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 1.65(m, 4H); 2.55(m, 4H); 2.85(t, 2H); 3.95(s, 3H); 4.25(t, 2H); 7.30(m, 3H); 7.38(s, 1H); 7.50(m, 2H); 7.55(s, 1H); 8.5(s, 1H)

MS - ESI: 366 $[MH]^+$

A mixture of 6-methoxy-4-phenoxy-7-(2-(pyrrolidin-1-yl)ethoxy)quinazoline (565mg, 1.55mmol) and 2M hydrochloric acid (5ml) was heated at 90°C for 90 minutes and allowed to cool. The solution was neutralised with aqueous sodium hydrogen carbonate, and the water removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol/ammonia (100/8/1) to give 6-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)-3,4-dihydroquinazolin-4-one (480mg). This material was used without further characterisation.

Example 22

1M Ethereal hydrogen chloride (0.72ml, 0.72mmol) was added to 4-chloro-6-methoxy-7-(2-morpholinoethoxy)quinazoline (210mg, 0.65mmol) and 4-chloro-2-fluoro-5-hydroxyaniline (115mg, 0.71mmol), (as described in EP 61741 A2), in isopropanol (5ml) and

the mixture heated at reflux for 2 hours and then allowed to cool. The mixture was diluted with acetone and the precipitated product collected by filtration. The impure product was dissolved in methylene chloride/ammonia (100/1) and methanol, the insolubles removed by filtration and the volatiles were removed from the filtrate by evaporation. The solid residue was washed with water and dried to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline** (60mg, 21%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.45-2.60(m, 4H); 2.78(t, 2H); 3.58(t, 4H); 3.94(s, 3H); 4.26(t, 2H); 7.17(d, 1H); 7.23(s, 1H); 7.38(d, 1H); 7.79(s, 1H); 8.37(s, 1H); 9.43(s, 1H); 10.17(s, 1H)

MS - ESI: 449 $[MH]^+$

Elemental analysis:	Found	C 53.5	H 5.2	N 11.6
$C_{21}H_{22}N_4O_4ClF\ 1.25H_2O$	Requires	C 53.5	H 5.3	N 11.9%

The starting material was prepared as follows:

1,2-Dibromoethane (1.6ml, 18.6mmol) was added to 7-hydroxy-6-methoxy-4-phenoxyquinazoline (0.5g, 1.86mmol), (prepared as described for the starting material in Example 16), and potassium carbonate (1.2g, 8.7mmol) in DMF (60ml) and the mixture was heated at 85°C for 2 hours, and was then allowed to cool. The insolubles were removed by filtration, and the volatiles were removed from the filtrate by evaporation to give a residue which was purified by column chromatography eluting with methylene chloride/methanol (97/3) to give 7-(2-bromoethoxy)-6-methoxy-4-phenoxyquinazoline (440mg, 63%).

MS - ESI: 375 $[MH]^+$

A mixture of morpholine (8ml) and 7-(2-bromoethoxy)-6-methoxy-4-phenoxyquinazoline (450mg, 1.2mmol) was stirred at ambient temperature for 3 hours. The excess morpholine was removed by evaporation and the residue was partitioned between aqueous sodium hydrogen carbonate and methylene chloride. The organic phase was separated, passed through phase separating paper and the solvent removed by evaporation. Trituration of the residue with isohexane gave a solid which was collected by filtration and dried to give 6-methoxy-7-(2-morpholinoethoxy)-4-phenoxyquinazoline (410mg, 90%).

MS - ESI: 382 $[MH]^+$

A mixture of 6-methoxy-7-(2-morpholinoethoxy)-4-phenoxyquinazoline (400mg, 1.05mmol) and 2M hydrochloric acid (10ml) was heated at 100°C for 2 hours and then

allowed to cool. The mixture was neutralised with solid sodium hydrogen carbonate. Addition of methylene chloride gave a white precipitate which was collected by filtration, washed with acetone and dried to give 6-methoxy-7-(2-morpholinoethoxy)-3,4-dihydroquinazolin-4-one (320mg, 100%).

MS - ESI: 306 $[MH]^+$

A mixture of 6-methoxy-7-(2-morpholinoethoxy)-3,4-dihydroquinazolin-4-one (310mg, 1.02mmol), thionyl chloride (10ml) and DMF (2 drops) was heated at reflux for 4 hours and allowed to cool. Excess thionyl chloride was removed by evaporation and the residue was azeotroped with toluene. The residue was partitioned between aqueous sodium hydrogen carbonate and methylene chloride. The organic layer was separated, washed with brine and filtered through phase separating paper. The volatiles were removed by evaporation and the residue purified by column chromatography eluting with methylene chloride/methanol (96/4) to give 4-chloro-6-methoxy-7-(2-morpholinoethoxy)quinazoline (225mg, 68%).

MS - ESI: 324 $[MH]^+$

Example 23

1M Ethereal hydrogen chloride (0.34ml, 0.34mmol) was added to 4-chloro-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline (115mg, 0.34mmol) and 4-chloro-2-fluoro-5-hydroxyaniline (61mg, 0.38mmol), (as described in EP 61741 A2), in isopropanol (5ml) and the mixture was heated at reflux for 90 minutes and then allowed to cool. The mixture was diluted with acetone, and the solid product collected by filtration. The impure solid was treated with methylene chloride/methanol/ammonia (100/8/1) (5ml), and water was added. The reprecipitated product was collected by filtration and dried to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline** (32%).

^1H NMR Spectrum: ($DMSOd_6$) 2.28(s, 3H); 2.53(m, 4H); 2.60(m, 4H); 2.81(t, 2H); 3.95(s, 3H); 4.25(t, 2H); 7.18(d, 1H); 7.20(s, 1H); 7.36(d, 1H); 7.80(s, 1H); 8.35(s, 1H); 9.43(s, 1H); 10.18(br s, 1H)

MS - ESI: 462 $[MH]^+$

Elemental analysis:	Found	C 54.1	H 5.3	N 14.0
$C_{22}H_{25}N_5O_3ClF\ 1.3H_2O$	Requires	C 54.4	H 5.7	N 14.4%

The starting material was prepared as follows:

A mixture of 1-methylpiperazine (7ml) and 7-(2-bromoethoxy)-6-methoxy-4-phenoxyquinazoline (1.0g, 2.67mmol), (prepared as described for the starting material in Example 22), was stirred at ambient temperature for 5 hours. The excess 1-methylpiperazine was removed by evaporation and the residue was partitioned between aqueous sodium hydrogen carbonate and methylene chloride. The organic phase was separated, passed through phase separating paper and the volatiles removed by evaporation to give 6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)-4-phenoxyquinazoline (970mg, 92%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.21(s, 3H); 2.38(m, 4H); 2.58(m, 4H); 2.85(t, 2H); 4.02(s, 3H); 4.35(t, 2H); 7.39(m, 3H); 7.46(s, 1H); 7.55(m, 2H); 7.61(s, 1H); 8.59(s, 1H)

A mixture of 6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)-4-phenoxyquinazoline (960mg, 2.4mmol) and 2M hydrochloric acid (20ml) was heated at 95°C for 2 hours and allowed to cool. The solution was basified with solid sodium hydrogen carbonate, the water removed by evaporation and the residue azeotroped with toluene. The residue was washed exhaustively with methylene chloride, the washings were combined, insolubles removed by filtration and the solvent removed by evaporation to give 6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)-3,4-dihydroquinazolin-4-one (500mg, 66%).

MS - ESI: 319 $[MH]^+$

A mixture of 6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)-3,4-dihydroquinazolin-4-one (500mg, 1.57mmol), thionyl chloride (20ml) and DMF (3 drops) was heated at reflux for 3 hours and allowed to cool. The excess thionyl chloride was removed by evaporation, and the residue was azeotroped with toluene. The residue was treated with aqueous sodium hydrogen carbonate and the product was extracted with methylene chloride. The combined extracts were washed with brine, passed through phase separating paper and the solvent removed by evaporation to give 4-chloro-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline (120mg, 23%).

MS - ESI: 337 $[MH]^+$

Example 24

A mixture of 6-methoxy-7-(2-piperidinoethoxy)-3,4-dihydroquinazolin-4-one (440mg, 1.45mmol), thionyl chloride (15ml) and DMF (3 drops) was heated at reflux for 3 hours then allowed to cool. The excess thionyl chloride was removed by evaporation and the residue was azeotroped with toluene to give a crude 4-chloro-6-methoxy-7-(2-piperidinoethoxy)quinazoline hydrochloride (640mg).

A sample (320mg, 0.89mmol) of this material was added to a solution of 4-chloro-2-fluoro-5-hydroxyquinazoline (130mg, 0.8mmol), (as described in EP 61741 A2), in isopropanol (10ml) and the mixture heated at reflux for 90 minutes and allowed to cool. The mixture was diluted with acetone, and the precipitated product was collected by filtration and dried. The residue was purified by column chromatography eluting with methylene chloride/methanol/ammonia, (100/8/1). The pure product was dissolved in acetone and 1M ethereal hydrogen chloride (1ml, 1mmol) added. The resulting precipitate was collected by filtration and dried to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-piperidinoethoxy)quinazoline** hydrochloride (137mg, 32%).

^{1}H NMR Spectrum: (DMSOd_6) 1.75(br m, 6H); 4.00(s, 3H); 4.65(t, 2H); 7.15(d, 1H); 7.35(s, 1H); 7.42(d, 1H); 8.15(s, 1H); 8.60(s, 1H); 10.4(s, 1H); 10.6(br s, 2H)

MS - ESI: 447 $[MH]^+$

Elemental analysis:	Found	C 51.0	H 5.4	N 10.6
$C_{22}H_{24}N_4O_3ClF$ 2HCl	Requires	C 50.8	H 5.0	N 10.8%

The starting material was prepared as follows:

1-(2-Chloroethyl)piperidine hydrochloride (0.83g, 4.5mmol) was added to 7-hydroxy-6-methoxy-4-phenoxyquinazoline (1.0g, 3.73mmol), (prepared as described for the starting material in Example 16), and potassium carbonate (2.6g, 18.8mmol) in DMF (30ml), and the mixture heated at 110°C for 2.5 hours and allowed to cool. The insolubles were removed by filtration, and the volatiles were removed from the filtrate by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (9/1) to give 6-methoxy-4-phenoxy-7-(2-piperidinoethoxy)quinazoline (1.2g, 85%).

^{1}H NMR Spectrum: (DMSOd_6) 1.38(m, 2H); 1.50(m, 4H); 2.4-2.5(m, 4H); 2.75(t, 2H); 3.95(s, 3H); 4.27(t, 2H); 7.30(m, 3H); 7.40(s, 1H); 7.46(m, 2H); 7.54(s, 1H); 8.52(s, 1H)

MS - ESI: 380 $[MH]^+$

A mixture of 6-methoxy-4-phenoxy-7-(2-piperidinoethoxy)quinazoline (1.15g, 3.0mmol) and 2M hydrochloric acid (20ml) was heated at 90°C for 2 hours and allowed to cool. The mixture was neutralised with solid sodium hydrogen carbonate and extracted with methylene chloride. The organic phase was separated, passed through phase separating paper and the volatiles removed by evaporation to give a solid product (230mg). The aqueous phase was adjusted to pH10, the resulting precipitate was collected by filtration, washed with water and dried to give a second crop of product (220mg). The products were combined to give 6-methoxy-7-(2-piperidinoethoxy)-3,4-dihydroquinazolin-4-one (450mg, 50%).

MS - ESI: 304 $[MH]^+$

Example 25

A mixture of 7-(2-cyclopentyloxyethoxy)-6-methoxy-3,4-dihydroquinazolin-4-one (260mg, 0.85mmol), thionyl chloride (5ml) and DMF (2 drops) was heated at reflux for 2 hours and allowed to cool. The excess thionyl chloride was removed by evaporation, and the residue was azeotroped with toluene. To the residue was added a solution of 4-chloro-2-fluoro-5-hydroxyaniline (140mg, 0.87mmol), (as described in EP 61741 A2), in isopropanol (5ml) and the mixture was heated at reflux for 1 hour and allowed to cool. The suspension was diluted with acetone, and the precipitate collected by filtration. The crude product was dissolved in methylene chloride/methanol/ammonia (100/8/1, 2ml), the insoluble material removed by filtration and the solvent removed from the filtrate by evaporation. The residue was dissolved in acetone, 1M ethereal hydrogen chloride (1ml, 1mmol) added and the resultant precipitate collected by filtration and dried to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-7-(2-cyclopentyloxyethoxy)-6-methoxyquinazoline** hydrochloride (50mg, 12%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.5-1.75(m, 8H); 3.75(m, 2H); 3.9-4.1(m, 1H); 4.00(s, 3H); 4.80(t, 2H); 7.20(m, 1H); 7.35(s, 1H); 7.50(d, 1H); 8.25(s, 1H); 8.75(s, 1H); 10.5(br s, 1H); 11.4(br s, 1H)

MS - ESI: 448 $[MH]^+$

Elemental analysis: Found C 54.1 H 4.8 N 8.5

$C_{22}H_{23}N_3O_4ClF$ 1HCl 0.1H_2O Requires C 54.4 H 5.0 N 8.6%

The starting material was prepared as follows:

2-Cyclopentyloxyethanol (4.3g, 33.1mmol) in pyridine (18ml) was added dropwise to a solution of 3-toluenesulphonyl chloride (6.8g, 35.7mmol) in pyridine (27ml) at 5°C. The mixture was allowed to warm to ambient temperature, and stirred overnight. The mixture was poured onto ice containing concentrated hydrochloric acid (46ml) and the product was extracted with ether. The organic phase was washed with 2M hydrochloric acid, dried ($MgSO_4$) and the solvent removed by evaporation to give 2-cyclopentyloxyethyl 4-toluenesulphonate (6.9g, 73%) which was used without further purification.

7-Hydroxy-6-methoxy-4-phenoxyquinazoline (1.11g, 4.2mmol), (prepared as described for the starting material in Example 16), in DMF (17ml) was added to a suspension of sodium hydride (184 mg of a 60% suspension in oil, 4.6mmol) in DMF (3ml). The mixture was stirred until evolution of gas ceased, and then 2-cyclopentyloxyethyl 4-toluenesulphonate (1.25g, 4.45mmol) in DMF (3ml) was added dropwise. The mixture was stirred at ambient temperature for 30 minutes, then heated at 60°C for 2 hours, and then at 80°C for a further 4 hours before being allowed to cool. The mixture was poured onto ice and extracted with methylene chloride. The combined extracts were washed with brine, passed through phase separating paper and the solvent removed by evaporation. The residue was purified by column chromatography eluting with ethyl acetate. The purified product was triturated with isohexane to give 7-(2-cyclopentyloxyethoxy)-6-methoxy-4-phenoxyquinazoline (480mg, 28%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.2-1.7m, (8H); 3.77(m, 2H); 3.95(s, 3H); 4.0(m, 1H); 4.25(m, 2H); 7.30(m, 3H); 7.38(s, 1H); 7.45(m, 2H); 7.55(s, 1H); 8.50 (s, 1H)

MS - ESI: 381 $[MH]^+$

A mixture of 7-(2-cyclopentyloxyethoxy)-6-methoxy-4-phenoxyquinazoline (470mg, 1.2mmol) and 2M hydrochloric acid (6ml) was heated at 90°C for 2 hours and allowed to cool. Water was added, and the product was extracted with methylene chloride. The combined extracts were washed with aqueous sodium hydrogen carbonate, passed through phase separating paper and the solvent was removed by evaporation. Trituration with ethyl acetate give 7-(2-cyclopentyloxyethoxy)-6-methoxy-3,4-dihydroquinazolin-4-one (270mg, 74%).

MS - ESI: 305 $[MH]^+$

Example 26

1M Aqueous sodium hydroxide solution (4ml, 4mmol) was added to a solution of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline (820mg, 2.2mmol) in methanol (20ml) and the mixture stirred for 1 hour at ambient temperature. Concentrated hydrochloric acid (0.8ml) was added, the volatiles removed by evaporation and the residue purified by column chromatography eluting with methylene chloride/methanol (60/40) to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline** (313mg, 45%).

m.p. 276-278°C

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.18(s, 3H); 4.0(s, 3H); 6.88(d, 1H); 7.12(d, 1H); 7.26(s, 1H); 8.08(s, 1H); 8.76(s, 1H)

MS - ESI: 316 $[MH]^+$

Elemental analysis:	Found	C 54.4	H 4.4	N 11.5
$C_{16}H_{14}N_3O_3F$ 1HCl 0.1H_2O	Requires	C 54.4	H 4.3	N 11.9%

The starting material was prepared as follows:

A solution of (4-fluoro-2-methyl-5-nitrophenyl) methyl carbonate (3g, 13mmol), (prepared as described in EP 0307777 A2), in ethanol (60ml) containing platinum(IV)oxide (300mg) was stirred under hydrogen at 0.3 atmosphere for 1 hour. After filtration and evaporation of the solvent, 2-fluoro-5-methoxycarbonyloxy-4-methylaniline was isolated as a solid (2.6g, 100%).

^{1}H NMR Spectrum: ($CDCl_3$) 2.07(s, 3H); 3.87(s, 3H); 6.52(d, 1H); 6.80(d, 1H)

A solution of 7-benzyloxy-4-chloro-6-methoxyquinazoline (800mg, 2.6mmol), (prepared as described for the starting material in Example 4 but with an aqueous work up), and 2-fluoro-5-methoxycarbonyloxy-4-methylaniline (570mg, 2.89 mmol) in isopropanol (20ml) was refluxed for 2 hours. After cooling to ambient temperature, the solid was filtered, washed with isopropanol and dried under vacuum to give 7-benzyloxy-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxyquinazoline (1.0g, 77%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.2(s, 3H); 3.85(s, 3H); 4.0(s, 3H); 5.37(s, 2H); 7.3-7.55(m, 8H); 8.13(s, 1H); 8.86(s, 1H)

MS - ESI: 464 $[MH]^-$

A solution of 7-benzyloxy-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxyquinazoline (700mg, 1.4mmol) in DMF (10ml), methanol (10ml) and trichloromethane (10ml) containing 10% palladium-on-charcoal (100 mg) was stirred under 1 atmosphere of hydrogen for 1 hour. After filtration and evaporation of the solvent, the residue was triturated with ether, filtered and dried under vacuum to give 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline (570mg, 98%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.23(s, 3H); 3.87(s, 3H); 4.01(s, 3H); 7.37(s, 1H); 7.45(d, 1H); 7.5(d, 1H); 8.20(s, 1H); 8.77(s, 1H); 11.35(s, 1H); 11.79(s, 1H)

MS - ESI: 374 $[MH]^+$

Example 27

A solution of 4-chloro-7-(2-methoxyethoxy)quinazoline hydrochloride (275mg, 1mmol) and 2-fluoro-5-hydroxy-4-methylaniline (170mg, 1.2mmol), (prepared as described for the starting material in Example 8), in 2-pentanol (5ml) was heated at reflux for 2 hours. The mixture was allowed to cool and the precipitate was collected by filtration, washed with isopropanol and ether, and dried under vacuum at 70°C to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-methoxyethoxy)quinazoline** hydrochloride(295mg, 78%) as a cream solid.

m.p. 217-220°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.17(s, 3H); 3.36(s, 3H); 3.75(t, 2H); 4.34(t, 2H); 6.89(d, 1H); 7.11(d, 1H); 7.30(d, 1H); 7.52(dd, 1H); 8.66(d, 1H); 8.82(s, 1H); 9.68(s, 1H); 11.40(s, 1H)

MS - ESI: 344 $[MH]^+$

Elemental analysis:	Found	C 56.8	H 5.2	N 11.1
$C_{18}H_{18}N_3O_3F$ 1HCl	Requires	C 56.9	H 5.0	N 11.1%

The starting material was prepared as follows:

A solution of 2-amino-4-fluorobenzoic acid (3g, 19.3mmol) in formamide (30ml) was heated at 150°C for 6 hours. The reaction mixture was poured onto ice/water 1/1 (250ml). The precipitated solid was collected by filtration, washed with water and dried to give 7-fluoro-3,4-dihydroquinazolin-4-one (2.6g, 82%).

Sodium (400mg, 17mmol) was added carefully to 2-methoxyethanol (10ml) and the mixture heated at reflux for 30 minutes. 7-Fluoro-3,4-dihydroquinazolin-4-one (750mg, 4.57mmol) was added to the resulting solution and the mixture heated at reflux for 15 hours. The mixture was cooled and poured into water (250ml). The mixture was acidified to pH4 with concentrated hydrochloric acid. The resulting solid product was collected by filtration, washed with water and then with ether, and dried under vacuum to give 7-(2-methoxyethoxy)-3,4-dihydroquinazolin-4-one (580mg, 58%).

A solution of 7-(2-methoxyethoxy)-3,4-dihydroquinazolin-4-one (500mg, 2.2mmol) in thionyl chloride (15ml) and DMF (0.1ml) was heated at reflux for 3 hours. The volatiles were removed by evaporation to give 4-chloro-7-(2-methoxyethoxy)quinazoline hydrochloride as a cream solid (520mg, 83%).

Example 28

A solution of 4-chloro-7-(2-methoxyethoxy)quinazoline hydrochloride (275mg, 1.0mmol), (prepared as described for the starting material in Example 27), and 4-chloro-2-fluoro-5-hydroxyaniline (193mg, 1.2mmol), (as described in EP 61741 A2), in 2-pentanol (5ml) was heated at reflux for 2 hours. The mixture was allowed to cool and the precipitate was collected by filtration, washed with isopropanol and ether, and dried under vacuum at 70°C to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-7-(2-methoxyethoxy)quinazoline** hydrochloride (178mg, 45%) as a cream solid.

m.p. 224-227°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.36(s, 3H); 3.76(t, 2H); 4.34(t, 2H); 7.14(d, 1H); 7.3(d, 1H); 7.53(m, 2H); 8.66(d, 1H); 8.85(s, 1H); 10.58(s, 1H); 11.40(s, 1H)

MS - ESI: 364 $[MH]^+$

Elemental analysis: Found C 50.8 H 4.1 N 10.4

$C_{17}H_{15}N_3O_3FCl$ 1HCl Requires C 51.0 H 4.0 N 10.5%

Example 29

A solution of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-methoxyacetamidoquinazoline (201mg, 0.5mmol) in methanol (5ml) and 2M aqueous sodium hydroxide solution (0.5ml) was stirred at ambient temperature for 1 hour. The mixture was

diluted with water and adjusted to pH6 with 2M hydrochloric acid. The precipitated solid was collected by filtration, washed with water, dried and then dissolved in a mixture of methylene chloride and methanol. A 5M solution of hydrogen chloride in isopropanol (0.3ml) was added and most of the solvent removed by evaporation. The precipitated solid was collected by filtration, washed with methylene chloride and dried under vacuum to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-7-methoxyacetamidoquinazoline** hydrochloride (70mg, 36%) as a yellow solid.

m.p. 213-215°C

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.18(s, 3H); 3.43(s, 3H); 4.16(s, 2H); 6.90(d, 1H); 7.12(d, 1H); 7.95(d, 1H); 8.56(s, 1H); 8.62(d, 1H); 8.86(s, 1H)

MS - ESI: 357 $[MH]^+$

Elemental analysis:	Found	C 53.7	H 4.9	N 13.6
$C_{18}H_{17}N_4O_3F$ 1HCl $0.5H_2O$	Requires	C 53.8	H 4.8	N 13.9%

The starting material was prepared as follows:

A mixture of 7-nitro-3,4-dihydroquinazolin-4-one (5g, 26mmol) in thionyl chloride (50ml) and DMF (1ml) was heated at reflux for 1.5 hours. Excess thionyl chloride was removed by evaporation and the residue azeotroped with toluene. The residue was suspended in ether, collected by filtration and dried under vacuum to give 4-chloro-7-nitroquinazoline hydrochloride (6.4 g ; 100 %).

^{1}H NMR Spectrum: ($DMSOd_6$) 8.26(dd, 1H); 8.36(d, 1H); 8.40(s, 1H); 8.42(dd, 1H)

MS - ESI: 209 $[MH]^+$

A solution of 4-chloro-7-nitroquinazoline hydrochloride (2.46g, 10mmol) and 2-fluoro-5-methoxycarbonyloxy-4-methylaniline (2.2g, 11mmol), (prepared as described for the starting material in Example 26), in isopropanol (25ml) was heated at 50°C for 1 hour. The mixture was allowed to cool, the precipitated solid was collected by filtration recrystallised from methylene chloride/methanol/isopropanol, to give 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-nitroquinazoline hydrochloride (1.8g, 45%) as a yellow solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 2.21(s, 3H); 3.86(s, 3H); 7.40(d, 1H); 7.46(d, 1H); 8.49(dd, 1H); 8.63(s, 1H); 8.84(s, 1H); 8.89(d, 1H)

MS - ESI: 373 $[MH]^+$

Elemental analysis:	Found	C 50.0	H 3.6	N 13.8
$C_{17}H_{13}N_4O_5F$ 1HCl	Requires	C 50.0	H 3.5	N 13.7%

A mixture of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-nitroquinazoline hydrochloride (5.3g, 13mmol) and 10% palladium-on-charcoal catalyst (1g) in ethanol (100ml), 7M ethanolic hydrogen chloride (1.8ml) and methanol (20ml) was stirred under hydrogen at 1.7atmospheres for 75 minutes. The catalyst was removed by filtration through diatomaceous earth and the filter pad thoroughly washed with methylene chloride, methanol and ether and the solvent was removed from the filtrate by evaporation to give 7-amino-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)quinazoline hydrochloride (4.8g, 97%) as a yellow solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 2.22(s, 3H); 3.87(s, 3H); 6.77(s, 1H); 7.08(dd, 1H); 7.15(m, 2H); 7.41(m, 2H); 8.35(d, 1H); 8.63(s, 1H); 11.03(s, 1H)

MS - ESI: 343 $[MH]^+$

Methoxyacetyl chloride (119mg, 1.1mmol) followed by triethylamine (232mg, 2.3mmol) were added to a suspension of 7-amino-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)quinazoline hydrochloride (415mg, 1.1mmol) in methylene chloride (10ml) and the mixture stirred for 1 hour. The solvent was removed by evaporation and the residue partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The resulting solid was purified by column chromatography eluting with methylene chloride/acetonitrile 50/50 followed by methylene chloride/acetonitrile/methanol 50/45/5 to give 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-methoxyacetamidoquinazoline (250mg, 60%) as a yellow solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 2.18(s, 3H); 3.41(s, 3H); 3.85(s, 3H); 4.09(s, 2H); 7.30(d, 1H); 7.44(d, 1H); 7.84(d, 1H); 8.22(s, 1H); 8.36(d, 1H); 8.44(s, 1H); 9.74(s, 1H); 10.21(s, 1H)

MS - ESI: 437 $[MNa]^+$

Example 30

1M Aqueous sodium hydroxide solution (2.1ml, 2.1mmol) was added to a solution of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxyquinazoline hydrochloride (400mg, 1.05mmol), in methanol (10ml) and the mixture stirred for 50 minutes at ambient

temperature. The solvent was removed by evaporation, the residue dissolved in water and adjusted to pH7 with hydrochloric acid. The aqueous mixture was extracted with ethyl acetate, the extracts washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol 95/5 and 80/20. The purified solid was dissolved in methanol and saturated methanolic hydrogen chloride was added. The volatiles were removed by evaporation, the residue was triturated with pentane to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-7-hydroxyquinazoline** (149mg, 44%) as a yellow solid.

m.p. 274-278°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.16(s, 3H); 6.87(d, 1H); 7.10(d, 1H); 7.22(d, 1H); 7.32(ss, 1H); 8.57(d, 1H); 8.76(s, 1H); 9.66(s, 1H); 11.24(s, 1H); 11.70(s, 1H)

MS - ESI: 285 $[MH]^+$

Elemental analysis:	Found	C 54.2	H 4.1	N 12.3
$C_{15}H_{12}N_3O_2F$ 1HCl 0.3H_2O 0.05NaCl	Requires	C 54.6	H 4.2	N 12.7%

The starting material was prepared as follows:

Sodium (368mg, 16mmol) was added to benzyl alcohol (10ml, 96mmol) and the mixture was heated at 148°C for 30 minutes, 7-fluoro-3,4-dihydroquinazolin-4-one (656mg, 4mmol), (J. Chem. Soc. section B 1967, 449), was added and the mixture maintained at 148°C for 24 hours. The reaction mixture was allowed to cool, the solution was poured on to water (170ml) and the aqueous mixture adjusted to pH3 with concentrated hydrochloric acid. The precipitate was collected by filtration, washed with water, ether and dried under vacuum to give 7-benzyloxy-3,4-dihydroquinazolin-4-one (890mg, 89%) as a white solid.

m.p. 267-269°C

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 5.32(s, 2H); 7.25(d, 1H); 7.32-7.52(m, 6H); 8.12(d, 1H); 8.99(s, 1H)

MS - ESI: 252 $[MH]^+$

Elemental analysis:	Found	C 71.4	H 4.9	N 10.7
$C_{15}H_{12}N_2O_2$ 0.04H_2O	Requires	C 71.2	H 4.8	N 11.1

A mixture of 7-benzyloxy-3,4-dihydroquinazolin-4-one (800mg, 3.17mmol) in thionyl chloride (20ml, 0.27mmol) and DMF (100µl) was heated at reflux for 3 hours. Excess

thionyl chloride was removed by evaporation and the residue azeotroped with toluene and dried under vacuum to give 7-benzyloxy-4-chloroquinazoline hydrochloride (835mg, 86%) as a cream solid.

m.p. 131-132°C

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 5.32(s, 2H); 7.29(d, 1H); 7.34-7.52(m, 6H); 8.12(d, 1H); 9.03(s, 1H)

MS - ESI: 270 $[MH]^+$

2-Fluoro-5-methoxycarbonyloxy-4-methylaniline (883mg, 4.4mmol), (prepared as described for the starting material in Example 26), was added to a solution of 7-benzyloxy-4-chloroquinazoline hydrochloride(1g, 3.7mmol) in 2-pentanol (15ml) at 120°C and the mixture was then heated at reflux for 4 hours. The precipitate was collected by filtration, washed with isopropanol followed by ether and dried under vacuum to give 7-benzyloxy-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)quinazoline hydrochloride (1.65g, 97%) as a cream solid.

m.p. 219-220°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.22(s, 3H); 3.86(s, 3H); 5.37(s, 2H); 7.30-7.60(m, 9H); 8.60(d, 1H); 8.80(s, 1H); 11.2(s, 1H)

MS - ESI: 434 $[MH]^-$

Elemental analysis:	Found	C 60.1	H 4.9	N 8.5
$C_{24}H_{20}N_3O_4F$ 1HCl 0.5H_2O	Requires	C 60.2	H 4.6	N 8.8

7-Benzyloxy-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)quinazoline hydrochloride (1.53g, 3.25mmol) and 10% palladium-on-charcoal catalyst (180mg) in a mixture of methanol/DMF/trichloromethane (75ml, 6ml, 30ml) was stirred under hydrogen at 1.5 atmospheres for 45 minutes. The catalyst was removed by filtration through diatomaceous earth and the solvent removed from the filtrate by evaporation. The residue was triturated with ether, the resulting solid collected by filtration and dried under vacuum to give 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxyquinazoline hydrochloride (1.23g, 84%) as an orange solid.

m.p. 205-210°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.22(s, 3H); 3.85(s, 3H); 7.24(d, 1H); 7.35(dd, 1H); 7.42(d, 1H); 7.45(d, 1H); 8.58(d, 1H); 8.81(s, 1H); 11.40(s, 1H); 11.76(s, 1H)

MS - ESI: 344 $[MH]^+$

Example 31

2M Aqueous sodium hydroxide solution (453µl, 0.9mmol) was added to a suspension of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-(3-morpholinopropionamido)quinazoline (219mg, 0.45mmol) in methanol (6ml) and the mixture stirred for 1 hour. The reaction mixture was diluted with water and then adjusted to pH6 with 2M hydrochloric acid. The resulting precipitate was collected by filtration, washed with water and ethanol and dried. The solid was dissolved in methylene chloride/methanol and a 5M solution of hydrogen chloride in isopropanol (0.3ml) added. The volatiles were removed by evaporation, the resulting solid was washed with ether, and dried under vacuum to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(3-morpholinopropionamido)quinazoline** (186mg, 80%) as a yellow solid.

m.p. 228-233°C

^1H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.21(s, 3H); 3.1(t, 2H); 3.22(t, 2H); 3.5-3.6(m, 4H); 3.8(t, 2H); 4.05(d, 2H); 6.94(d, 1H); 7.10(d, 1H); 7.88(d, 1H); 8.55(s, 1H); 8.7(d, 1H); 8.9(s, 1H)

MS - ESI: 426 $[MH]^+$

Elemental analysis:	Found	C 52.1	H 5.8	N 13.4
$C_{22}H_{24}N_5O_3F$	Requires	C 52.5	H 5.6	N 13.5

1.9HCl $0.6H_2O$ 0.2isopropanol

The starting material was prepared as follows:

Potassium hydroxide (485mg, 8.6mmol) was added to a solution of methyl 3-morpholinopropionate (1g, 5.7mmol) in ethanol (20ml) and the mixture stirred for 2 hours at 80°C. The solution was allowed to cool and adjusted to pH1 with 6M hydrochloric acid. Insoluble material was removed by filtration and the volatiles removed from the filtrate by evaporation. The resulting oil was triturated with ether, the solid product collected by filtration, washed with methylene chloride and dried under vacuum to give 3-morpholinopropionic acid (993mg, 89%) as a white solid.

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.83(t, 2H); 3.13(t, 2H); 3.36(t, 2H); 3.46(d, 2H); 3.73(t, 2H); 3.97(d, 2H)

MS - ESI: 159 $[MH]^+$

1,3-Dicyclohexylcarbodiimide (343mg, 1.6mmol) was added to a suspension of 3-morpholinopropionic acid (325mg, 1.6mmol) in pyridine (12ml) and the mixture stirred for 10 minutes. 7-Amino-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)quinazoline hydrochloride (370mg, 0.97mmol), (prepared as described for the starting material in Example 29), was added and the mixture stirred for 32 hours. 3-Morpholinopropionic acid (57mg, 0.29mmol) followed by 1,3-dicyclohexylcarbodiimide (100mg, 0.48mmol) was added and the mixture stirred for a further 18 hours. The solvent was removed by evaporation, the residue partitioned between water and ethyl acetate and the aqueous layer adjusted to pH8 with a saturated solution of sodium hydrogen carbonate. The organic layer was separated, washed with brine, dried ($MgSO_4$), and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (95/5) to give 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-(3-morpholinopropionamido)quinazoline (226mg, 48%) as a white solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 2.18(s, 3H); 2.4-2.5(m, 4H); 2.5-2.6(m, 2H); 2.62-2.7(m, 2H); 3.58(t, 4H); 3.85(s, 3H); 7.30(d, 1H); 7.44(d, 1H); 7.7(d, 1H); 8.13(s, 1H); 8.35(d, 1H); 8.41(s, 1H); 9.7(s, 1H); 10.46(s, 1H)

Example 32

2M Aqueous sodium hydroxide solution (760µl, 1.5mmol) was added to a solution of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-(2-methoxyethylamino)quinazoline (304mg, 0.76mmol) in methanol (8ml) at 5°C and the mixture then stirred for 30 minutes at ambient temperature. The mixture was diluted with water and adjusted to pH6 with 2M hydrochloric acid. The precipitated solid was collected by filtration and then suspended in methylene chloride/methanol. A 5M solution of hydrogen chloride in isopropanol (0.4ml) was added and the volatiles were removed from the resulting solution by evaporation. The residue was triturated with ether, the solid product collected by filtration, washed with ether and dried under vacuum to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-methoxyethylamino)quinazoline** hydrochloride (260mg, 90%) as yellow solid.

m.p. 192-197°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.16(s, 3H); 3.32(s, 3H); 3.38(m, 2H); 3.58(m, 2H); 6.71(bs, 1H); 6.88(d, 1H); 7.1(d, 1H); 7.2(d, 1H); 7.73(m, 1H); 8.37(d, 1H); 8.61(s, 1H); 9.66(s, 1H); 10.95(s, 1H)

MS - ESI: 343 $[MH]^+$

The starting material was prepared as follows:

A solution of methoxyacetaldehyde dimethyl acetal (1.27g, 10mol) in water (7ml) and 2M hydrochloric acid (76µl) was heated at 50-60°C for 2 hours. The mixture was allowed to cool and adjusted to pH7.5 with saturated aqueous sodium hydrogen carbonate solution. This solution was added to a suspension of 7-amino-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)quinazoline hydrochloride (400mg, 1mmol), (prepared as described for the starting material in Example 30), in ethanol (32ml) and acetic acid (95µl, 1.5mmol). The mixture was then stirred for 5 minutes, sodium cyanoborohydride (133mg, 2mmol) added and the solution adjusted to pH5.5 with glacial acetic acid. The mixture was stirred for 18 hours and the organic solvents removed by evaporation and the resulting aqueous mixture partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (96/4 followed by 12/8) to give 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-(2-methoxyethylamino)quinazoline (308mg, 77%) as a yellow foam.

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.22(s, 3H); 3.33(s, 3H); 3.41(t, 2H); 3.60(t, 2H); 3.87(s, 3H); 6.68(br s, 1H); 7.22(dd, 1H); 7.37(d, 1H); 7.43(d, 1H); 8.30(d, 1H); 8.7(s, 1H)

Example 33

2M Aqueous sodium hydroxide solution (620µl) was added dropwise to a suspension of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxy-7-methoxyacetamidoquinazoline (275mg, 0.62mmol) in methanol (8ml) at 5°C and the mixture then stirred for 90 minutes at ambient temperature. The reaction mixture was diluted with water and adjusted to pH7 with 2M hydrochloric acid. The precipitated solid was collected by filtration, resuspended in ethanol and a 5M solution of hydrogen chloride in isopropanol

(0.3ml) added. The volatiles were removed from the resulting solution by evaporation and the solid washed with ether collected by filtration and dried under vacuum to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-methoxyacetamidoquinazoline** hydrochloride (216mg, 82%).

m.p. 300-306°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.18(s, 3H); 3.47(s, 2H); 4.13(s, 3H); 4.21(s, 3H); 6.92(d, 1H); 7.13(d, 1H); 8.41(s, 1H); 8.80(s, 1H); 8.90(s, 1H); 9.54(s, 1H); 9.72(s, 1H); 11.49(s, 1H)

MS - ESI: 387 $[MH]^+$

Elemental analysis:	Found	C 52.3	H 4.8	N 12.7
$C_{19}H_{19}N_4O_4F$ 1HCl 0.6H_2O	Requires	C 52.6	H 4.9	N 12.9

The starting material was prepared as follows:

Acetic anhydride (50ml) was added dropwise to a solution of 4-methoxy-2-methylaniline (49.7g, 360mmol) in DMA (200ml) at 5°C and the mixture stirred for 4.5 hours at ambient temperature. The solvent was removed by evaporation and the resulting solid washed with water and dried under vacuum to give N-(4-methoxy-2-methylphenyl)acetamide (57.3g, 88%).

^{1}H NMR Spectrum: ($CDCl_3$) 2.16(s, 3H); 2.21(s, 3H); 3.77(s, 3H); 6.7-6.75(m, 2H); 7.42(d, 1H)

A mixture of tin(IV)chloride (19.3ml) and 69.5% nitric acid (10.3ml) in methylene chloride (140ml) was added dropwise to a solution of N-(4-methoxy-2-methylphenyl)acetamide (28g, 0.14mol) in methylene chloride (500ml) cooled to and maintained at -30°C. The reaction mixture was stirred at -30°C for 1.5 hours, allowed to warm to ambient temperature then poured on to ice/water. The organic layer was separated and the aqueous layer extracted with ethyl acetate. The combined extracts were dried ($MgSO_4$), the solvent removed by evaporation and the residue purified by column chromatography eluting with petroleum ether/ethyl acetate (2/8) to give N-(4-methoxy-2-methyl-5-nitrophenyl)acetamide (17.8g, 51%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.06(s, 3H); 2.29(s, 3H); 3.9(s, 3H); 7.24(s, 1H); 7.99(s, 1H); 9.41(s, 1H)

Potassium permanganate (68g) was added portionwise to a solution of N-(4-methoxy-2-methyl-5-nitrophenyl)acetamide (35g, 0.156mol) and magnesium sulphate (38.5g) in water (2.3l) at 75°C. The mixture was maintained at 75°C for 3.5 hours, further magnesium sulphate (4g) and potassium permanganate (12g) were added and stirring continued for 30 minutes at 75°C. The insolubles were removed from the hot reaction mixture by filtration through diatomaceous earth, the filtrate cooled and was acidified to pH1 with concentrated hydrochloric acid. The precipitated solid was collected by filtration, washed with water and the aqueous filtrate extracted with ethyl acetate. The solid product and the ethyl acetate extract were combined and extracted with 2M aqueous sodium hydroxide solution. The basic aqueous layer was separated, washed with ethyl acetate, acidified with concentrated hydrochloric acid and re-extracted with ethyl acetate. The ethyl acetate extract was washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation to give 2-acetamido-5-methoxy-4-nitrobenzoic acid (21.6g, 54%) as a yellow solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 2.12(s, 3H); 3.93(s, 3H); 7.74(s, 1H); 8.75(s, 1H)

A solution of 2-acetamido-5-methoxy-4-nitrobenzoic acid (21.6g, 85mmol) in water (76ml) and concentrated hydrochloric acid (30.5ml) was heated at reflux for 3 hours. The reaction mixture was cooled to 0°C, the resulting solid was collected by filtration, washed with water and dried under vacuum to give 2-amino-5-methoxy-4-nitrobenzoic acid (16.6g, 92%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.79(s, 3H); 7.23(s, 1H); 7.52(s, 1H); 8.8(br s, 2H)

A solution of 2-amino-5-methoxy-4-nitrobenzoic acid (16.6g, 78mmol) in formamide (250ml) was heated at reflux for 4.5 hours. The reaction mixture was cooled to 0°C, diluted with water and the resulting precipitate collected by filtration, washed with water and dried under vacuum to give 6-methoxy-7-nitro-3,4-dihydroquinazolin-4-one (11.56g, 67%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 4.02(s, 3H); 7.8(s, 1H); 8.12(s, 1H); 8.18(s, 1H)

A suspension of 6-methoxy-7-nitro-3,4-dihydroquinazolin-4-one (8g, 36mmol) in thionyl chloride (150ml) and DMF (0.8ml) was heated at reflux for 3 hours. Excess thionyl chloride was removed by evaporation and the residue azeotroped with toluene. The resulting solid was triturated with ether, collected by filtration and dried under vacuum to give 4-chloro-6-methoxy-7-nitroquinazoline hydrochloride(7.5g, 75%).

^{1}H NMR Spectrum: ($DMSOd_6$) 4.13(s, 3H); 7.8(s, 1H); 8.7(s, 1H); 9.13(s, 1H)

A mixture of 4-chloro-6-methoxy-7-nitroquinazoline hydrochloride (784mg, 2.8mmol) and 2-fluoro-5-methoxycarbonyloxy-4-methylaniline (621mg, 3.1mmol), (prepared as described for the starting material in Example 26), in isopropanol (10ml) was heated at reflux for 2 hours. The mixture was allowed to cool, the precipitated product collected by filtration, washed with isopropanol, ether and dried under vacuum to give 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxy-7-nitroquinazoline hydrochloride (1.12g, 90%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.22(s, 3H); 3.86(s, 3H); 4.10(s, 3H); 7.41(d, 1H); 7.46(d, 1H); 8.40(s, 1H); 8.55(s, 1H); 8.77(s, 1H); 11.4(br s, 1H)

MS - ESI: 403 $[MH]^+$

A mixture of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxy-7-nitroquinazoline hydrochloride (1.1g, 25mmol) and 10% palladium-on-charcoal catalyst (220mg) in methanol (200ml) and ethanol (10ml) was stirred under hydrogen at 2.7 atmospheres for 7 hours. The catalyst was removed by filtration through diatomaceous earth, the solvent removed fom the filtrate by evaporation and the solid residue washed with ether, collected by filtration and dried under vacuum to give 7-amino-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxyquinazoline hydrochloride (930mg, 91%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.22(s, 3H); 3.87(s, 3H); 4.02(s, 3H); 6.9(s, 1H); 7.4-7.5(m, 2H); 7.99(s, 1H); 8.62(s, 1H)

MS - ESI: 372 $[MH]^+$

Methoxyacetyl chloride (62µl, 0.68mmol) was added dropwise to a solution of 7-amino-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxyquinazoline hydrochloride (215mg, 0.52mmol) in methylene chloride (5ml) and pyridine (1.5ml) at 0°C and the mixture stirred for 2 hours at 0°C. Further methoxyacetyl chloride (14µl, 0.15mmol) was added and the mixture stirred for 20 minutes at 0°C. The reaction mixture was partitioned between ethyl acetate and water and the aqueous layer adjusted to pH9 with saturated aqueous sodium hydrogen carbonate solution. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/acetonitrile/methanol

(60/38/2) to give 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxy-7-methoxyacetamidoquinazoline (175mg, 75%) as a white solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 2.21(s, 3H); 3.47(s, 2H); 3.87(s, 3H); 4.07(s, 3H); 4.15(s, 3H); 7.35(d, 1H); 7.45(d, 1H); 7.96(s, 1H); 8.40(s, 1H); 8.65(s, 1H); 9.28(s, 1H); 9.65(s, 1H)

Example 34

A solution of ethereal hydrogen chloride (1.0ml of a 1.0M solution, 1.0mmol) was added to 4-chloro-6-methoxy-7-(2-thiomorpholinoethoxy)quinazoline (340mg, 1.0mmol) and 4-chloro-2-fluoro-5-hydroxyaniline (200mg, 1.2mmol), (as described in EP 61741 A2), in t-butanol (15ml). The mixture was heated at 95°C for 1 hour and then stirred for 18 hours at ambient temperature. The reaction mixture was diluted with acetone and the precipitated product collected by filtration, washed with acetone and dried to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-thiomorpholinoethoxy)quinazoline** hydrochloride hemihydrate (480mg, 88%) as beige powder.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.67(t, 2H); 4.04(s, 3H); 4.70(t, 2H); 7.18(d, 1H); 7.4-7.5(m, 2H); 7.51(dd, 1H); 8.44(s, 1H); 8.82(s, 1H); 10.6(br s, 1H); 11.7(br s, 1H)

MS - ESI: 465 $[MH]^+$

Elemental analysis :	Found	C 45.8	H 4.4	N 10.0
$C_{21}H_{22}N_4ClFO_3S$ 2HCl 0.5H_2O	Requires	C 46.1	H 4.6	N 10.2%

The starting material was prepared as follows:

1,2-Dibromoethane (19.2ml, 286mmol) was added to 7-hydroxy-6-methoxy-4-phenoxyquinazoline (6.0g, 22mmol), (prepared as described for the starting material in Example 16), and potassium carbonate (14.4g, 107mmol) in DMF. The mixture was stirred at 85°C for 2.5 hours, allowed to cool and insoluble material was removed by filtration. The solvent was removed by evaporation and the residue purified by column chromatography eluting with methylene chloride/methanol (93/7). The product was trituated with ethyl acetate to give 7-(2-bromoethoxy)-6-methoxy-4-phenoxyquinazoline (5.3g, 63%).

A mixture of 7-(2-bromoethoxy)-6-methoxy-4-phenoxyquinazoline (2.0g, 5.3mmol) in thiomorpholine (15ml) was stirred at ambient temperature for 5 hours. The mixture was diluted with water and the resulting precipitate collected by filtration. The solid product was

dissolved in methylene chloride, washed with brine and passed through phase separating paper. The solvent was removed by evaporation to give 6-methoxy-4-phenoxy-7-(2-thiomorpholinoethoxy)quinazoline (2.0g, 94%) as a pale yellow solid.

MS - ESI: 398 $[MH]^+$

A mixture of 6-methoxy-4-phenoxy-7-(2-thiomorpholinoethoxy)quinazoline (2.0g, 5mmol) in 2M hydrochloric acid (25ml) was heated at 90°C for 1.5 hours. The mixture was allowed to cool and adjusted to pH7 with solid sodium hydrogen carbonate. Methylene chloride was added and the resulting semi-solid product was isolated by decanting and filtering the aqueous mixture. This product was dissolved in acetone and insoluble material was removed by filtration. The solvent was removed by evaporation and the residue azeotroped with toluene to give 6-methoxy-7-(2-thiomorpholinoethoxy)-3,4-dihydroquinazolin-4-one (1.5g, 92%) as a white solid.

MS - ESI: 322 $[MH]^+$

A mixture of 6-methoxy-7-(2-thiomorpholinoethoxy)-3,4-dihydroquinazolin-4-one (1.5g, 4.6mmol), thionyl chloride (25ml) and DMF (0.2 ml) was heated at reflux for 2 hours. Excess thionyl chloride was removed by evaporation and the residue azeotroped with toluene. The resulting gum was partitioned between aqueous sodium hydrogen carbonate solution and methylene chloride. The organic layer was separated and the aqueous layer extracted with methylene chloride (4x40ml). The combined extracts were passed through phase separating paper, the solvent removed by evaporation and the residue purified by column chromatography eluting with methylene chloride/methanol (95/5). The purified product was triturated with acetone to give 4-chloro-6-methoxy-7-(2-thiomorpholinoethoxy)quinazoline (400mg, 25%) as an orange/brown solid.

MS - ESI: 342 $[MH]^+$

Example 35

A solution of ethereal hydrogen chloride (1.0ml of a 1.0M solution, 1.0mmol) was added to 4-chloro-6-methoxy-7-(2-(2-methoxyethylamino)ethoxy)quinazoline (110mg, 3.5mmol) and 4-chloro-2-fluoro-5-hydroxyaniline (72mg, 4.5mmol), (as described in EP 61741 A2), in t-butanol (5ml). The mixture was heated at 95°C for 1 hour, allowed to cool and diluted with acetone. The precipitated product was collected by filtration, washed with

methylene chloride and acetone and dried to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-(2-methoxyethylamino)ethoxy)quinazoline** hydrochloride hydrate (110mg, 59%) as a beige powder.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.2-3.6(m, 4H); 3.38(s, 3H); 3.73(t, 2H); 4.09(s, 3H); 4.58(t, 2H); 7.24(d, 1H); 7.52(d, 1H); 7.55(s, 1H); 8.48(s, 1H); 7.85(s, 1H); 9.35(br s, 1H); 10.65(br s, 1H); 11.75(br s, 1H)

MS - ESI: 437 $[MH]^+$

Elemental analysis : Found C 45.1 H 4.6 N 10.1

$C_{20}H_{22}N_4ClFO_4$ 2HCl $1.2H_2O$ Requires C 45.2 H 5.0 N 10.5%

The starting material was prepared as follows:

A mixture of 7-(2-bromoethoxy)-6-methoxy-4-phenoxyquinazoline (1.1g, 2.9mmol), (prepared as described for the starting material in Example 22), in 2-methoxyethylamine (8ml) was stirred at ambient temperature for 4 hours. The mixture was diluted with water and extracted with methylene chloride (5x25ml). The combined extracts were washed with brine and passed through phase separating paper. The solvent was removed by evaporation and the residue purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (100/8/1) to give 6-methoxy-4-phenoxy-7-(2-(2-methoxyethylamino)ethoxy)quinazoline (760mg, 70%) as a white solid.

MS - ESI: 370 $[MH]^+$

A mixture of 6-methoxy-4-phenoxy-7-(2-(2-methoxyethylamino)ethoxy)quinazoline (760mg, 2mmol) in 2M hydrochloric acid (5ml) was heated at 90°C for 1.5 hours. The mixture was allowed to cool and adjusted to pH7 with solid sodium hydrogen carbonate. The water was removed by evaporation and the residue extracted with methylene chloride/methanol/aqueous ammonia (100/8/1). The volatiles were removed from the extract by evaporation, the residue dissolved in methylene chloride, passed through phase separating paper and the solvent removed by evaporation to give 6-methoxy-7-(2-(2-methoxyethylamino)ethoxy)-3,4-dihydroquinazolin-4-one (600mg, 99%) as a white solid.

A mixture of 6-methoxy-7-(2-(2-methoxyethylamino)ethoxy)-3,4-dihydroquinazolin-4-one (300mg, 1mmol), thionyl chloride (5ml) and DMF (0.1ml) was heated at reflux for 45 minutes. Excess thionyl chloride was removed by evaporation and the

residue azeotroped with toluene. The resulting gum was partitioned between aqueous sodium hydrogen carbonate solution and methylene chloride. The organic layer was separated and the aqueous layer extracted with methylene chloride (4x40ml). The combined extracts were passed through phase separating paper and the solvent removed by evaporation to give 4-chloro-6-methoxy-7-(2-(2-methoxyethylamino)ethoxy)quinazoline (120mg, 38%) as a yellow solid.

Example 36

A solution of 4-chloro-6-methoxy-7-(3-morpholinopropoxy)quinazoline (202mg, 0.6mmol) and 5M isopropanolic hydrogen chloride (1.5ml) in isopropanol (5ml) was heated at 80°C for 18 hours. The mixture was allowed to cool and the volatiles were removed by evaporation. The residue was partitioned between methylene chloride and water and the aqueous layer was adjusted to pH6.5 with 0.1M aqueous sodium hydroxide. The organic layer was separated, washed with water and brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (95/5). The purified solid was dissolved in methylene chloride/methanol and 2.2M ethereal hydrogen chloride was added. The volatiles were removed by evaporation, the solid residue was suspended in ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-bromo-2,6-difluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline** hydrochloride (91mg, 26%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.3-2.4(m, 2H); 3.1-3.2(m, 2H); 3.3-3.4(m, 2H); 3.55(d, 2H); 3.75(t, 2H); 4.01(d, 2H); 4.03(s, 3H); 4.35(t, 2H); 7.43(s, 1H); 7.76(d, 2H); 8.21(s, 1H); 8.93(s, 1H)

MS - ESI: 511 $[MH]^+$

Elemental Analysis:	Found	C 45.4	H 4.7	N 9.2
$C_{22}H_{23}N_4O_3BrF_2$ 0.3H_2O 1.85 HCl 0.09 ether 0.05 CH_2Cl_2	Requires	C 45.4	H 4.5	N 9.4%

The starting material was prepared as follows:

Diethyl azodicarboxylate (2.67ml, 17mmol) was added dropwise to a solution of 3-morpholinopropan-1-ol (1.54g, 10mmol), 7-hydroxy-3,4-dihydro-6-methoxy-3-

((pivaloyloxy)methyl)quinazolin-4-one (2.6g, 8.5mmol) and triphenylphosphine (4.45g, 17mmol) in methylene chloride (40ml). After stirring for 2 hours at ambient temperature, the volatiles were removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (97/3 followed by 95/5) to give 3,4-dihydro-6-methoxy-3-((pivaloyloxy)methyl)-7-(3-morpholinopropoxy)quinazolin-4-one (3.6g, 97%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.12(s, 9H); 2.2-2.3(m, 2H); 3.1-3.2(m, 2H); 3.32(t, 2H); 3.55(d, 2H); 3.65-3.75(m, 2H); 3.92(s, 3H); 4.05(d, 2H); 4.25(t, 2H); 5.93(s, 2H); 7.23(s, 1H); 7.54(s, 1H); 8.41(s, 1H)

A solution of 3,4-dihydro-6-methoxy-3-((pivaloyloxy)methyl)-7-(3-morpholinopropoxy)quinazolin-4-one (4.93g, 11.4mmol) in a saturated solution of methanolic ammonia (70ml) was stirred at ambient temperature for 2 days. The volatiles were removed by evaporation. The solid residue was suspended in ether, collected by filtration, washed with ether and dried under vacuum to give 4-hydroxy-6-methoxy-7-(3-morpholinopropoxy)quinazoline (2.87g, 79%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.2-2.3(m, 2H); 3.15(t, 2H); 3.35(t, 2H); 3.55(d, 2H); 3.7(t, 2H); 3.94(s, 3H); 4.05(d, 2H); 4.26(t, 2H); 7.29(s, 1H); 7.56(s, 1H); 8.96(s, 1H)

A solution of 4-hydroxy-6-methoxy-7-(3-morpholinopropoxy)quinazoline (2.87g, 9mmol) and DMF (1ml) in thionyl chloride (35ml) was refluxed for 45 minutes. After addition of toluene, the volatiles were removed by evaporation. The residue was partitioned between ethyl acetate and water and the aqueous layer was adjusted to pH8 with 2M aqueous sodium hydroxide. The organic layer was washed with water and brine, dried ($MgSO_4$) and the volatiles were removed by evaporation. The solid residue was purified by column chromatography eluting with a mixture of methylene chloride, acetonitrile and methanol (50/47.5/2.5) to give 4-chloro-6-methoxy-7-(3-morpholinopropoxy)quinazoline (2g, 66%).

^{1}H NMR Spectrum: ($CDCl_3$) 2.13(m, 2H); 2.48(br s, 4H); 2.56(t, 2H); 3.72(t, 4H); 4.05(s, 3H); 4.29(t, 2H); 7.37(d, 2H); 8.86(s, 1H)

Example 37

The following illustrate representative pharmaceutical dosage forms containing the compound of formula I, or a pharmaceutically acceptable salt thereof (hereafter compound X), for therapeutic or prophylactic use in humans:

(a)	Tablet I	mg/tablet
	Compound X	100
	Lactose Ph.Eur	182.75
	Croscarmellose sodium	12.0
	Maize starch paste (5% w/v paste)	2.25
	Magnesium stearate	3.0

(b)	Tablet II	mg/tablet
	Compound X	50
	Lactose Ph.Eur	223.75
	Croscarmellose sodium	6.0
	Maize starch	15.0
	Polyvinylpyrrolidone (5% w/v paste)	2.25
	Magnesium stearate	3.0

(c)	Tablet III	mg/tablet
	Compound X	1.0
	Lactose Ph.Eur	93.25
	Croscarmellose sodium	4.0
	Maize starch paste (5% w/v paste)	0.75
	Magnesium stearate	1.0

(d)	Capsule	mg/capsule
	Compound X	10
	Lactose Ph.Eur	488.5
	Magnesium stearate	1.5

(e)	Injection I	(50 mg/ml)
	Compound X	5.0% w/v
	1N Sodium hydroxide solution	15.0% v/v
	0.1N Hydrochloric acid	

(to adjust pH to 7.6)

Polyethylene glycol 400 ..... 4.5% w/v

Water for injection to 100%

(f) Injection II (10 mg/ml)

Compound X ..... 1.0% w/v

Sodium phosphate BP ..... 3.6% w/v

0.1N Sodium hydroxide solution..... 15.0% v/v

Water for injection to 100%

(g) Injection III (1mg/ml,buffered to pH6)

Compound X ..... 0.1% w/v

Sodium phosphate BP ..... 2.26% w/v

Citric acid ..... 0.38% w/v

Polyethylene glycol 400 ..... 3.5% w/v

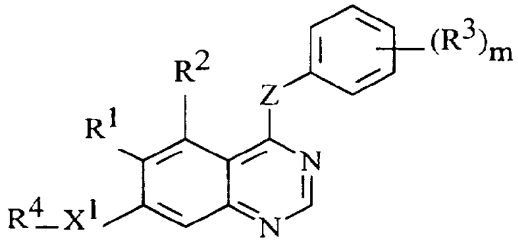
Water for injection to 100%

Note

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate.

CLAIMS

1. A quinazoline derivative of the formula I:



(I)

[wherein:

Z represents -O-, -NH- or -S-;

m is an integer from 1 to 5 with the proviso that where Z is -NH- m is an integer from 3 to 5;

R^1 represents hydrogen, hydroxy, halogeno, nitro, trifluoromethyl, cyano, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkylthio, or $-NR^5R^6$ (wherein R^5 and R^6, which may be the same or different, each represents hydrogen or C_{1-3}alkyl);

R^2 represents hydrogen, hydroxy, halogeno, methoxy, amino or nitro;

R^3 represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkanoyloxy, trifluoromethyl, cyano, amino or nitro;

X^1 represents -O-, $-CH_2-$, -S-, -SO-, $-SO_2-$, $-NR^7-$, $-NR^8CO-$, $-CONR^9-$, $-SO_2NR^{10}-$ or $-NR^{11}SO_2-$, (wherein R^7, R^8, R^9, R^{10} and R^{11} each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

R^4 is selected from one of the following seven groups:

1) hydrogen, C_{1-5}alkyl, C_{1-5}hydroxyalkyl, (preferably C_{2-5}hydroxyalkyl), C_{1-5}fluoroalkyl, C_{1-5}aminoalkyl;

2) C_{1-5}alkylX^2COR^{12} (wherein X^2 represents -O- or $-NR^{13}-$ (in which R^{13} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{12} represents C_{1-3}alkyl, $-NR^{14}R^{15}$ or $-OR^{16}$ (wherein R^{14}, R^{15} and R^{16} which may be the same or different each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl));

3) C_{1-5}alkylX^3R^{17} (wherein X^3 represents -O-, -S-, -SO-, $-SO_2-$, -OCO-, $-NR^{18}CO-$, $-CONR^{19}-$, $-SO_2NR^{20}-$, $-NR^{21}SO_2-$ or $-NR^{22}-$ (wherein R^{18}, R^{19}, R^{20}, R^{21} and R^{22} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{17} represents hydrogen, C_{1-3}alkyl, cyclopentyl, cyclohexyl or a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which C_{1-3}alkyl group may bear one or two substituents selected from oxo, hydroxy, halogeno and C_{1-4}alkoxy and which cyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy);

4) C_{1-5}alkylR^{23} (wherein R^{23} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy);

5) C_{2-5}alkenylR^{23} (wherein R^{23} is as defined hereinbefore);

6) C_{2-5}alkynylR^{23} (wherein R^{23} is as defined hereinbefore); and

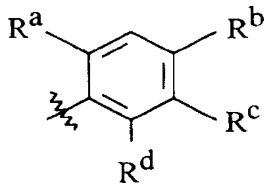
7) C_{1-5}alkylX^4C_{1-5}alkylX^5R^{24} (wherein X^4 and X^5 which may be the same or different are each -O-, -S-, -SO-, $-SO_2-$, $-NR^{25}CO-$, $-CONR^{26}-$, $-SO_2NR^{27}-$, $-NR^{28}SO_2-$ or $-NR^{29}-$ (wherein R^{25}, R^{26}, R^{27}, R^{28} and R^{29} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{24} represents hydrogen or C_{1-3}alkyl)];

and salts thereof.

2. A quinazoline derivative as claimed in claim 1 wherein R^1 is hydrogen, hydroxy, cyano, nitro, trifluoromethyl, methyl, ethyl, methoxy, or ethoxy.

3. A quinazoline derivative as claimed in claim 1 or claim 2 wherein R^2 is hydrogen.

4. A quinazoline derivative as claimed in any one of the preceding claims wherein the phenyl group bearing $(R^3)_m$ is of the formula II:



(II)

wherein:

R^a represents hydrogen, methyl, fluoro or chloro;

R^b represents hydrogen, methyl, methoxy, bromo, fluoro or chloro;

R^c represents hydrogen or hydroxy; and

R^d represents hydrogen, fluoro or chloro.

5. A quinazoline derivative as claimed in any one of the preceding claims wherein Z is NH.

6. A quinazoline derivative as claimed in any one of the preceding claims wherein X^1 represents -O-, -S-, $-NR^8CO-$, $-NR^{11}SO_2-$ (wherein R^8 and R^{11} each independently represents hydrogen or C_{1-2}alkyl) or NH.

7. A quinazoline derivative as claimed in any one of the preceding claims wherein R^4 is selected from one of the following nine groups:

1) C_{1-5}alkyl, C_{2-5}hydroxyalkyl, C_{1-5}fluoroalkyl, C_{2-4}aminoalkyl;

2) C_{2-3}alkylX^2COR^{12} (wherein X^2 is as defined in claim 1 and R^{12} represents C_{1-3}alkyl, $-NR^{14}R^{15}$ or $-OR^{16}$ (wherein R^{14}, R^{15} and R^{16} which may be the same or different are each C_{1-2}alkyl or C_{1-2}alkoxyethyl));

3) C_{2-4}alkylX^3R^{17} (wherein X^3 is as defined in claim 1 and R^{17} is a group selected from C_{1-3}alkyl, cyclopentyl, cyclohexyl, pyrrolidinyl and piperidinyl which group is linked to X^3 through a carbon atom and which C_{1-3}alkyl group may bear one or two substituents selected from oxo, hydroxy, halogeno and C_{1-2}alkoxy and which cyclopentyl, cyclohexyl, pyrrolidinyl or piperidinyl group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy);

4) C_{1-4}alkylR^{30} (wherein R^{30} is a group selected from pyrrolidinyl, piperazinyl, piperidinyl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl and 1,3-dithian-2-yl, which group is linked to C_{1-4}alkyl through a carbon atom and which group may carry one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy) or C_{2-4}alkylR^{31} (wherein R^{31} is a group selected from morpholino, thiomorpholino, pyrrolidin-1-yl, piperazin-1-yl and piperidino which group may carry one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy);

5) C_{3-4}alkenylR^{30} (wherein R^{30} is as defined herein);

6) C_{3-4}alkynylR^{30} (wherein R^{30} is as defined herein);

7) C_{3-4}alkenylR^{31} (wherein R^{31} is as defined herein);

8) C_{3-4}alkynylR^{31} (wherein R^{31} is as defined herein); and

9) C_{2-3}alkylX^4C_{2-3}alkylX^5R^{24} (wherein X^4 and X^5 are as defined in claim 1 and R^{24} represents hydrogen or C_{1-3}alkyl).

8. A quinazoline derivative as claimed in any one of the preceding claims wherein R^4 is selected from one of the following five groups:

1) C_{1-3}alkyl, C_{2-3}hydroxyalkyl, C_{1-3}fluoroalkyl, C_{2-3}aminoalkyl;

2) 2-(3,3-dimethylureido)ethyl, 3-(3,3-dimethylureido)propyl, 2-(3-methylureido)ethyl, 3-(3-methylureido)propyl, 2-ureidoethyl, 3-ureidopropyl, 2-(N,N-dimethylcarbamoyloxy)ethyl, 3-(N,N-dimethylcarbamoyloxy)propyl, 2-(N-methylcarbamoyloxy)ethyl, 3-(N-methylcarbamoyloxy)propyl, 2-(carbamoyloxy)ethyl, 3-(carbamoyloxy)propyl;

3) C_{2-3}alkylX^3R^{17} (wherein X^3 is as defined in claim 1 and R^{17} is a group selected from C_{1-2}alkyl, cyclopentyl, cyclohexyl, pyrrolidinyl and piperidinyl which group is linked to X^3 through a carbon atom and which C_{1-2}alkyl group may bear one or two substituents selected from hydroxy, halogeno and C_{1-2}alkoxy and which cyclopentyl, cyclohexyl, pyrrolidinyl or piperidinyl group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy);

4) C_{1-2}alkylR^{30} (wherein R^{30} is a group selected from pyrrolidinyl, piperazinyl, piperidinyl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl and 1,3-dithian-2-yl, which group is

linked to C_{1-2}alkyl through a carbon atom and which group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy) or C_{2-3}alkylR^{31} (wherein R^{31} is a group selected from morpholino, thiomorpholino, piperidino, piperazin-1-yl and pyrrolidin-1-yl which group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy); and

5) C_{2-3}alkylX^4C_{2-3}alkylX^5R^{24} (wherein X^4 and X^5 are as defined in claim 1 and R^{24} represents hydrogen or C_{1-2}alkyl).

9. A quinazoline derivative as claimed in any one of the preceding claims wherein R^4 represents methyl, ethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 2-hydroxyethyl, 3-hydroxypropyl, 2-methoxyethyl, 3-methoxypropyl, 2-(methylsulphinyl)ethyl, 2-(methylsulphonyl)ethyl, 2-(N,N-dimethylsulphamoyl)ethyl, 2-(N-methylsulphamoyl)ethyl, 2-sulphamoylethyl, 2-(N,N-dimethylamino)ethyl, 3-(N,N-dimethylamino)propyl, 2-morpholinoethyl, 3-morpholinopropyl, 2-piperidinoethyl, 3-piperidinopropyl, 2-(piperazin-1-yl)ethyl, 3-(piperazin-1-yl)propyl, 2-(pyrrolidin-1-yl)ethyl, 3-(pyrrolidin-1-yl)propyl, (1,3-dioxolan-2-yl)methyl, 2-(1,3-dioxolan-2-yl)ethyl, 2-(2-methoxyethylamino)ethyl, 2-(2-hydroxyethylamino)ethyl, 3-(2-methoxyethylamino)propyl, 3-(2-hydroxyethylamino)propyl, 2-thiomorpholinoethyl, 3-thiomorpholinopropyl, 2-(4-methylpiperazin-1-yl)ethyl, 3-(4-methylpiperazin-1-yl)propyl or 2-(2-methoxyethoxy)ethyl.

10. A quinazoline derivative as claimed in any one of the preceding claims wherein R^4 represents 2-hydroxyethyl, 3-hydroxypropyl, 2-methoxyethyl, 3-methoxypropyl, 2-(methylsulphinyl)ethyl, 2-(methylsulphonyl)ethyl, 2-(N,N-dimethylamino)ethyl, 3-(N,N-dimethylamino)propyl, 2-morpholinoethyl, 3-morpholinopropyl, 2-piperidinoethyl, 3-piperidinopropyl, 2-(piperazin-1-yl)ethyl, 3-(piperazin-1-yl)propyl, 2-(pyrrolidin-1-yl)ethyl, 3-(pyrrolidin-1-yl)propyl, (1,3-dioxolan-2-yl)methyl, 2-(1,3-dioxolan-2-yl)ethyl, 2-(2-methoxyethylamino)ethyl, 2-(2-hydroxyethylamino)ethyl, 3-(2-methoxyethylamino)propyl, 3-(2-hydroxyethylamino)propyl, 2-thiomorpholinoethyl, 3-thiomorpholinopropyl, 2-(4-methylpiperazin-1-yl)ethyl, 3-(4-methylpiperazin-1-yl)propyl or 2-(2-methoxyethoxy)ethyl.

11. A quinazoline derivative as claimed in claim 1 selected from:
4-(2-fluoro-5-hydroxy-4-methylanilino)-6,7-dimethoxyquinazoline,
4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,
4-(2-fluoro-5-hydroxy-4-methylanilino)-7-methoxyacetamidoquinazoline
4-(4-bromo-2,6-difluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline
and salts thereof.

12. A quinazoline derivative as claimed in claim 1 selected from:
4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)quinazoline,
4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-methylthioethoxy)quinazoline,
4-(4-chloro-2-fluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline,
4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,
7-(2-acetoxyethoxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline,
4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline,
4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-piperidinoethoxy)quinazoline,
4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-methoxyethylamino)quinazoline,
4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-cyclopentyloxyethoxy)quinazoline,
4-(2,4-difluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline,
4-(2,4-difluoro-5-hydroxyanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,
and salts thereof.

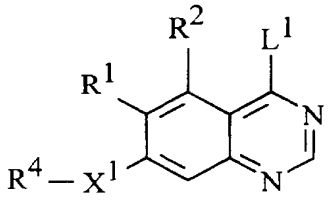
13. A quinazoline derivative as claimed in claim 1 selected from:
4-(4-bromo-2,6-difluoroanilino)-6,7-dimethoxyquinazoline,
4-(4-bromo-2-fluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline,
4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-thiomorpholinoethoxy)quinazoline,
6,7-dimethoxy-4-(3-hydroxy-4-methylphenoxy)quinazoline,

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-hydroxyethoxy)-6-methoxyquinazoline,
4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,
4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-methoxyethoxy)quinazoline,
4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-(methylsulphinyl)ethoxy)quinazoline,
and salts thereof.

14. A quinazoline derivative as claimed in any one of the preceding claims in the form of a pharmaceutically acceptable salt.

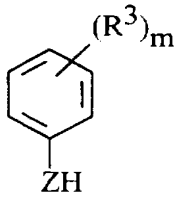
15. A process for the preparation of a quinazoline derivative of formula I or salt thereof (as defined in claim 1) which comprises:

(a) the reaction of a compound of the formula III:



(III)

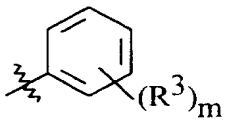
(wherein R^1, R^2, X^1 and R^4 are as defined in claim 1 and L^1 is a displaceable moiety), with a compound of the formula IV:



(IV)

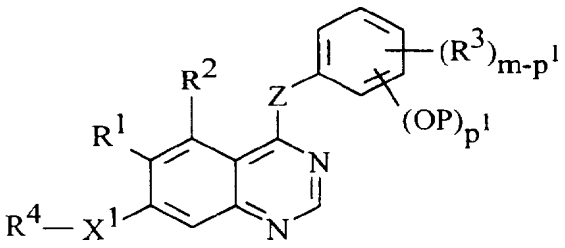
(wherein Z, R^3 and m are as defined in claim 1) whereby to obtain compounds of the formula I and salts thereof;

(b) for the preparation of compounds of formula I and salts thereof in which the group of formula IIa:



(IIa)

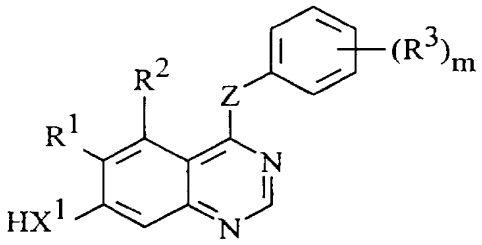
(wherein R^3 and m are as defined in claim 1) represents a phenyl group carrying one or more hydroxy groups, the deprotection of a compound of formula V:



(V)

(wherein X^1, m, R^1, R^2, R^3, R^4 and Z are as defined in claim 1, P represents a phenolic hydroxy protecting group and p^1 is an integer from 1 to 5 equal to the number of protected hydroxy groups and such that m-p^1 is equal to the number of R^3 substituents which are not protected hydroxy);

(c) for the preparation of those compounds of formula I and salts thereof wherein the substituent X^1 is -O-, -S- or -NR^7-, (wherein R^7 is as defined in claim 1), the reaction of a compound of the formula VI:



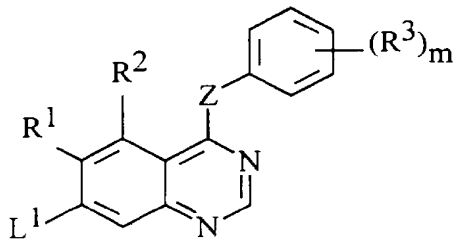
(VI)

(wherein m, X^1, R^1, R^2, R^3, and Z are as defined in claim 1) with a compound of formula VII:

$$R^4\text{-}L^1 \qquad \text{(VII)}$$

(wherein R^4 is as defined in claim 1 and L^1 is as herein defined);

(d) the reaction of a compound of the formula VIII:



(VIII)

(wherein R^1, R^2, R^3, Z and m are all as defined in claim 1 and L^1 is as herein defined) with a compound of the formula IX:

$$R^4\text{-}X^1\text{-}H \qquad \text{(IX)}$$

(wherein R^4 and X^1 are as defined in claim 1);

(e) for the preparation of compounds of formula I and salts thereof wherein R^4 is C_{1-5}alkylR^{32}, [wherein R^{32} is selected from one of the following four groups:

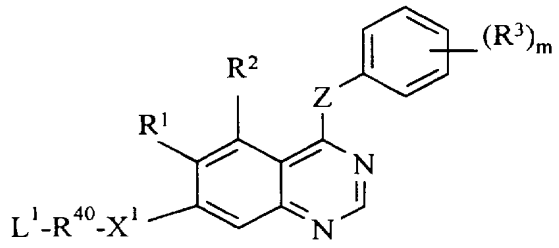
1) X^6C_{1-3}alkyl (wherein X^6 represents -O-, -S-, $-SO_2-$, $-NR^{33}CO-$ or $-NR^{34}SO_2-$ (wherein R^{33} and R^{34} are each independently hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

2) $NR^{35}R^{36}$ (wherein R^{35} and R^{36} which may be the same or different are each hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

3) X^7C_{1-5}alkylX^5R^{24} (wherein X^7 represents -O-, -S-, $-SO_2-$, $-NR^{37}CO-$, $-NR^{38}SO_2-$ or $-NR^{39}-$ (wherein R^{37}, R^{38} and R^{39} are each independently hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and X^5 and R^{24} are as defined in claim 1); and

4) R^{31} (wherein R^{31} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to C_{2-5}alkyl through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy);]

the reaction of a compound of the formula X:



(X)

(wherein X^1, R^1, R^2, R^3, Z and m are as defined in claim 1, L^1 is as defined herein and R^{40} is C_{1-5}alkyl) with a compound of the formula XI:

$$R^{32}\text{-}H \qquad \text{(XI)}$$

(wherein R^{32} is as defined herein);

(f) for the preparation of those compounds of formula I and salts thereof wherein the substituent R^1 is represented by NR^5R^6, where one or both of R^5 and R^6 are C_{1-3}alkyl and/or the substituent R^4-X^1 is an alkyamino or dialkylamino group, the reaction of compounds of

formula I wherein the substituent R^1 and/or the substituent R^4-X^1 is an amino group with an alkylating agent;

(g) for the preparation of those compounds of formula I and salts thereof wherein one or more of the substituents R^1, R^2 or R^3 is an amino group or where R^4-X^1 is an amino group, the reduction of a corresponding compound of formula I wherein the substituent(s) at the corresponding position(s) of the quinazoline and/or phenyl ring is/are a nitro group(s);

and when a salt of a quinazoline derivative of formula I is required, reaction of the compound obtained with an acid or base whereby to obtain the desired salt.

16. A pharmaceutical composition which comprises as active ingredient a quinazoline derivative of formula I as defined in claim 1 or a pharmaceutically acceptable salt thereof in association with a pharmaceutically acceptable excipient or carrier.

17. A method for producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal in need of such treatment which comprises administering to said animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof as defined in claim 1.

18. A quinazoline derivative as claimed in any one of claims 1 to 14 for use as a medicament.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/00365

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D239/94 A61K31/505 C07D239/88 C07D239/93 C07D413/12
A61K31/535 C07D403/12 C07D401/12 C07D417/12 A61K31/54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 15758 A (RHONE POULENC RORER PHARMA ;MYERS MICHAEL R (US); SPADA ALFRED P () 15 June 1995 See first compound of claim 12 see claims 3-6,12 ---	1
X	EP 0 635 498 A (ZENECA LTD) 25 January 1995 see claim 1 ---	1
X	WO 92 20642 A (RHONE POULENC RORER INT) 26 November 1992 see claim 19 ---	1
X	EP 0 326 330 A (LILLY CO ELI) 2 August 1989 cited in the application see page 20; claim 1; examples 127,128 --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
12 May 1997	13.06.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Gettins, M

Form PCT/ISA/210 (second sheet) (July 1992)

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
19/043,275	**02/05/2025 07:37:02 PM Z ET**	**50535-710.304**

Title of Invention

METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	5486	FILED BY	Jennifer Vail
PATENT CENTER #	69068752	FILING DATE	-
CUSTOMER #	21971	FIRST NAMED INVENTOR	Alexis HOWERTON
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Victoria Cheng

Documents

TOTAL DOCUMENTS: 54

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Spruce 50535-710_304_IDS-IDS.pdf	(5-14)	10	Information Disclosure Statement (IDS) Form (SB08)	440 KB
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(71) Applicant *(for all designated States except US)*: ANGIOGENE PHARMACEUTICALS LTD. [GB/GB]; 14 Plowden Park, Aston Rowant, Watlington, Oxfordshire OX9 5SW (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: DAVIS, Peter, David [GB/GB]; 10 Aston Park, Aston Rowant, Watlington OX9 5SW (GB).

(74) Agents: BAILLIE, Iain, C. et al.; Langner Parry, 52–54 High Holborn, London WC1V 6RR (GB).

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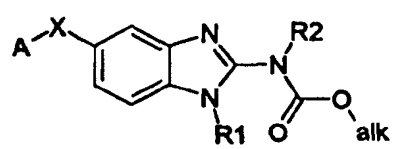

(I)

(57) Abstract

A group of vascular damaging agents which can be used in the preparation of medicaments for the treatment of diseases involving neovascularisation are provided. These are 5(6)–substituted benzimidazole–2–carbamates of formula (I) wherein Alk is an alkyl group, X is oxygen, sulphur, sulphinyl, sulphonyl, carbonyl (CO), thiocarbonyl (CS), sulphonyloxy, NH, iminomethylene (C=NH), N–hydroxyiminomethylene, N–alkoxyiminomethylene, dialkoxymethylene, 1,3–dioxolan–2yl, 1,1–ethenyl, a group CHR^3 or a bond, R^1 is hydrogen, alkylaminocarbonyl or alkoxycarbonyl, R^2 is hydrogen, alkoxycarbonyl, cyanomethyl, cyanoethyl, alkoxymethyl or acetoxymethyl. R^3 is hydrogen, hydroxy, alkoxy or amino, A is an optionally substituted aromatic, optionally substituted heteroaromatic, optionally substituted heterocycloalkyl, optionally substituted alkyl or optionally substituted cycloalkyl group and the pharmaceutically acceptable salts, solvates and hydrates thereof. Most of the compounds of this group are novel, in particular those in which A is an aromatic or heteroaromatic ring with substituents, particularly substituents which are phosphates or alkylphosphates. The invention therefore provides both novel compounds and pharmacological compositions with compounds within the broad definition.

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BENZIMIDAZOLE VASCULAR DAMAGING AGENTS

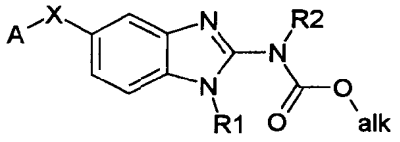
This invention relates to vascular damaging agents and particularly to the use of new and known substituted benzimidazoles in the preparation of medicaments for the treatment of diseases involving neovascularisation.

Formation of new vasculature by angiogenesis is a key pathological feature of several diseases (J Folkman, New England Journal of Medicine 333, 1757-1763 (1995)). For example, for a solid tumour to grow it must develop its own blood supply upon which it depends critically for the provision of oxygen and nutrients; if this blood supply is mechanically shut off the tumour undergoes necrotic death. Neovascularisation is also a clinical feature of skin lesions in psoriasis, of the invasive pannus in the joints of rheumatoid arthritis patients and of atherosclerotic plaques. Retinal neovascularisation is pathological in macular degeneration and in diabetic retinopathy. In all these diseases reversal of neovascularisation by damaging the newly-formed vascular endothelium is expected to have a beneficial therapeutic effect.

5(6)-Substituted benzimidazole-carbamates are known and have found use as antiparasitic agents (P. J. Islip in Burgers Medicinal Chemistry (M. E. Wolff ed.), Fourth Edition, Part II, p481, (1979)). Examples of such compounds include mebendazole, fenbendazole, oxibendazole, flubendazole, albendazole, cyclobendazole, parbendazole, dribendazole, luxabendazole, and etibendazole. Their mode of action for their antiparasitic action is believed to involve selective binding to tubulin of the target parasite while having little effect due to binding tubulin of the mammalian host (Biochim. Biophys. Acta 630, 271-278, (1980)). Some of these compounds have been shown to be antimitotic for cancer cells and one particular 5(6)-substituted benzimidazole-2-carbamate, nocodazole, has therefore been studied as an anticancer agent (Cancer Research, 36, 905-916 (1976)). No effects on neovasculature have been reported for any of these compounds.

Some structurally-unrelated compounds which bind tubulin have been shown to have anti-vascular effects when given at their maximum tolerated dose (MTD) (S. A. Hill et al. Eur. J Cancer, 29A, 1320-1324 (1993)) but other tubulin-binding agents, such as docetaxel, have no vascular-damaging activity even when administered at the MTD. The presence of tubulin-binding properties is then not predictive for antivascular activity.

According to the present invention there is provided the use of 5(6)-substituted benzimidazole-2-carbamates for the preparation of compositions for the treatment of diseases involving angiogenesis in which the 5(6)-substituted benzimidazole carbamate has the formula



I

wherein

Alk is an alkyl group

X is oxygen, sulphur, sulphinyl, sulphonyl, carbonyl (CO), thiocarbonyl (CS) , sulphonyloxy, NH, iminomethylene (C=NH), N-hydroxyiminomethylene, N-alkoxyiminomethylene, dialkoxymethylene, 1,3-dioxolan-2yl, 1,1-ethenyl, a group CHR^3 or a bond

R^1 is hydrogen, alkylaminocarbonyl or alkoxycarbonyl

R^2 is hydrogen, alkoxycarbonyl, cyanomethyl, cyanoethyl, alkoxymethyl or acetoxymethyl.

R^3 is hydrogen, hydroxy, alkoxy or amino

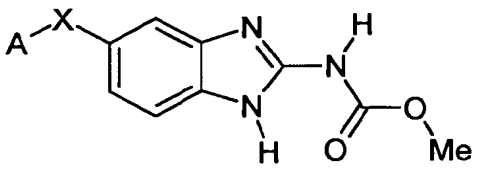
A is an optionally substituted aromatic, optionally substituted heteroaromatic, optionally substituted heterocycloalkyl, optionally substituted alkyl or optionally substituted cycloalkyl group

and the pharmaceutically acceptable salts, solvates and hydrates thereof.

Particular substituents that may be present on the group A include one or more substituents selected from a group Y, optionally substituted alkyl,(where substituents on such alkyl group may include one or more selected from hydroxy, amino, alkylamino, dialkylamino, halogen, carboxyl, SO_3H, sulphate, phosphate, alkoxycarbonyl, aralkoxycarbonyl, alkoxycarbonylamino, aminoalkylaminocarbonyl and cyano), halogen, hydroxy, amino, alkoxy, alkylthio, cyano, nitro, sulphate, isothiocyanate, aryl, heteroaryl and heterocycloalkyl.

Y is a group selected from phosphate, alkylphosphate, $C(O)R^4$, $OC(O)R^4$, SO_2R^4, $NHC(O)R^4$, $NR^5C(O)R^4$, SR^4, $S(O)R^4$, OSO_2R^4, $NHSO_2R^4$, $NR^5SO_2R^4$, SO_3H, CO_2H and CO_2R^5 where R^4 is a group selected from hydrogen, R^5, OR^5, NHR^5, NR^5R^6, aryl, heteroaryl or heterocycloalkyl such aryl, heteroaryl or heterocycloalkyl groups being optionally substituted with one or more substituents selected from alkyl, heterocycloalkyl, haloalkyl, hydroxy, nitro, cyano, amino, alkylamino, dialkylamino, halogen, carboxyl, SO_3H, sulphate and phosphate. R^5 and R^6, which may be the same or different, are each an alkyl group optionally substituted with one or more substituents selected from hydroxy, amino, alkylamino, dialkylamino, guanidino, halogen, carboxyl, SO_3H, sulphate, phosphate, aryl and heteroaryl.

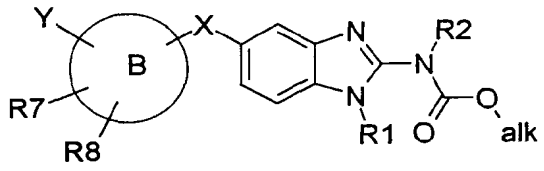
Some of the compounds usable in the invention are known, for example the following compounds within the following formula:



These compounds are

	X	A
Fenbendazole	S	Ph
Mebendazole	CO	Ph
Albendazole	S	nPr
Oxibendazole	O	nPr
Nocodazole	CO	2-thienyl

Certain of these compounds are novel. In one embodiment the novel compounds are those of formula I in which at least one of the substituents on the group A is a group Y where Y is as hereinbefore defined. Particularly preferred are compounds defined by the formula



II

wherein

alk is an alkyl group

B is an aromatic or heteroaromatic ring

X is oxygen, sulphur, sulphinyl, sulphonyl, carbonyl (CO), thiocarbonyl (CS) , sulphonyloxy, NH, iminomethylene (C=NH), N-hydroxyiminomethylene, N-alkoxyiminomethylene, dialkoxymethylene, 1,3-dioxolan-2yl, 1,1-ethenyl, a group CHR^3 or a bond

R^1 is hydrogen, alkylaminocarbonyl or alkoxycarbonyl

R^2 is hydrogen, alkoxycarbonyl, cyanomethyl, cyanoethyl, alkoxymethyl or acetoxymethyl

R^3 is hydrogen, hydroxy, alkoxy or amino

Y is as hereinbefore defined

R^7 and R^8 are each independently H, alkyl, halogen, hydroxy, amino, alkylamino, dialkylamino, alkoxy, alkylthio, cyano, nitro, or trifluoromethyl

with the proviso that Y is not NHC(O)Me and when B is a thiophene ring then Y is not $C(O)CF_3$ and when B is a 5(6)-benzimidazole ring then Y is not $NHCO_2Me$ or $NHCO_2Et$

and the pharmaceutically acceptable salts, solvates, hydrates and prodrugs thereof.

As used herein the term "alkyl", alone or in combinations, means a straight or branched-chain alkyl group containing from one to seven, preferably a maximum of four, carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, t-butyl and pentyl. Examples of alkoxy groups are methoxy, ethoxy, propoxy, isopropoxy, butoxy and t-butoxy. The term "halogen" means fluorine, chlorine, bromine or iodine.

The term "aryl" as used herein unless otherwise stated includes reference to a C_{6-10} aryl group which may, if desired, carry one or more substituents selected from halogeno, alkyl, haloalkyl, alkoxy, hydroxy, amino, nitro and cyano. The term "aralkoxy" means an alkoxy group substituted with an aryl group.

The term heteroaryl is defined herein as a mono- or bi-cyclic aromatic group containing one to four heteroatoms selected in any combination from N, S or O atoms and a maximum of 9 carbon atoms. Examples of heteroaryl groups include pyridyl, pyrimidyl, furyl, thienyl, pyrrolyl , pyrazolyl, indolyl, benzofuryl, benzothienyl, benzothiazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, imidazolyl, triazolyl, quinolyl and isoquinolyl groups.

The term heterocycloalkyl includes heterocycloalkyl groups containing 3-6 carbon atoms and one or two oxygen, sulphur or nitrogen atoms. Particular examples of such groups include azetidinyl, pyrrolidinyl, piperidinyl, homopiperidinyl, piperazinyl, homopiperazinyl, morpholinyl or thiomorpholinyl groups.

The term cycloalkyl means a cycloaliphatic group containing 3-10 carbon atoms such as, for example, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl.

One particularly preferred group of compounds are those of formula II in which Y is a phosphate group.

Another particularly preferred group of compounds are those of formula II in which Y is a group $NR^5C(O)R^4$, R^5 is hydrogen and R^4 is a 1-aminoalkyl group which can be further substituted for example by a hydroxy group.

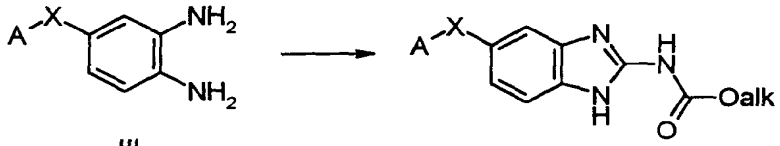
Where one or more functional groups in compounds of formula I or II are sufficiently basic or acidic the formation of salts is possible. Suitable salts include pharmaceutically acceptable salts for example acid addition salts including hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates and tartrates, salts derived from inorganic bases including alkali metal salts such as sodium or potassium salts, alkaline earth metal salts such as magnesium or calcium salts, and salts derived from organic amines such as morpholine, piperidine or dimethylamine salts.

Those skilled in the art will recognise that compounds of formulae I and II may exist as stereoisomers and/or geometrical isomers and accordingly the present invention includes all such isomers and mixtures thereof. The substituted benzimidazole group is capable of existing in tautomeric forms and the formulae I and II are intended to represent all tautomeric forms and the substituent AX- is in the 5(6) position.

Compounds of Formula I or II may be prepared by any process known to a person skilled in the art. Compounds of Formula I or II may be prepared by a number of processes as generally described below and more specifically in the Examples hereinafter. In the following process description, the symbols A, X and alk when used in the formulae depicted are to be understood to represent those groups described above in relation to formula I or II unless otherwise indicated. In the schemes described below it may be necessary to employ protecting groups which are then

removed during the final stages of the synthesis. The appropriate use of such protecting groups and processes for their removal will be readily apparent to those skilled in the art.

Thus according to a further aspect of the invention compounds of formulae I and II in which R^1 and R^2 are hydrogen may be prepared by treatment of a diamine of formula III with a 1,3-bis(alkoxycarbonyl)- S-alkyl isothiourea, for example 1,3-bis(methoxycarbonyl)-S-methyl isothiourea or 1,3-bis(ethoxycarbonyl)-S-methyl isothiourea, in a solvent such as an alcohol, for example methanol or ethanol, optionally mixed with water, at from about room temperature to the reflux temperature of the solvent for about 5 minutes to 6 hours. The reaction medium is preferably made acidic by the addition of for example an organic acid such as acetic acid or p-toluenesulphonic acid.



III I or II

Compounds of formula III are either known or can be prepared by conventional procedures.

Compounds of formulae I and II can also be prepared from other compounds of formulae I and II by chemical modification. Example of such chemical modifications that may be applied are standard alkylation, acylation, reduction, oxidation, sulphation, aromatic halogenation, aromatic nitration, phosphorylation, hydrolysis, condensation, cleavage and coupling reactions. These reactions may be used to add new substituents, to modify existing substituents or to modify other parts of the molecule.

Thus for example a compound of formula I or II in which R^1 is hydrogen can be converted into the corresponding compounds where R^1 is alkylaminocarbonyl by

treatment with an alkyl isocyanate in a solvent such as tetrahydrofuran at a temperature in the range 0° to 40°C, typically room temperature.

In another general example a thioether group in a compound of formula I or II can be converted into a sulphinyl group by treatment with periodate in an aqueous alcohol solvent such as aqueous methanol or in aqeuous acetonitrile at about -20° to 50°C, for about 1 to 16 h. Alternatively this conversion can be effected by treatment with one equivalent of a peracid such as 3-chloroperbenzoic acid in a chlorinated solvent such as dichloromethane or chloroform, at a temperature of about -30°C to room temperature.

In a further general example a thioether group in a compound of formula I or II can be converted into a sulphonyl group by treatment with two or more equivalents of a peracid such as 3-chloroperbenzoic acid in a chlorinated solvent such as dichloromethane or chloroform, at a temperature of about -30°C to room temperature.

In a further general example a keto group in a compound of formula I or II can be converted into a carbinol group by treatment with a reducing agent for example a hydride such as lithium aluminium hydride in an ether solvent such as diethyl ether or tetrahydrofuran at a temperature of from about 0° to the reflux temperature of the solvent.

In a further general example a keto group in a compound of formula I or II can be converted into an imine by treatment with ammonia in an alcoholic solvent such as ethanol at around room temperature for an extended period, for example three weeks.

In a further general example a keto group in a compound of formula I or II can be converted into an oxime by treatment with hydroxylamine in an alcoholic solvent such as ethanol at around room temperature to around the reflux temperature of the solvent.

In a further general example a compound of formula I or II containing a hydroxy group can be converted into the corresponding dihydrogenphosphate ester by treatment with

for example di-tert-butyl diethylphosphoramidite in the presence of a suitable catalyst for example tetrazole in a solvent such as an ether solvent for example tetrahydrofuran at a temperature in the range -40 to 40°C , conveniently at or near room temperature, followed by treatment with an oxidising agent for example 3-chloroperoxy benzoic acid or magnesium monoperoxyphthalate at a temperature in the range -78°C to 40°C preferably -65 to -10°C. The resulting intermediate phosphate triester is treated with an acid for example trifluoroacetic acid in a solvent such as a chlorinated solvent e.g. dichloromethane at a temperature in the range -30 to 40°C conveniently at or near 0°C to give the compound of formula I or II containing a dihydrogenphosphate ester.

In another general example an O-alkyl group may be cleaved to the corresponding alcohol (OH) by reaction with boron tribromide in a solvent such as a chlorinated solvent e.g. dichloromethane at a low temperature e.g. around -78°C.

In a further general example compounds of formula I or II may be alkylated by reaction with a suitable alkylating agent such as an alkyl halide, an alkyl toluenesulphonate, an alkyl methanesulphonate or an alkyl triflate. The alkylation reaction can be carried out in the presence of a base for example an inorganic base such as a carbonate e.g. caesium or potassium carbonate, a hydride such as sodium hydride or an alkoxide such as potassium t-butoxide in a suitable solvent such as an aprotic solvent e.g. dimethylformamide or an ether solvent such as tetrahydrofuran at a temperature of around -10 to 80°C.

In a further general example compounds of formula I or II containing an amine group may be acylated by treatment with a carboxylic acid and a coupling reagent, for example dicyclohexylcarbodiimide, in a sutable solvent for example an aprotic solvent such as dimethylformamide, an ether solvent such as tetrahydrofuran, a chlorinated solvent for example dichloromethane or a solvent mixture at a temperature in the range 0° to 60°, preferably about room temperature.

In a further general example a compound of formula I or II containing an OH group can be converted into a carbamate by reaction with an alkyl isocyanate or a carbamoyl chloride in an aprotic solvent such as dimethylformamide, an ether solvent such as tetrahydrofuran, a chlorinated solvent for example dichloromethane or a solvent mixture in the presence of a base such as a tertiary amine base for example triethylamine at a temperature in the range -20° to the reflux temperature of the solvent, conveniently at or around room temperature.

In a further general example a compound of formula I or II containing an amino group can be converted into a urea by reaction with an isocyanate or a carbamoyl chloride in an aprotic solvent such as dimethylformamide, an ether solvent such as tetrahydrofuran, a chlorinated solvent for example dichloromethane or a solvent mixture in the presence of a base such as a tertiary amine base for example triethylamine at a temperature in the range -20°C to the reflux temperature of the solvent, conveniently at or around room temperature.

In a further general example a compound of formula I or II containing a hydroxy group can be converted into a carbonate by reaction with an chloroformate in an aprotic solvent such as dimethylformamide, an ether solvent such as tetrahydrofuran, a chlorinated solvent for example dichloromethane or a solvent mixture in the presence of a base such as a tertiary amine base for example triethylamine at a temperature in the range -20°C to the reflux temperature of the solvent, preferably at or around 0°C.

Preparation of a compound of formula I or II as a single enantiomer or, where appropriate, diastereomer may be effected by synthesis from an enantiomerically pure starting material or intermediate or by resolution of the final product in a conventional manner.

Acid addition salts of the compounds of formula I or II are prepared in a conventional manner by treating a solution or suspension of the free base I or II with about one

equivalent of a pharmaceutically acceptable acid. Salts of compounds of formula I or II derived from inorganic or organic bases are prepared in a conventional manner by treating a solution or suspension of the free acid I or II with about one equivalent of a pharmaceutically acceptable organic or inorganic base. Alternatively both acid addition salts and salts derived from bases may be prepared by treatment of the parent compound with the appropriate ion-exchange resin in a standard fashion. Conventional concentration and recrystallisation techniques are employed in isolating the salts.

Compounds according to the invention are able to destroy vasculature that has been newly formed, for example tumour vasculature, while leaving unaffected normal, mature vasculature. The ability of the compounds to act in this way may be determined by the tests described hereinafter.

The compounds according to the invention are thus of particular use in the prophylaxis and treatment of cancers involving solid tumours and in the prophylaxis and treatment of diseases where inappropriate angiogenesis occurs such as diabetic retinopathy, psoriasis, rheumatoid arthritis, atherosclerosis and macular degeneration.

The compounds of the invention may be administered as a sole therapy or in combination with other treatments. For the treatment of solid tumours compounds of the invention may be administered in combination with radiotherapy or in combination with other anti-tumour substances for example those selected from mitotic inhibitors, for example vinblastine, paclitaxel and docetaxel; alkylating agents, for example cisplatin, carboplatin and cyclophosphamide; antimetabolites, for example 5-fluorouracil, cytosine arabinoside and hydroxyurea; intercalating agents for example adriamycin and bleomycin; enzymes, for example aspariginase; topoisomerase inhibitors for example etoposide, topotecan and irinotecan; thymidylate synthase inhibitors for example raltitrexed; biological response modifiers for example interferon; antibodies for example edrecolomab and antibodies against the EGFr, HER2 receptor or VEGF receptor: and anti-hormones for example tamoxifen. Such combination

treatment may involve simultaneous or sequential application of the individual components of the treatment.

For the prophylaxis and treatment of disease the compounds according to the invention may be administered as pharmaceutical compositions selected with regard to the intended route of administration and standard pharmaceutical practice. Such pharmaceutical compositions may take a form suitable for oral, buccal, nasal, topical, rectal or parenteral administration and may be prepared in a conventional manner using conventional excipients. For example for oral administration the pharmaceutical compositions may take the form of tablets or capsules. For nasal administration or administration by inhalation the compounds may be conveniently delivered as a powder or in solution. Topical administration may be as an ointment or cream and rectal administration may be as a suppository. For parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) the composition may take the form of, for example, a sterile solution, suspension or emulsion.

The dose of a compound of the invention required for the prophylaxis or treatment of a particular condition will vary depending on the compound chosen, the route of administration, the form and severity of the condition and whether the compound is to be administered alone or in combination with another drug. Thus the precise dose will be determined by the administering physician but in general daily dosages may be in the range 0.001 to 100mg/kg preferably 0.1 to 50mg/kg.

BIOLOGICAL ACTIVITY

The following test was used to demonstrate the activity and selectivity of compounds according to the invention.

Activity against tumour vasculature measured by fluorescent dye.

The following experiment demonstrates the ability of the compounds to damage tumour vasculature.

Tumour functional vascular volume in CaNT tumour-bearing mice was measured using the fluorescent dye Hoechst 33342 according to the method of Smith *et al* (Brit J Cancer **57**, 247-253, 1988). The fluorescent dye was dissolved in saline at 6.25 mg/ml and injected intravenously at 10 mg/kg 6 hours or 24 hours after intraperitoneal drug treatment. One minute later, animals were killed and tumours excised and frozen; 10 μm sections were cut at 3 different levels and observed under UV illumination using an Olympus microscope equipped with epifluorescence. Blood vessels were identified by their fluorescent outlines and vascular volume was quantified using a point scoring system based on that described by Chalkley, (J Natl Cancer Inst, 4, 47-53, 1943). All estimates were based on counting a minimum of 100 fields from sections cut at the 3 different levels. Results are expressed as percentage reduction in vascular volume compared to control.

The activity of compounds of the invention in this assay is shown in Table 1.

Table 1: Reduction in tumour vascular volume measured by fluorescent dye

Compound	Dose (mg/kg) (i.p.)	Time (h)	% Reduction in vascular volume
Fenbendazole	500	6	44
Mebendazole	500	6	56
Albendazole	500	6	51
Oxibendazole	100	6	43
Nocodazole	100	24	23
Compound of Example:			
1	500	6	81
2	500	6	80
3	50	24	22
4	50	24	53
5	50	24	99
12	50	24	51
14	50	24	39

15	50	24	61
16	50	24	56
17	50	24	62
18	50	24	47
19	50	24	88
20	50	24	69
21	50	24	74

The following non-limiting Examples illustrate the invention:

Example 1

Methyl [5(6)-(4-hydroxybenzoyl)-1*H*-benzimidazol-2yl]carbamate.

A solution of 3,4-diamino-4`-hydroxybenzophenone (49.6mg, 0.21mmol) and 1,3-bis(methoxycarbonyl)-S-methyl isothiourea (98mg, 0.44mmol) in ethanol (2.5ml) was treated with p-toluenesulphonic acid (7mg) and the mixture heated at reflux for 10 minutes. The mixture was cooled and the precipitate collected by filtration and washed with ethanol and hexane to give the title compound (16mg) as a white solid m.p. >258°C, ^{1}H–NMR (400 MHz, d6–DMSO) δ 7.80 (s, 1H), 7.65 (d, 2H, J=8Hz), 7.50 (s, 1H), 7.49 (s, 1H), 6.89 (d, 2H, J=8Hz), 3.77 (s, 3H) ppm. m/e 311 (M+). Anal. Calculated for C16H13N3O4: C, 61.73; H, 4.21; N 13.49. Found: C, 61.68; H, 4.18; N 13.36.

Example 2

Methyl [5(6)-(4-phosphonooxybenzoyl)-1*H*-benzimidazol-2yl]carbamate.

A solution of methyl [5-(4-(di-tert-butylphosphonooxy)benzoyl)-1*H*-benzimidazol-2yl]carbamate (1.5g, 3.0mmol) in dichloromethane (55ml) was cooled in an ice bath and treated with trifluoroacetic acid (6ml) dropwise. The mixture was allowed to warm to room temperature and stirred for 1 hour before solvents were removed under reduced pressure. The residue was triturated with ether to give the title compound (1.13g) as a white solid m.p. >258°C, ^{1}H–NMR (300 MHz, d6–DMSO) δ: 7.84 (s, 1H), 7.75 (d, 1H, J=9Hz), 7.57 (d, 1H, J=8Hz), 7.52 (d, J=8Hz), 7.33 (d, 1H, J=9Hz), 3.78 (s, 3H) ppm.

The methyl [5(6)-(4-(di-*tert*-butylphosphonooxy)benzoyl)-1*H*-benzimidazol-2yl]carbamate used as starting material was prepared as follows:

A solution of methyl [5(6)-(4-hydroxybenzoyl)-1*H*-benzimidazol-2yl]carbamate (100mg, 0.3mmol) in anhydrous tetrahydrofuran (1ml) stirred under a nitrogen atmosphere was treated with di-*tert*-butyl diethylphosphoramidite (74mg, 0.29mmol) and 1*H*-tetrazole (54mg, 0.78mmol) and the mixture stirred until the reaction was shown to be complete by TLC (about 1h). The cooled (-40°C) mixture was treated with 3-chloroperbenzoic acid (79mg, 0.39mmol) in dichloromethane (1ml) and stirred 10minutes before being allowed to warm to room temperature. The mixture was washed with saturated aqueous sodium bicarbonate followed by brine and the dried (MgSO4) organic phase concentrated under reduced pressure. The residue was chromatographed on silica gel eluting first with 4% methanol/dichloromethane then with 5% methanol/dichloromethane. Methyl [5(6)-(4-(di-*tert*-butylphosphonooxy)benzoyl)-1*H*-benzimidazol-2yl]carbamate (35mg) was obtained as a white solid m.p. 99-101°C.

Example 3

Methyl [5(6)-(4-phosphonooxyphenylthio)-1*H*-benzimidazol-2yl]carbamate

A solution of methyl [5(6)-(4-(di-*tert*-butylphosphonooxy)phenylthio)-1*H*-benzimidazol-2yl]carbamate (180mg) in anhydrous dichloromethane (10ml) at 4°C was treated with trifluoroacetic acid (1ml) and stirred for 1.5h. The mixture was allowed to warm to room temperature and concentrated under reduced pressure. Ethyl acetate was added and the mixture concentrated again. The residue was triturated with diethyl ether, washed with diethyl ether followed by acetone/water 9:1 and dried to give the title compound (134mg) as a white solid m.p. 190-193°C. Anal. Calculated for $C_{15}H_{14}N_3O_6PS.3H_2O$: C, 40.1; H, 4.5; N 9.3. Found: C, 40.1; H, 3.9; N 9.2.

The methyl [5(6)-(4-(di-*tert*-butylphosphonooxy)phenylthio)-1*H*-benzimidazol-2yl]carbamate used as starting material was prepared as follows:

A solution of methyl [5-(4-hydroxyphenylthio)-1*H*-benzimidazol-2yl]carbamate (200mg) in a mixture of anhydrous dimethylformamide (2ml) and anhydrous

tetrahydrofuran (2ml) was treated with di-*tert*-butyl diethylphosphoramidite (350mg) and the mixture stirred for 48h at room temperature. The mixture was cooled to -65°C and treated gradually with magnesium monoperoxyphthalate (850mg) so that the temperature remained below -50°C. A saturated aqueous solution of sodium bicarbonate was added, keeping the temperature below -40°C during the addition then allowing the mixture to warm to room temperature. The mixture was extracted with three portions (50ml each) of ethyl acetate and the combined extracts washed with brine (50ml), dried (MgSO4) and concentrated under reduced pressure. The residue was purified by radial chromatography on silica gel eluting with dichloromethane/methanol 9:1 to give methyl [5(6)-(4-(di-*tert*-butylphosphonooxy)phenylthio)-1*H*-benzimidazol-2yl]carbamate (180mg)as a white foam.

Example 4

Methyl [5(6)-(4-aminophenylthio)-1*H*-benzimidazol-2yl]carbamate

Methyl [5(6)-(4-(acetylamino)phenylthio)-1*H*-benzimidazol-2yl]carbamate (602 mg, 1.78 mmol) was dissolved in mixture of methanol (24 ml) and hydrochloric acid (10%, 6 ml) and heated under reflux for 16h. The solution was neutralised with ammonia solution and the methanol removed under reduced pressure. The white precipitate was collected by filtration, washed with water and dried in vacuo to give 392 mg of a pale yellow solid m.p. 282-284°C. m/e 298 (M^+).

Example 5

Methyl [5(6)-(4-alanylaminophenylthio)-1*H*-benzimidazol-2yl]carbamate

A suspension of methyl [5(6)-(4-(Nα-*tert*-butoxycarbonylalanylamino)phenylthio)-1*H*-benzimidazol-2yl]carbamate (250mg) in dichloromethane (20ml) was treated with trifluoroacetic acid (4ml). The mixture was allowed to warm to room temperature and concentrated under reduced pressure. Ethyl acetate was added and the mixture concentrated again. The residue was triturated with diethyl ether to afford the trifluoroacetic acid salt of the title compound (105mg) as a white solid m.p. 178-

182°C. m/e 485 (M+). Anal. Calculated for $C_{18}H_{19}N_5O_3S.2C_2HF_3O_2$ C; 43.1, H; 3.5, N; 11.4 Found C; 42.8, H; 3.8, N; 11.3

The methyl [5(6)-(4-(Nα-*tert*-butoxycarbonylalanylamino)phenylthio)-1*H*-benzimidazol-2yl]carbamate used as starting material was prepared as follows:

A suspension of methyl [5(6)-(4-aminophenylthio)-1*H*-benzimidazol-2yl]carbamate (150mg) in anhydrous tetrahydrofuran (4ml) was treated with N-*tert*-butoxycarbonylalanine (100mg), cooled to -35°C and treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (100mg). The mixture was allowed to warm to room temperature and stir for 16h. Water (40ml) and ethyl acetate (20ml) were added and the mixture extracted with four portions of ethyl acetate (50ml each). The combined extracts were washed with brine (50ml), dried (MgSO4) and concentrated under reduced pressure. The residue was purified on silica gel eluting with ethyl acetate to give methyl [5(6)-(4-(Nα-*tert*-butoxycarbonylalanylamino)phenylthio)-1*H*-benzimidazol-2yl]carbamate (258mg) as a white solid m.p. 222-224°C. m/e 485 (M^+).

Example 6

Methyl [5(6)-(4-(methoxycarbonylamino)phenylthio)-1*H*-benzimidazol-2yl]carbamate

Methyl [5(6)-(4-aminophenylthio)-1*H*-benzimidazol-2yl]carbamate(150 mg, 048 mmol) was dissolved in dimethylformamide (2 ml) and tetrahydrofuran (2 ml) and methyl chloroformate (40 mg, 0.42 mmol) added together with triethylamine (43 mg, 0.42 mmol). The solution was stirred at 20°C for 72 h and then evaporated to dryness under reduced pressure. The residue was purified by chromatography on silica gel eluting with 5 % methanol/dichloromethane to give the title compound as a white solid; mp >350°C (dec.).

Example 7

Methyl [5(6)-(4-(phenylaminocarbonylamino)phenylthio)-1*H*-benzimidazol-2yl]carbamate

Methyl [5(6)-(4-aminophenylthio)-1*H*-benzimidazol-2yl]carbamate (150 mg, 0.48 mmol) was dissolved in dimethylformamide (2 ml) and tetrahydrofuran (2 ml) and

phenylisocyanate (56.8 mg, 0.48 mmol) added together with triethylamine (50 mg, ca.0.5 mmol). The solution was stirred at 20°C for 12 h and the solvents removed in vacuo. The residue was purified on silica (ethyl acetate/hexane, 2:1) to give the title compound as a white solid; mp 335–340°C (dec.).m/e 433 (M^+).

Example 8

Methyl [5(6)-(4-(methoxycarbonyloxy)benzoyl)-1*H*-benzimidazol-2yl]carbamate

Methyl [5(6)-(4-hydroxybenzoyl)-1*H*-benzimidazol-2yl]carbamate (110 mg, 0.35 mmol) was dissolved in dimethylformamide (3.5 ml) and triethylamine (0.5 ml). The solution was cooled to 0°C and methyl chloroformate (50 mg, 0.52 mmol) added with stirring. The solution was stirred for 0.5 h at 0°C and the 1 h at 20°C and evaporated to dryness. The residue was dissolved in ethyl acetate (50 ml) and washed with sodium bicarbonate (sat., aq., 50 ml) and brine (50 ml), dried and evaporated. The residue was purified by radial chromatography on silica gel eluting with ethyl acetate/hexane, 1:1 followed by ethyl acetate to give the title compound as a white solid; mp 224–226°C (dec).m/e 369 (M^+).

Prepared in an analogous fashion to Example 1 were:

Example 9

Methyl [5(6)-(2-methoxycarbonylphenylthio)-1*H*-benzimidazol-2yl]carbamate from methyl 2-(3,4-diaminophenylthio)benzoate (1.5g) and 1,3-bis(methoxycarbonyl)-S-methyl isothiourea (2.25g) there was obtained the title compound (1.51g) as a white solid m.p. 228-230 m/e 357 (M+). Anal. Calculated for $C_{17}H_{15}N_3O_4S$: C, 57.13; H, 4.23; N 11.75. Found: C, 57.39; H, 4.13; N 11.81.

Example 10

Methyl [5(6)-(3-methoxycarbonylphenylthio)-1*H*-benzimidazol-2yl]carbamate from methyl 3-(3,4-diaminophenylthio)benzoate (246mg) and 1,3-bis(methoxycarbonyl)-S-methyl isothiourea (371mg) there was obtained the title compound (183mg) as an off-

white solid m.p. 226-228 m/e 357 (M+). Anal. Calculated for $C_{17}H_{15}N_3O_4S$: C, 57.13; H, 4.23; N 11.75. Found: C, 56.58; H, 4.31; N 11.86.

Example 11

Methyl [5(6)-(4-methoxycarbonylphenylthio)-1*H*-benzimidazol-2yl]carbamate from methyl 4-(3,4-diaminophenylthio)benzoate (830mg) and 1,3-bis(methoxycarbonyl)-S-methyl isothiourea (1.25g) there was obtained the title compound (694mg) as an off-white solid m.p. 280-282 m/e 357 (M+). Anal. Calculated for $C_{17}H_{15}N_3O_4S$: C, 57.13; H, 4.23; N 11.75. Found: C, 57.21; H, 4.31; N 11.73.

Example 12

Methyl [5(6)-(4-hydroxyphenylthio)-1*H*-benzimidazol-2yl]carbamate from 4–(4–hydroxyphenylthio)–1,2–phenylenediamine (4g) and 1,3-bis(methoxycarbonyl)-S-methyl isothiourea (6g) there was obtained the title compound (2.4g) as a white solid m.p. 202–204°C. m/e 315 (M^+). ^{1}H–NMR (400 MHz, d6–DMSO) δ 3.74 (s, 3H,), 6.76 and 7.2 (AA'BB', 4H, J = 8.6 Hz), 7.03 (dd, 1H, J = 1.7, 6.6 Hz,), 7.29 (d, 1H, J = 1.4 Hz,), 7.34 (d, 1H, J = 8.3 Hz,), 9.68 (b, 1H,), 11.67 (b, 2H) ppm.

Example 13

Methyl [5(6)-(4-(acetylamino)phenoxy)-1*H*-benzimidazol-2yl]carbamate from 4–(4-(acetylamino)phenoxy)–1,2–phenylenediamine (1.02g) and 1,3-bis(methoxycarbonyl)-S-methyl isothiourea (2.67g) there was obtained the title compound (0.93g) as a white solid m.p. 304-306°C m/e 340 (M^+). Anal. Calculated for $C_{17}H_{16}N_4O_4$: C, 60.00; H, 4.74; N 16.46. Found: C, 60.03; H, 4.72; N 16.42.

Prepared in an analogous fashion to Example 3 was:

Example 14

Methyl [5(6)-(4-phosphonooxyphenoxy)-1*H*-benzimidazol-2yl]carbamate from methyl [5(6)-(4-(di-*tert*-butylphosphonooxy)phenoxy)-1*H*-benzimidazol-2yl]carbamate

(200mg) there was obtained the title compound (140mg) as a white solid m.p. 272-275°C.

Example 15

Methyl [5(6)-(4-hydroxy-α-hydroxyiminobenzyl)-1*H*-benzimidazol-2yl]carbamate

[5(6)-(4-hydroxybenzoyl)-1*H*-benzimidazol-2yl]carbamate (100 mg, 0.32 mmol) was added to a solution of hydroxylamine (0.8 mmol) in MeOH (20 mL, prepared by treatment of hydroxylamine hydrochloride (0.64 g, 1.6 mmol) and NaOH (0.14g, 1.6 mmol) followed by filtration). The solution was heated at 70°C for 36 h, cooled and water (30 ml) added. The solution was filtered and washed with ether and the solid triturated with methanol to yield the title compound (40 mg) as a white solid; mp 288–290°C.

The following known compounds were prepared by literature methods:

Example 16: Methyl [5(6)-(4-(acetylamino)phenoxy)-1*H*-benzimidazol-2yl]carbamate

Example 17: Methyl [5(6)-(4-aminophenoxy)-1*H*-benzimidazol-2yl]carbamate

Example 18: Methyl [5(6)-(3-aminophenoxy)-1*H*-benzimidazol-2yl]carbamate

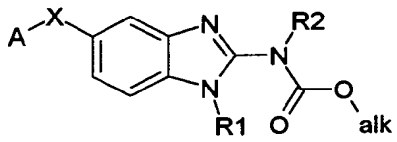
Example 19: [5(6)-(4-hydroxybenzoyl)-1*H*-benzimidazol-2yl]carbamate

Example 20: [5(6)-(2-hydroxybenzoyl)-1*H*-benzimidazol-2yl]carbamate

Example 21: [5(6)-(3-hydroxybenzoyl)-1*H*-benzimidazol-2yl]carbamate

CLAIMS

1. Use of vascular damaging agents in the preparation of compositions for the treatment of diseases involving angiogenesis, characterised in that the agents are 5(6)-substituted benzimidazole-2-carbamates of formula:



I

wherein

Alk is an alkyl group

X is oxygen, sulphur, sulphinyl, sulphonyl, carbonyl (CO), thiocarbonyl (CS) , sulphonyloxy, NH, iminomethylene (C=NH), N-hydroxyiminomethylene, N-alkoxyiminomethylene, dialkoxymethylene, 1,3-dioxolan-2yl, 1,1-ethenyl, a group CHR^3 or a bond

R^1 is hydrogen, alkylaminocarbonyl or alkoxycarbonyl

R^2 is hydrogen, alkoxycarbonyl, cyanomethyl, cyanoethyl, alkoxymethyl or acetoxymethyl.

R^3 is hydrogen, hydroxy, alkoxy or amino

A is an optionally substituted aromatic, optionally substituted heteroaromatic, optionally substituted heterocycloalkyl, optionally substituted alkyl or optionally substituted cycloalkyl group

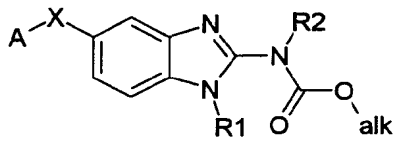
and the pharmaceutically acceptable salts, solvates and hydrates thereof.

2. A use according to claim 1 wherein the substituent on A is selected from (a) alkyl substituted by one or more of hydroxy, amino, alkylamino, dialkylamino, halogen, carboxyl, SO_3H, sulphate, phosphate, alkoxycarbonyl, aralkoxycarbonyl, alkoxycarbonylamino, aminoalkylaminocarbonyl and cyano; or halogen, hydroxy,

amino, alkoxy, alkylthio, cyano, nitro, sulphate, isothiocyanate, aryl, heteroaryl or heterocycloalkyl; or (b) a group Y selected from

phosphate, alkylphosphate, $C(O)R^4$, $OC(O)R^4$, SO_2R^4, $NHC(O)R^4$, $NR^5C(O)R^4$, SR^4, $S(O)R^4$, OSO_2R^4, $NHSO_2R^4$, $NR^5SO_2R^4$, SO_3H, CO_2H and CO_2R^5 where R^4 is selected from hydrogen, R^5, OR^5, NHR^5, NR^5R^6, aryl, heteroaryl or heterocycloalkyl such aryl, heteroaryl or heterocycloalkyl groups being optionally substituted with one or more substituents selected from alkyl, heterocycloalkyl, haloalkyl, hydroxy, nitro, cyano, amino, alkylamino, dialkylamino, halogen, carboxyl, SO_3H, sulphate and phosphate wherein R^5 and R^6, which may be the same or different, are each an alkyl group optionally substituted with one or more substituents selected from hydroxy, amino, alkylamino, dialkylamino, guanidino, halogen, carboxyl, SO_3H, sulphate, phosphate, aryl and heteroaryl.

3. A 5(6)-substituted benzimidazole–2-carbamate of formula



I

wherein

Alk is an alkyl group

X is oxygen, sulphur, sulphinyl, sulphonyl, carbonyl (CO), thiocarbonyl (CS) , sulphonyloxy, NH, iminomethylene (C=NH), N-hydroxyiminomethylene, N-alkoxyiminomethylene, dialkoxymethylene, 1,3-dioxolan-2yl, 1,1-ethenyl, a group CHR^3 or a bond

R^1 is hydrogen, alkylaminocarbonyl or alkoxycarbonyl

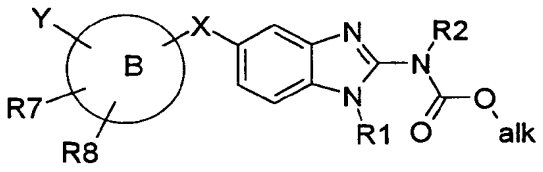
R^2 is hydrogen, alkoxycarbonyl, cyanomethyl, cyanoethyl, alkoxymethyl or acetoxymethyl.

R^3 is hydrogen, hydroxy, alkoxy or amino

A is an optionally substituted aromatic, optionally substituted heteroaromatic, optionally substituted heterocycloalkyl, optionally substituted alkyl or optionally substituted cycloalkyl group

in which at least one substituent is Y selected from phosphate, alkylphosphate, $C(O)R^4$, $OC(O)R^4$, SO_2R^4, $NHC(O)R^4$, $NR^5C(O)R^4$, SR^4, $S(O)R^4$, OSO_2R^4, $NHSO_2R^4$, $NR^5SO_2R^4$, SO_3H, CO_2H and CO_2R^5 where R^4 is selected from hydrogen, R^5, OR^5, NHR^5, NR^5R^6, aryl, heteroaryl or heterocycloalkyl such aryl, heteroaryl or heterocycloalkyl groups being optionally substituted with one or more substituents selected from alkyl, heterocycloalkyl, haloalkyl, hydroxy, nitro, cyano, amino, alkylamino, dialkylamino, halogen, carboxyl, SO_3H, sulphate and phosphate wherein R^5 and R^6, which may be the same or different, are each an alkyl group optionally substituted with one or more substituents selected from hydroxy, amino, alkylamino, dialkylamino, guanidino, halogen, carboxyl, SO_3H, sulphate, phosphate, aryl and heteroaryl and the pharmaceutically acceptable salts, solvates and hydrates thereof.

4. A carbamate according to claim 3 of formula:



II

wherein

alk is an alkyl group

B is an aromatic or heteroaromatic ring

X is oxygen, sulphur, sulphinyl, sulphonyl, carbonyl (CO), thiocarbonyl (CS), sulphonyloxy, NH, iminomethylene (C=NH), N-hydroxyiminomethylene, N-alkoxyiminomethylene, dialkoxymethylene, 1,3-dioxolan-2yl, 1,1-ethenyl, a group CHR^3 or a bond

R^1 is hydrogen, alkylaminocarbonyl or alkoxycarbonyl

R^2 is hydrogen, alkoxycarbonyl, cyanomethyl, cyanoethyl, alkoxymethyl or acetoxymethyl

R^3 is hydrogen, hydroxy, alkoxy or amino

Y is as hereinbefore defined

R^7 and R^8 are each independently H, alkyl, halogen, hydroxy, amino, alkylamino, dialkylamino, alkoxy, alkylthio, cyano, nitro, or trifluoromethyl

with the proviso that Y is not NHC(O)Me and when B is a thiophene ring then Y is not $C(O)CF_3$ and when B is a 5(6)-benzimidazole ring then Y is not $NHCO_2Me$ or $NHCO_2Et$

and the pharmaceutically acceptable salts, solvates, hydrates and prodrugs thereof.

5. A carbamate according to claim 4 in which Y is a phosphate group.

6. A carbamate according to claim 4 in which Y is $NHC(O)R^4$ wherein R^4 is a 1-aminoalkyl group.

7. A composition for treatment of diseases involving angiogenesis comprising at least one 5(6)-substituted benzimidazole-2-carbamate of formula I as defined in claim 1 in an amount sufficient to damage new vasculature.

8. A composition according to claim 7, wherein the carbamate is as defined in claim 2.

9. A composition according to claim 7, wherein the carbamate has a formula II as defined in claim 3.

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(71) Applicants *(for all designated States except US)*: ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB). ZENECA PHARMA S.A. [FR/FR]; "Le Galien", 1, rue des Chauffours, Boîte postale 127, F-95022 Cergy Cédex (FR).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: THOMAS, Andrew, Peter [GB/GB]; Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). HENNEQUIN, Laurent, François, André [FR/FR]; Zeneca Pharma S.A., Centre de Recherches Z.I. La Pompelle, Chemin de Vrilly, Boîte postale 1050, F-51689 Reims Cédex (FR). JOHNSTONE, Craig [GB/GB]; Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).

(74) Agent: MACK, John, Richard; Zeneca Pharmaceuticals, Intellectual Property Dept., Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).

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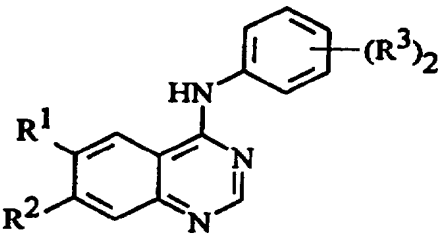
(57) Abstract

The invention relates to quinazoline derivatives of formula (I) (wherein: R^1 represents hydrogen or methoxy; R^2 represents methoxy, ethoxy, 2-methoxyethoxy, 3-methoxypropoxy, 2-ethoxyethoxy, trifluoromethoxy, 2,2,2-trifluoroethoxy, 2-hydroxyethoxy, 3-hydroxypropoxy, 2-(N,N-dimethylamino)ethoxy, 3-(N,N-dimethylamino)propoxy, 2-morpholinoethoxy, 3-morpholinopropoxy, 4-morpholinobutoxy, 2-piperidinoethoxy, 3-piperidinopropoxy, 4-piperidinobutoxy, 2-(piperazin-1-yl)ethoxy, 3-(piperazin-1-yl)propoxy, 4-(piperazin-1-yl)butoxy, 2-(4-methylpiperazin-1-yl)ethoxy, 3-(4-methylpiperazin-1-yl)propoxy or 4-(4-methylpiperazin-1-yl)butoxy; the phenyl group bearing $(R^3)_2$ is selected from: 2-fluoro-5-hydroxyphenyl, 4-bromo-2-fluorophenyl, 2,4-difluorophenyl, 4-chloro-2-fluorophenyl, 2-fluoro-4-methylphenyl, 2-fluoro-4-methoxyphenyl, 4-bromo-3-hydroxyphenyl, 4-fluoro-3-hydroxyphenyl, 4-chloro-3-hydroxyphenyl, 3-hydroxy-4-methylphenyl, 3-hydroxy-4-methoxyphenyl and 4-cyano-2-fluorophenyl); and salts thereof, processes for their preparation and pharmaceutical compositions containing a compound of formula (I) or a pharmaceutically acceptable salt thereof as active ingredient. The compounds of formula (I) and the pharmaceutically acceptable salts thereof inhibit the effects of VEGF, a property of value in the treatment of a number of disease states including cancer and rheumatoid arthritis.



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4-ANILINOQUINAZOLINE DERIVATIVES

The present invention relates to quinazoline derivatives, processes for their preparation, pharmaceutical compositions conta ining them as active ingredient, methods for the treatment of disease states associated with angiogenesis and/or increased vascular permeability, to their use as medicaments and to their use in the manufacture of medicaments for use in the production of antiangiogenic and/or vascular permeability reducing effects in warm-blooded animals such as humans.

Normal angiogenesis plays an important role in a variety of processes including embryonic development, wound healing and several components of female reproductive function. Undesirable or pathological angiogenesis has been associated with disease states including diabetic retinopathy, psoriasis, cancer, rheumatoid arthritis, atheroma, Kaposi's sarcoma and haemangioma (Fan et al, 1995, Trends Pharmacol. Sci. 16: 57-66; Folkman, 1995, Nature Medicine 1: 27-31). Alteration of vascular permeability is thought to play a role in both normal and pathological physiological processes (Cullinan-Bove et al, 1993, Endocrinology 133: 829-837; Senger et al, 1993, Cancer and Metastasis Reviews, 12: 303-324). Several polypeptides with in vitro endothelial cell growth promoting activity have been identified including, acidic and basic fibroblast growth factors (aFGF & bFGF) and vascular endothelial growth factor (VEGF). By virtue of the restricted expression of its receptors, the growth factor activity of VEGF, in contrast to that of the FGFs, is relatively specific towards endothelial cells. Recent evidence indicates that VEGF is an important stimulator of both normal and pathological angiogenesis (Jakeman et al, 1993, Endocrinology, 133: 848-859; Kolch et al, 1995, Breast Cancer Research and Treatment, 36:139-155) and vascular permeability (Connolly et al, 1989, J. Biol. Chem. 264: 20017-20024). Antagonism of VEGF action by sequestration of VEGF with antibody can result in inhibition of tumour growth (Kim et al, 1993, Nature 362: 841-844).

Receptor tyrosine kinases (RTKs) are important in the transmission of biochemical signals across the plasma membrane of cells. These transmembrane molecules characteristically consist of an extracellular ligand-binding domain connected through a segment in the plasma membrane to an intracellular tyrosine kinase domain. .Binding of ligand to the receptor results in stimulation of the receptor-associated tyrosine kinase activity which

leads to phosphorylation of tyrosine residues on both the receptor and other intracellular molecules. These changes in tyrosine phosphorylation initiate a signalling cascade leading to a variety of cellular responses. To date, at least nineteen distinct RTK subfamilies, defined by amino acid sequence homology, have been identified. One of these subfamilies is presently comprised by the fms-like tyrosine kinase receptor, Flt or Flt1, the kinase insert domain-containing receptor, KDR (also referred to as Flk-1), and another fms-like tyrosine kinase receptor, Flt4. Two of these related RTKs, Flt and KDR, have been shown to bind VEGF with high affinity (De Vries et al, 1992, Science 255: 989-991; Terman et al, 1992, Biochem. Biophys. Res. Comm. 1992, 187: 1579-1586). Binding of VEGF to these receptors expressed in heterologous cells has been associated with changes in the tyrosine phosphorylation status of cellular proteins and calcium fluxes.

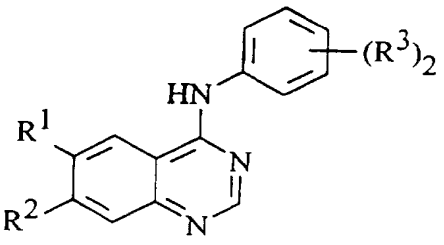
European Patent Publication No. 0326330 discloses certain quinoline, quinazoline and cinnoline plant fungicides. Certain of these plant fungicides are also stated to possess insecticidal and miticidal activity. There is however no disclosure or any suggestion that any of the compounds disclosed may be used for any purpose in animals such as humans. In particular, the European Patent Publication contains no teaching whatsoever concerning angiogenesis and/or increased vascular permeability mediated by growth factors such as VEGF.

European Patent Publication No. 0566226 describes compounds having activity against epidermal growth factor (EGF) receptor tyrosine kinase. Whilst the compounds of the present invention fall within the broad general disclosure of EP 0566226, we have found, surprisingly, that the compounds of the present invention possess very good inhibitory activity against VEGF, a property nowhere disclosed in EP 0566226. Moreover compounds of EP 0566226, outside the scope of the present invention, which have been tested, do not show significant inhibtory activity against VEGF receptor tyrosine kinase.

The present invention is thus based on the discovery of compounds that surprisingly inhibit the effects of VEGF, a property of value in the treatment of disease states associated with angiogenesis and/or increased vascular permeability such as cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation and ocular diseases with retinal vessel proliferation. Compounds of the present invention possess higher potency

against VEGF receptor tyrosine kinase whilst possessing some activity against EGF receptor tyrosine kinase. Furthermore, compounds of the present invention, possess substantially higher potency against VEGF receptor tyrosine kinase than against EGF receptor tyrosine kinase or FGF R1 receptor tyrosine kinase.

According to one aspect of the present invention there is provided a quinazoline derivative of the formula I:



(I)

(wherein:

R^1 represents hydrogen or methoxy;

R^2 represents methoxy, ethoxy, 2-methoxyethoxy, 3-methoxypropoxy, 2-ethoxyethoxy, trifluoromethoxy, 2,2,2-trifluoroethoxy, 2-hydroxyethoxy, 3-hydroxypropoxy, 2-(N,N-dimethylamino)ethoxy, 3-(N,N-dimethylamino)propoxy, 2-morpholinoethoxy, 3-morpholinopropoxy, 4-morpholinobutoxy, 2-piperidinoethoxy, 3-piperidinopropoxy, 4-piperidinobutoxy, 2-(piperazin-1-yl)ethoxy, 3-(piperazin-1-yl)propoxy, 4-(piperazin-1-yl)butoxy, 2-(4-methylpiperazin-1-yl)ethoxy, 3-(4-methylpiperazin-1-yl)propoxy or 4-(4-methylpiperazin-1-yl)butoxy;

the phenyl group bearing $(R^3)_2$ is selected from: 2-fluoro-5-hydroxyphenyl, 4-bromo-2-fluorophenyl, 2,4-difluorophenyl, 4-chloro-2-fluorophenyl, 2-fluoro-4-methylphenyl, 2-fluoro-4-methoxyphenyl, 4-bromo-3-hydroxyphenyl, 4-fluoro-3-hydroxyphenyl, 4-chloro-3-hydroxyphenyl, 3-hydroxy-4-methylphenyl, 3-hydroxy-4-methoxyphenyl and 4-cyano-2-fluorophenyl);

and salts thereof.

R^1 is preferably methoxy.

Advantageously R^2 represents methoxy, ethoxy, 2-methoxyethoxy, 3-methoxypropoxy, trifluoromethoxy, 2,2,2-trifluoroethoxy, 2-hydroxyethoxy, 3-hydroxypropoxy, 2-(N,N-dimethylamino)ethoxy, 3-(N,N-dimethylamino)propoxy, 2-morpholinoethoxy, 3-morpholinopropoxy, 2-piperidinoethoxy, 3-piperidinopropoxy, 2-(piperazin-1-yl)ethoxy or 3-(piperazin-1-yl)propoxy. Further advantageous values of R^2 are 2-(4-methylpiperazin-1-yl)ethoxy and 3-(4-methylpiperazin-1-yl)propoxy.

Preferably R^2 is methoxy, ethoxy, 2-methoxyethoxy, 3-methoxypropoxy, 2,2,2-trifluoroethoxy, 2-hydroxyethoxy, 3-hydroxypropoxy, 2-(N,N-dimethylamino)ethoxy, 3-(N,N-dimethylamino)propoxy, 2-morpholinoethoxy, 3-morpholinopropoxy, 2-piperidinoethoxy, 3-piperidinopropoxy, 2-(piperazin-1-yl)ethoxy or 3-(piperazin-1-yl)propoxy. Additional preferred values of R^2 are 2-(4-methylpiperazin-1-yl)ethoxy and 3-(4-methylpiperazin-1-yl)propoxy.

More preferably R^2 is 2-methoxyethoxy, 2-hydroxyethoxy, 3-(N,N-dimethylamino)propoxy, 2-morpholinoethoxy, 3-morpholinopropoxy or 3-(piperazin-1-yl)propoxy, and additional more preferred values of R^2 are 2-(4-methylpiperazin-1-yl)ethoxy and 3-(4-methylpiperazin-1-yl)propoxy.

Particularly preferred values of R^2 are 2-methoxyethoxy, 2-morpholinoethoxy, 3-morpholinopropoxy and 2-(4-methylpiperazin-1-yl)ethoxy.

Especially preferred values of R^2 are 2-methoxyethoxy and 3-morpholinopropoxy.

In a particular aspect of the invention the phenyl group bearing $(R^3)_2$ is selected from: 2-fluoro-5-hydroxyphenyl, 4-bromo-2-fluorophenyl, 2,4-difluorophenyl, 4-chloro-2-fluorophenyl, 2-fluoro-4-methylphenyl, 2-fluoro-4-methoxyphenyl, 4-bromo-3-hydroxyphenyl, 4-fluoro-3-hydroxyphenyl, 4-chloro-3-hydroxyphenyl, 3-hydroxy-4-methylphenyl and 3-hydroxy-4-methoxyphenyl.

The phenyl group bearing $(R^3)_2$ is preferably 3-hydroxy-4-methylphenyl or 4-chloro-2-fluorophenyl especially 4-chloro-2-fluorophenyl. A further especially preferred value for the phenyl group bearing $(R^3)_2$ is 4-bromo-2-fluorophenyl.

Preferred compounds are

4-(4-chloro-2-fluoroanilino)-7-(2-methoxyethoxy)quinazoline,

6,7-dimethoxy-4-(2-fluoro-5-hydroxyanilino)quinazoline,

4-(4-chloro-3-hydroxyanilino)-6,7-dimethoxyquinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6,7-dimethoxyquinazoline,
4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-7-(3-hydroxypropoxy)-6-methoxyquinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline
and salts thereof especially hydrochloride salts thereof,
and other preferred compounds are
4-(4-bromo-2-fluoroanilino)-6,7-dimethoxyquinazoline,
4-(2-fluoro-4-methylanilino)-6,7-dimethoxyquinazoline,
6,7-dimethoxy-4-(3-hydroxy-4-methylanilino)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,
4-(2-fluoro-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,
4-(3-hydroxy-4-methylanilino)-7-(2-methoxyethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-7-(3-morpholinopropoxy)quinazoline,
4-(4-cyano-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-methoxypropoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-ethoxyethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-7-(2-hydroxyethoxy)-6-methoxyquinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-morpholinobutoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(4-methylpiperazin-1yl)propoxy)quinazoline
and salts thereof especially hydrochloride salts thereof.
More preferred compounds are
4-(4-chloro-2-fluoroanilino)-7-(2-methoxyethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6,7-dimethoxyquinazoline,

4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-7-(3-hydroxypropoxy)-6-methoxyquinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,
4-(2-fluoro-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,
4-(3-hydroxy-4-methylanilino)-7-(2-methoxyethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-7-(3-morpholinopropoxy)quinazoline,
4-(4-cyano-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-methoxypropoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-ethoxyethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-7-(2-hydroxyethoxy)-6-methoxyquinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-morpholinobutoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(4-methylpiperazin-1yl)propoxy)quinazoline
and salts thereof especially hydrochloride salts thereof.

Particularly preferred compounds are

4-(4-chloro-2-fluoroanilino)-7-(2-methoxyethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6,7-dimethoxyquinazoline,
4-(4-chloro-2-fluoroanilino)-7-(3-hydroxypropoxy)-6-methoxyquinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline
and salts thereof especially hydrochloride salts thereof, other particularly preferred compounds are

4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,

4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline
and salts thereof especially hydrochloride salts thereof.

More particularly preferred compounds are
4-(4-chloro-2-fluoroanilino)-7-(2-methoxyethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline.
4-(4-chloro-2-fluoroanilino)-7-(3-hydroxypropoxy)-6-methoxyquinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline.
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline
and salts thereof especially hydrochloride salts thereof.

Especially preferred compounds are
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline.
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline.
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline
and salts thereof especially hydrochloride salts thereof.

More especially preferred compounds are
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline.
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,

4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline and salts thereof especially hydrochloride salts thereof, of which 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline, 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline and salts thereof especially hydrochloride salts thereof are preferred.

For the avoidance of doubt it is to be understood that where in this specification a group is qualified by 'hereinbefore defined' or 'defined hereinbefore' the said group encompasses the first occurring and broadest definition as well as each and all of the preferred definitions for that group.

In this specification the term "alkyl" includes both straight and branched chain alkyl groups but references to individual alkyl groups such as "propyl" are specific for the straight chain version only. An analogous convention applies to other generic terms. Unless otherwise stated the term "alkyl" advantageously refers to chains with 1-6 carbon atoms, preferably 1-4 carbon atoms.

In this specification the term "alkoxy" means an alkyl group as defined hereinbefore linked to an oxygen atom.

In this specification the term "aryl" includes C_{6-10}aromatic groups which may, if desired, carry one or more substituents selected from halogeno, alkyl, alkoxy, cyano, nitro or trifluoromethyl (wherein alkyl and alkoxy are as hereinbefore defined). The term "aryloxy" means an aryl group as defined hereinbefore linked to an oxygen atom.

In this specification the term "sulphonyloxy" includes alkylsulphonyloxy and arylsulphonyloxy wherein "alkyl" and "aryl" are as defined hereinbefore.

In formula I, as hereinbefore defined, hydrogen will be present at positions 2, 5 and 8 of the quinazoline group.

Within the present invention it is to be understood that a quinazoline of the formula I or a salt thereof may exhibit the phenomenon of tautomerism and that the formulae drawings within this specification can represent only one of the possible tautomeric forms. It is to be understood that the invention encompasses any tautomeric form which inhibits VEGF receptor tyrosine kinase activity and is not to be limited merely to any one tautomeric form utilised within the formulae drawings..

It is also to be understood that certain quinazolines of the formula I and salts thereof can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms which inhibit VEGF receptor tyrosine kinase activity.

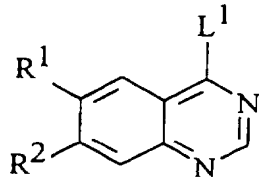
The present invention relates to the compounds of formula I as hereinbefore defined as well as to the salts thereof. Salts for use in pharmaceutical compositions will be pharmaceutically acceptable salts, but other salts may be useful in the production of the compounds of formula I and their pharmaceutically acceptable salts. Pharmaceutically acceptable salts of the invention may, for example, include acid addition salts of the compounds of formula I as hereinbefore defined which are sufficiently basic to form such salts. Such acid addition salts include for example salts with inorganic or organic acids affording pharmaceutically acceptable anions such as with hydrogen halides (especially hydrochloric or hydrobromic acid of which hydrochloric acid is particularly preferred) or with sulphuric or phosphoric acid, or with trifluoroacetic, citric or maleic acid. In addition where the compounds of formula I are sufficiently acidic, pharmaceutically acceptable salts may be formed with an inorganic or organic base which affords a pharmaceutically acceptable cation. Such salts with inorganic or organic bases include for example an alkali metal salt, such as a sodium or potassium salt, an alkaline earth metal salt such as a calcium or magnesium salt, an ammonium salt or for example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

A compound of the formula I, or salt thereof, and other compounds of the invention (as hereinafter defined) may be prepared by any process known to be applicable to the preparation of chemically-related compounds. Such processes include, for example, those illustrated in European Patent Applications, Publication Nos. 0520722, 0566226, 0602851 and 0635498. Such processes, are provided as a further feature of the invention and are as described hereinafter. Necessary starting materials may be obtained by standard procedures of organic chemistry. The preparation of such starting materials is described within the accompanying non-limiting Examples. Alternatively necessary starting materials are obtainable by analogous procedures to those illustrated which are within the ordinary skill of an organic chemist.

Thus the following processes (a) to (e) and (i) to (v) constitute further features of the present invention.

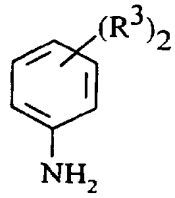
Synthesis of Compounds of Formula I

(a) Compounds of the formula I and salts thereof may be prepared by the reaction of a compound of the formula III:



(III)

(wherein R^1 and R^2 are as defined hereinbefore and L^1 is a displaceable moiety), with a compound of the formula IV:



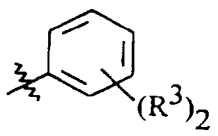
(IV)

(wherein R^3 is as defined hereinbefore) whereby to obtain compounds of the formula I and salts thereof. A convenient displaceable moiety L^1 is, for example, a halogeno, alkoxy (preferably C_{1-4}alkoxy), aryloxy or sulphonyloxy group, for example a chloro, bromo, methoxy, phenoxy, methanesulphonyloxy or toluene-4-sulphonyloxy group.

The reaction is advantageously effected in the presence of either an acid or a base. Such an acid is, for example, an anhydrous inorganic acid such as hydrogen chloride. Such a base is, for example, an organic amine base such as, for example, pyridine, 2,6-lutidine, collidine, 4-dimethylaminopyridine, triethylamine, morpholine, N-methylmorpholine or diazabicyclo[5.4.0]undec-7-ene, or for example, an alkali metal or alkaline earth metal carbonate or hydroxide, for example sodium carbonate, potassium carbonate, calcium carbonate, sodium hydroxide or potassium hydroxide. Alternatively such a base is, for example, an alkali metal hydride, for example sodium hydride, or an alkali metal or alkaline earth metal amide, for example sodium amide or sodium bis(trimethylsilyl)amide. The reaction is preferably effected in the presence of an inert solvent or diluent, for example an alkanol or ester such as methanol, ethanol, isopropanol or ethyl acetate, a halogenated solvent such as methylene chloride, trichloromethane or carbon tetrachloride, an ether such as tetrahydrofuran or 1,4-dioxan, an aromatic hydrocarbon solvent such as toluene, or a dipolar aprotic solvent such as N,N-dimethylformamide, N,N-dimethylacetamide, N-methylpyrrolidin-2-one or dimethylsulphoxide. The reaction is conveniently effected at a temperature in the range, for example, 10 to 150°C, preferably in the range 20 to 80°C.

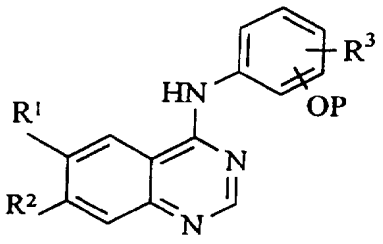
The compound of the invention may be obtained from this process in the form of the free base or alternatively it may be obtained in the form of a salt with the acid of the formula H-L^1 wherein L^1 has the meaning defined hereinbefore. When it is desired to obtain the free base from the salt, the salt may be treated with a base as defined hereinbefore using a conventional procedure.

(b) Where the group of formula II:



(II)

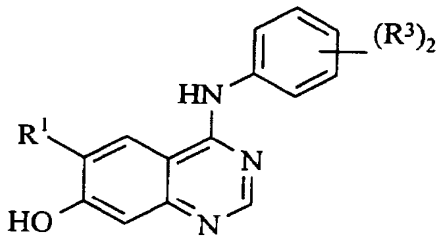
(wherein R^3 is as hereinbefore defined) represents a phenyl group carrying a hydroxy group, a compound of the formula I and salts thereof can be prepared by the deprotection of a compound of formula V:



(V)

(wherein R^1, R^2 and R^3 are as hereinbefore defined and P represents a phenolic hydroxy protecting group). The choice of phenolic hydroxy protecting group P is within the standard knowledge of an organic chemist, for example those included in standard texts such as "Protective Groups in Organic Synthesis" T.W. Greene and R.G.M.Wuts, 2nd Ed. Wiley 1991, including ethers (for example, methyl, methoxymethyl, allyl and benzyl), silyl ethers (for example, t-butyldiphenylsilyl and t-butyldimethylsilyl), esters (for example, acetate and benzoate) and carbonates (for example, methyl and benzyl). The removal of such a phenolic hydroxy protecting group may be effected by any of the procedures known for such a transformation, including those reaction conditions indicated in standard texts such as that indicated hereinbefore, or by a related procedure. The reaction conditions preferably being such that the hydroxy derivative is produced without unwanted reactions at other sites within the starting or product compounds. For example, where the protecting group P is acetate, the transformation may conveniently be effected by treatment of the quinazoline derivative with a base as defined hereinbefore and including ammonia, and its mono and di-alkylated derivatives, preferably in the presence of a protic solvent or co-solvent such as water or an alcohol, for example methanol or ethanol. Such a reaction can be effected in the presence of an additional inert solvent or diluent as defined hereinbefore and at a temperature in the range 0 to 50°C, conveniently at about 20°C.

(c) Production of compounds of formula I and salts thereof can be achieved by the reaction, conveniently in the presence of a base as defined hereinbefore, of a compound of the formula VI:



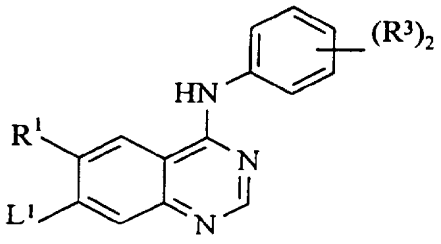
(VI)

(wherein R^1 and R^3 are as hereinbefore defined) with a compound of formula VII:

$$R^4\text{-}L^1 \qquad \text{(VII)}$$

(wherein L^1 is as hereinbefore defined and R^4 is methyl, ethyl, 2-methoxyethyl, 3-methoxypropyl, 2-ethoxyethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 2-hydroxyethyl, 3-hydroxypropyl, 2-(N,N-dimethylamino)ethyl, 3-(N,N-dimethylamino)propyl, 2-morpholinoethyl, 3-morpholinopropyl, 4-morpholinobutyl, 2-piperidinoethyl, 3-piperidinopropyl, 4-piperidinobutyl, 2-(piperazin-1-yl)ethyl, 3-(piperazin-1-yl)propyl, 4-(piperazin-1-yl)butyl, 2-(4-methylpiperazin-1-yl)ethyl, 3-(4-methylpiperazin-1-yl)propyl or 4-(4-methylpiperazin-1-yl)butyl); L^1 is a displaceable moiety for example a halogeno or sulphonyloxy group such as a bromo or methanesulphonyloxy group. The reaction is preferably effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), advantageously at a temperature in the range, for example 10 to 150°C, conveniently at about 50°C.

(d) Compounds of the formula I and salts thereof may be prepared by the reaction of a compound of the formula VIII:



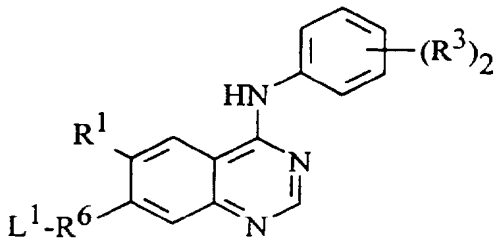
(VIII)

with a compound of the formula IX:

$$R^2\text{-}H \qquad \text{(IX)}$$

(wherein L^1, R^1, R^2 and R^3 are all as hereinbefore defined). The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), advantageously at a temperature in the range, for example 10 to 150°C, conveniently at about 100°C.

(e) Compounds of the formula I and salts thereof wherein R^2 is R^5C_{1-4}alkoxy, in particular R^5C_{1-3}alkoxy, (wherein R^5 is selected from methoxy, ethoxy, hydroxy, N,N-dimethylamino, morpholino, piperidino, piperazin-1-yl or 4-methylpiperazin-1-yl) may be prepared by reacting a compound of the formula X:



(X)

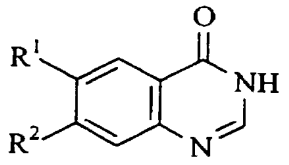
(wherein L^1, R^1 and R^3 are as hereinbefore defined and R^6 is C_{1-4}alkoxy, in particular C_{1-3}alkoxy) with a compound of the formula XI:

R^5-H (XI)

(wherein R^5 is as defined hereinbefore) to give a compound of the formula I. The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), and at a temperature in the range, for example 0 to 150°C, conveniently at about 50°C.

Synthesis of Intermediates

(i) Compounds of formula III and salts thereof constitute a further feature of the present invention. Such compounds in which L^1 is halogeno may for example be prepared by halogenating a compound of the formula XII:

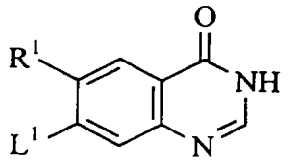


(XII)

(wherein R^1 and R^2 are as hereinbefore defined).

Convenient halogenating agents include inorganic acid halides, for example thionyl chloride, phosphorus(III)chloride, phosphorus(V)oxychloride and phosphorus(V)chloride. The halogenation reaction is conveniently effected in the presence of an inert solvent or diluent such as for example a halogenated solvent such as methylene chloride, trichloromethane or carbon tetrachloride, or an aromatic hydrocarbon solvent such as benzene or toluene. The reaction is conveniently effected at a temperature in the range, for example 10 to 150°C, preferably in the range 40 to 100°C.

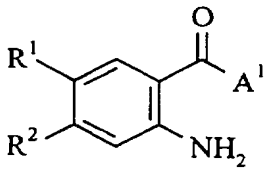
The compounds of formula XII and salts thereof which constitute a further feature of the present invention may for example be prepared by reacting a compound of the formula XIII:



(XIII)

(wherein R^1 and L^1 are as hereinbefore defined) with a compound of the formula IX as hereinbefore defined. The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), advantageously at a temperature in the range, for example 10 to 150°C, conveniently at about 100°C.

The compounds of formula XII and salts thereof may also be prepared by cyclising a compound of the formula XIV:

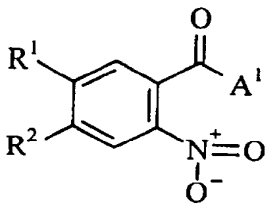


(XIV)

(wherein R^1 and R^2 are as hereinbefore defined, and A^1 is an hydroxy, alkoxy (preferably C_{1-4}alkoxy) or amino group) whereby to form a compound of formula XII or salt thereof. The cyclisation may be effected by reacting a compound of the formula XIV, where A^1 is an hydroxy or alkoxy group, with formamide or an equivalent thereof effective to cause cyclisation whereby a compound of formula XII or salt thereof is obtained, such as [3-(dimethylamino)-2-azaprop-2-enylidene]dimethylammonium chloride. The cyclisation is conveniently effected in the presence of formamide as solvent or in the presence of an inert solvent or diluent such as an ether for example 1,4-dioxan. The cyclisation is conveniently effected at an elevated temperature, preferably in the range 80 to 200°C. The compounds of formula XII may also be prepared by cyclising a compound of the formula XIV, where A^1 is an amino group, with formic acid or an equivalent thereof effective to cause cyclisation whereby a

compound of formula XII or salt thereof is obtained. Equivalents of formic acid effective to cause cyclisation include for example a tri-C_{1-4}alkoxymethane, for example triethoxymethane and trimethoxymethane. The cyclisation is conveniently effected in the presence of a catalytic amount of an anhydrous acid, such as a sulphonic acid for example p-toluenesulphonic acid, and in the presence of an inert solvent or diluent such as for example a halogenated solvent such as methylene chloride, trichloromethane or carbon tetrachloride, an ether such as diethylether or tetrahydrofuran, or an aromatic hydrocarbon solvent such as toluene. The cyclisation is conveniently effected at a temperature in the range, for example 10 to 100°C, preferably in the range 20 to 50°C.

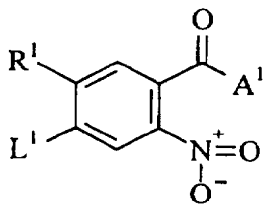
Compounds of formula XIV and salts thereof, which constitute a further feature of the present invention, may for example be prepared by the reduction of the nitro group in a compound of the formula XV:



(XV)

(wherein R^1, R^2 and A^1 are as hereinbefore defined) to yield a compound of formula XIV as hereinbefore defined. The reduction of the nitro group may conveniently be effected by any of the procedures known for such a transformation. The reduction may be carried out, for example, by the hydrogenation of a solution of the nitro compound in the presence of an inert solvent or diluent as defined hereinbefore in the presence of a metal effective to catalyse hydrogenation reactions such as palladium or platinum. A further reducing agent is, for example, an activated metal such as activated iron (produced for example by washing iron powder with a dilute solution of an acid such as hydrochloric acid). Thus, for example, the reduction may be effected by heating the nitro compound and the activated metal in the presence of a solvent or diluent such as a mixture of water and alcohol, for example methanol or ethanol, to a temperature in the range, for example 50 to 150°C, conveniently at about 70°C.

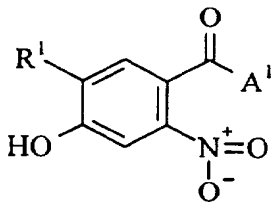
Compounds of the formula XV and salts thereof which constitute a further feature of the present invention, may for example be prepared by the reaction of a compound of the formula XVI:



(XVI)

(wherein R^1, L^1 and A^1 are as hereinbefore defined) with a compound of the formula IX as hereinbefore defined to give a compound of the formual XV. The reaction of the compounds of formulae XVI and IX is conveniently effected under conditions as described for process (d) hereinbefore.

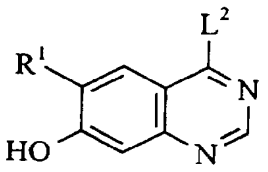
Compounds of formula XV and salts thereof, may for example also be prepared by the reaction of a compound of the formula XVII:



(XVII)

(wherein R^1 and A^1 are as hereinbefore defined) with a compound of the formula VII as hereinbefore defined to yield a compound of formula XV as hereinbefore defined. The reaction of the compounds of formulae XVII and VII is conveniently effected under conditions as described for process (c) hereinbefore.

The compounds of formula III and salts thereof may also be prepared for example by reacting a compound of the formula XVIII:

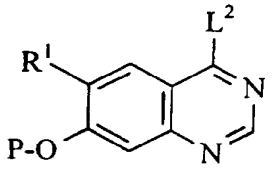


(XVIII)

(wherein R^1 is as hereinbefore defined and L^2 represents a displaceable protecting moiety) with a compound of the formula VII as hereinbefore defined, whereby to obtain a compound of formula III in which L^1 is represented by L^2.

A compound of formula XVIII is conveniently used in which L^2 represents a phenoxy group which may if desired carry up to 5 substituents, preferably up to 2 substituents, selected from halogeno, nitro and cyano. The reaction may be conveniently effected under conditions as described for process (c) hereinbefore.

The compounds of formula XVIII and salts thereof as hereinbefore defined may for example be prepared by deprotecting a compound of the formula XIX:



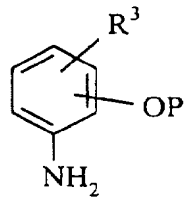
(XIX)

(wherein R^1, P and L^2 are as hereinbefore defined). Deprotection may be effected by techniques well known in the literature, for example where P represents a benzyl group deprotection may be effected by hydrogenolysis or by treatment with trifluoroacetic acid.

One compound of formula III may if desired be converted into another compound of formula III in which the moiety L^1 is different. Thus for example a compound of formula III in which L^1 is other than halogeno, for example optionally substituted phenoxy, may be converted to a compound of formula III in which L^1 is halogeno by hydrolysis of a compound of formula III (in which L^1 is other than halogeno) to yield a compound of formula XII as hereinbefore

defined, followed by introduction of halide to the compound of formula XII, thus obtained as hereinbefore defined, to yield a compound of formula III in which L^1 represents halogen.

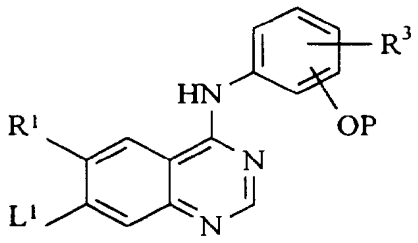
(ii) The compounds of formula V and salts thereof, constitute a further feature of the present invention, and may for example be prepared by the reaction of a compound of formula III as hereinbefore defined with a compound of the formula XX:



(XX)

(wherein R^3 and P are as hereinbefore defined). The reaction may for example be effected as described for process (a) hereinbefore.

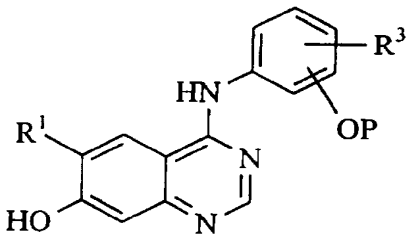
The compounds of formula V and salts thereof may also be prepared by reacting a compound of formula XXI:



(XXI)

(wherein R^1, L^1, R^3 and P are as hereinbefore defined) with a compound of formula IX as hereinbefore defined. The reaction may for example be effected as described for process (d) above.

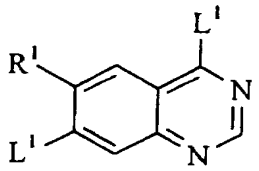
The compounds of formula V and salts thereof may also be prepared by reacting a compound of formula XXII:



(XXII)

(wherein R^1, R^3 and P are as hereinbefore defined) with a compound of the formula VII as hereinbefore defined. The reaction may for example be effected as described for process (c) hereinbefore.

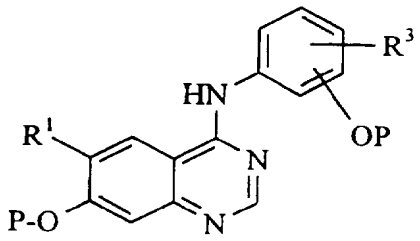
The compounds of formula XXI and salts thereof may for example be prepared by reaction of a compound of formula XXIII:



(XXIII)

(wherein R^1 and L^1 are as hereinbefore defined, and L^1 in the 4- and 7- positions may be the same or different) with a compound of the formula XX as hereinbefore defined. The reaction may be effected for example by a process as described in (a) above.

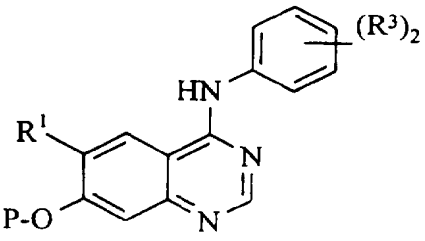
Compounds of the formula XXII and salts thereof may be made by reacting compounds of the formulae XIX and XX as hereinbefore defined, under conditions described in (a) hereinbefore, to give a compound of formula XXIV:



(XXIV)

(wherein R^1, R^3 and P are as hereinbefore defined) and then deprotecting the compound of formula XXIV for example as described in (i) above.

(iii) Compounds of the formula VI as hereinbefore defined and salts thereof may be made by deprotecting the compound of formula XXV:



(XXV)

(wherein R^1, R^3 and P are as hereinbefore defined) by a process for example as described in (i) above.

Compounds of the formula XXV and salts thereof may be made by reacting compounds of the formulae XIX and IV as hereinbefore defined, under the conditions described in (a) hereinbefore, to give a compound of the formula XXV or salt thereof.

(iv) Compounds of the formula VIII and salts thereof as hereinbefore defined may be made by reacting compounds of the formulae XXIII and IV as hereinbefore defined, the reaction for example being effected by a process as described in (a) above.

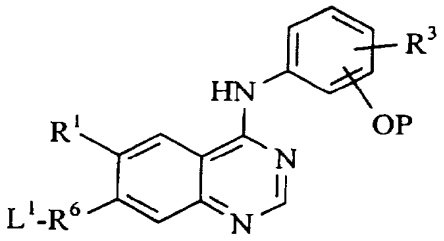
(v) Compounds of the formula X as defined hereinbefore and salts thereof may for example be made by the reaction of a compound of formula VI as defined hereinbefore with a compound of the formula XXVI:

L^1-R^6-L^1

(XXVI)

(wherein L^1 and R^6 are as hereinbefore defined) to give a compound of the formula X. The reaction may be effected for example by a process as described in (c) above.

Compounds of the formula X and salts thereof may also be made for example by deprotecting a compound of the formula XXVII:



(XXVII)

(wherein L^1, R^6, R^1, R^3 and P are as defined hereinbefore) by a process for example as described in (b) above.

Compounds of the formula XXVII and salts thereof may be made for example by reacting compounds of the formulae XXII and XXVI as defined hereinbefore, under the conditions described in (c) above.

When a pharmaceutically acceptable salt of a compound of the formula I is required, it may be obtained, for example, by reaction of said compound with, for example, an acid using a conventional procedure, the acid having a pharmaceutically acceptable anion.

Many of the intermediates defined herein are novel, for example, those of the formulae III, V, XII, XIV and XV, and these are provided as a further feature of the invention.

Intermediates of the formulae VI, VIII, X, XXI, XXII, XXIV, XXV and XXVII are also provided as a further feature of the invention.

The identification of compounds which potently inhibit the tyrosine kinase activity associated with the VEGF receptors such as Flt and/or KDR and which inhibit angiogenesis and/or increased vascular permeability is desirable and is the subject of the present invention.

These properties may be assessed, for example, using one or more of the procedures set out below:

(a) In Vitro Receptor Tyrosine Kinase Inhibition Test

This assay determines the ability of a test compound to inhibit tyrosine kinase activity. DNA encoding VEGF or epidermal growth factor (EGF) receptor cytoplasmic domains may be obtained by total gene synthesis (Edwards M, International Biotechnology Lab 5(3), 19-25, 1987) or by cloning. These may then be expressed in a suitable expression system to obtain polypeptide with tyrosine kinase activity. For example VEGF and EGF receptor cytoplasmic domains, which were obtained by expression of recombinant protein in insect cells, were found to display intrinsic tyrosine kinase activity. In the case of the VEGF receptor Flt (Genbank accession number X51602), a 1.7kb DNA fragment encoding most of the cytoplasmic domain, commencing with methionine 783 and including the termination codon, described by Shibuya et al (Oncogene, 1990, 5: 519-524), was isolated from cDNA and cloned into a baculovirus transplacement vector (for example pAcYM1 (see The Baculovirus Expression System: A Laboratory Guide, L.A. King and R. D. Possee, Chapman and Hall, 1992) or pAc360 or pBlueBacHis (available from Invitrogen Corporation)). This recombinant construct was co-transfected into insect cells (for example Spodoptera frugiperda 21(Sf21)) with viral DNA (eg Pharmingen BaculoGold) to prepare recombinant baculovirus. (Details of the methods for the assembly of recombinant DNA molecules and the preparation and use of recombinant baculovirus can be found in standard texts for example Sambrook et al, 1989, Molecular cloning - A Laboratory Manual, 2nd edition, Cold Spring Harbour Laboratory Press and O'Reilly et al, 1992, Baculovirus Expression Vectors - A Laboratory Manual, W. H. Freeman and Co, New York). For other tyrosine kinases for use in assays, cytoplasmic fragments starting from methionine 806 (KDR, Genbank accession number L04947) and methionine 668 (EGF receptor, Genbank accession number X00588) may be cloned and expressed in a similar manner.

For expression of cFlt tyrosine kinase activity, Sf21 cells were infected with plaque-pure cFlt recombinant virus at a multiplicity of infection of 3 and harvested 48 hours later. Harvested cells were washed with ice cold phosphate buffered saline solution (PBS) (10mM sodium phosphate pH7.4, 138mM NaCl, 2.7mM KCl) then resuspended in ice cold

HNTG/PMSF (20mM Hepes pH7.5, 150mM NaCl, 10% v/v glycerol, 1% v/v Triton X100, 1.5mM $MgCl_2$, 1mM ethylene glycol-bis(βaminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), 1mM PMSF (phenylmethylsulphonyl fluoride); the PMSF is added just before use from a freshly-prepared 100mM solution in methanol) using 1ml HNTG/PMSF per 10 million cells. The suspension was centrifuged for 10 minutes at 13,000 rpm at 4°C, the supernatant (enzyme stock) was removed and stored in aliquots at -70°C. Each new batch of stock enzyme was titrated in the assay by dilution with enzyme diluent (100mM Hepes pH 7.4, 0.2mM Na_3VO_4, 0.1% v/v Triton X100, 0.2mM dithiothreitol). For a typical batch, stock enzyme is diluted 1 in 2000 with enzyme diluent and 50µl of dilute enzyme is used for each assay well.

A stock of substrate solution was prepared from a random copolymer containing tyrosine, for example Poly (Glu, Ala, Tyr) 6:3:1 (Sigma P3899), stored as 1 mg/ml stock in PBS at -20°C and diluted 1 in 500 with PBS for plate coating.

On the day before the assay 100µl of diluted substrate solution was dispensed into all wells of assay plates (Nunc maxisorp 96-well immunoplates) which were sealed and left overnight at 4°C.

On the day of the assay the substrate solution was discarded and the assay plate wells were washed once with PBST (PBS containing 0.05% v/v Tween 20) and once with 50mM Hepes pH7.4.

Test compounds were diluted with 10% dimethylsulphoxide (DMSO) and 25µl of diluted compound was transferred to wells in the washed assay plates. "Total" control wells contained 10% DMSO instead of compound. Twenty five microlitres of 40mM $MnCl_2$ containing 8µM adenosine-5'-triphosphate (ATP) was added to all test wells except "blank" control wells which contained $MnCl_2$ without ATP. To start the reactions 50µl of freshly diluted enzyme was added to each well and the plates were incubated at room temperature for 20 minutes. The liquid was then discarded and the wells were washed twice with PBST. One hundred microlitres of mouse IgG anti-phosphotyrosine antibody (Upstate Biotechnology Inc. product 05-321), diluted 1 in 6000 with PBST containing 0.5% w/v bovine serum albumin (BSA), was added to each well and the plates were incubated for 1 hour at room temperature before discarding the liquid and washing the wells twice with PBST. One hundred microlitres of horse radish peroxidase (HRP)-linked sheep anti-mouse Ig antibody (Amersham product NXA 931), diluted 1 in 500 with PBST containing 0.5% w/v BSA, was added and the plates

were incubated for 1 hour at room temperature before discarding the liquid and washing the wells twice with PBST. One hundred microlitres of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) solution, freshly prepared using one 50mg ABTS tablet (Boehringer 1204 521) in 50ml freshly prepared 50mM phosphate-citrate buffer pH5.0 + 0.03% sodium perborate (made with 1 phosphate citrate buffer with sodium perborate (PCSB) capsule (Sigma P4922) per 100ml distilled water), was added to each well. Plates were then incubated for 20-60 minutes at room temperature until the optical density value of the "total" control wells, measured at 405nm using a plate reading spectrophotometer, was approximately 1.0. "Blank" (no ATP) and "total" (no compound) control values were used to determine the dilution range of test compound which gave 50% inhibtion of enzyme activity.

(b) In Vitro HUVEC Proliferation Assay

This assay determines the ability of a test compound to inhibit the growth factor-stimulated proliferation of human umbilical vein endothelial cells (HUVEC).

HUVEC cells were isolated in MCDB 131 (Gibco BRL) + 7.5% v/v foetal calf serum (FCS) and were plated out (at passage 2 to 8), in MCDB 131 + 2% v/v FCS + 3μg/ml heparin + 1μg/ml hydrocortisone, at a concentration of 1000 cells/well in 96 well plates. After a minimum of 4 hours they were dosed with the appropriate growth factor (i.e. VEGF 3ng/ml, EGF 3ng/ml or b-FGF 0.3ng/ml) and compound. The cultures were then incubated for 4 days at 37°C with 7.5% CO_2. On day 4 the cultures were pulsed with 1μCi/well of tritiated-thymidine (Amersham product TRA 61) and incubated for 4 hours. The cells were harvested using a 96-well plate harvester (Tomtek) and then assayed for incorporation of tritium with a Beta plate counter. Incorporation of radioactivity into cells, expressed as cpm, was used to measure inhibition of growth factor-stimulated cell proliferation by compounds.

(c) In Vivo Rat Uterine Oedema Assay

This test measures the capacity of compounds to reduce the acute increase in uterine weight in rats which occurs in the first 4-6 hours following oestrogen stimulation. This early increase in uterine weight has long been known to be due to oedema caused by increased permeability of the uterine vasculature and recently Cullinan-Bove and Koos (Endocrinology, 1993,133:829-837) demonstrated a close temporal relationship with increased expression of

VEGF mRNA in the uterus. We have found that prior treatment of the rats with a neutralising monoclonal antibody to VEGF significantly reduces the acute increase in uterine weight, confirming that the increase in weight is substantially mediated by VEGF.

Groups of 20 to 22-day old rats were treated with a single subcutaneous dose of oestradiol benzoate (2.5μg/rat) in a solvent, or solvent only. The latter served as unstimulated controls. Test compounds were orally administered at various times prior to the administration of oestradiol benzoate. Five hours after the administration of oestradiol benzoate the rats were humanely sacrificed and their uteri were dissected, blotted and weighed. The increase in uterine weight in groups treated with test compound and oestradiol benzoate and with oestradiol benzoate alone was compared using a Student T test. Inhibition of the effect of oestradiol benzoate was considered significant when $p<0.05$.

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises a compound of the formula I as defined hereinbefore or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable excipient or carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) for example as a sterile solution, suspension or emulsion, for topical administration for example as an ointment or cream or for rectal administration for example as a suppository. In general the above compositions may be prepared in a conventional manner using conventional excipients.

The compositions of the present invention are advantageously presented in unit dosage form. The compound will normally be administered to a warm-blooded animal at a unit dose within the range 5-5000mg per square metre body area of the animal, i.e. approximately 0.1-100mg/kg. A unit dose in the range, for example, 1-100mg/kg, preferably 1-50mg/kg is envisaged and this normally provides a therapeutically-effective dose. A unit dose form such as a tablet or capsule will usually contain, for example 1-250mg of active ingredient.

According to a further aspect of the present invention there is provided a compound of the formula I or a pharmaceutically acceptable salt thereof as defined hereinbefore for use in a method of treatment of the human or animal body by therapy.

We have found that compounds of the present invention inhibit VEGF receptor tyrosine kinase activity and are therefore of interest for their antiangiogenic effects and/or their ability to cause a reduction in vascular permeability.

A further feature of the present invention is a compound of formula I, or a pharmaceutically acceptable salt thereof, for use as a medicament, conveniently a compound of formula I, or a pharmaceutically acceptable salt thereof, for use as a medicament for producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal such as a human being.

Thus according to a further aspect of the invention there is provided the use of a compound of the formula I, or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for use in the production of an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal such as a human being.

According to a further feature of the invention there is provided a method for producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal, such as a human being, in need of such treatment which comprises administering to said animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof as defined hereinbefore.

As stated above the size of the dose required for the therapeutic or prophylactic treatment of a particular disease state will necessarily be varied depending on the host treated, the route of administration and the severity of the illness being treated. Preferably a daily dose in the range of 1-50mg/kg is employed. However the daily dose will necessarily be varied depending upon the host treated, the particular route of administration, and the severity of the illness being treated. Accordingly the optimum dosage may be determined by the practitioner who is treating any particular patient.

The antiangiogenic and/or vascular permeability reducing treatment defined hereinbefore may be applied as a sole therapy or may involve, in addition to a compound of the invention, one or more other substances and/or treatments. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate administration of the individual components of the treatment. In the field of medical oncology it is normal practice to use a combination of different forms of treatment to treat each patient with cancer. In medical oncology the other component(s) of such conjoint treatment in addition to the

antiangiogenic and/or vascular permeability reducing treatment defined hereinbefore may be: surgery, radiotherapy or chemotherapy. Such chemotherapy may cover three main categories of therapeutic agent:

(i) other antiangiogenic agents that work by different mechanisms from those defined hereinbefore (for example linomide, inhibitors of integrin $\alpha v\beta 3$ function, angiostatin, razoxin, thalidomide);

(ii) cytostatic agents such as antioestrogens (for example tamoxifen,toremifene, raloxifene, droloxifene, iodoxyfene), progestogens (for example megestrol acetate), aromatase inhibitors (for example anastrozole, letrazole, vorazole, exemestane), antiprogestogens, antiandrogens (for example flutamide, nilutamide, bicalutamide, cyproterone acetate), LHRH agonists and antagonists (for example goserelin acetate, luprolide), inhibitors of testosterone 5α-dihydroreductase (for example finasteride), anti-invasion agents (for example metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator receptor function) and inhibitors of growth factor function, (such growth factors include for example platelet derived growth factor and hepatocyte growth factor such inhibitors include growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors and serine/threonine kinase inhibitors); and

(iii) antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as antimetabolites (for example antifolates like methotrexate, fluoropyrimidines like 5-fluorouracil, purine and adenosine analogues, cytosine arabinoside); antitumour antibiotics (for example anthracyclines like doxorubicin, daunomycin, epirubicin and idarubicin, mitomycin-C, dactinomycin, mithramycin); platinum derivatives (for example cisplatin, carboplatin); alkylating agents (for example nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide, nitrosoureas, thiotepa); antimitotic agents (for example vinca alkaloids like vincrisitine and taxoids like taxol, taxotere); topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan).

As stated above the compounds defined in the present invention are of interest for their antiangiogenic and/or vascular permeability reducing effects. Such compounds of the invention may therefore be useful in a wide range of disease states including cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic

nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation and ocular diseases with retinal vessel proliferation. In particular such compounds of the invention are expected to slow advantageously the growth of primary and recurrent solid tumours of, for example, the colon, breast, prostate, lungs and skin. More particularly such compounds of the invention are expected to inhibit the growth of those primary and recurrent solid tumours which are associated with VEGF, especially those tumours which are significantly dependent on VEGF for their growth and spread, including for example, certain tumours of the colon, breast, prostate, lung, vulva and skin.

Furthermore compounds of the present invention may be particularly useful in any of the disease states listed above such as cancer in which VEGF is a significant contributing factor to angiogenesis and in which EGF is contributing less than VEGF to angiogenesis.

In addition to their use in therapeutic medicine, the compounds of formula I and their pharmaceutically acceptable salts are also useful as pharmacological tools in the development and standardisation of in vitro and in vivo test systems for the evaluation of the effects of inhibitors of VEGF receptor tyrosine kinase activity in laboratory animals such as cats, dogs, rabbits, monkeys, rats and mice, as part of the search for new therapeutic agents.

It is to be understood that where the term "ether" is used anywhere in this specification it refers to diethyl ether.

The invention will now be illustrated in the following non-limiting Examples in which, unless otherwise stated:-

[(i) evaporations were carried out by rotary evaporation in vacuo and work-up procedures were carried out after removal of residual solids such as drying agents by filtration;

(ii) operations were carried out at ambient temperature, that is in the range 18-25°C and under an atmosphere of an inert gas such as argon;

(iii) column chromatography (by the flash procedure) and medium pressure liquid chromatography (MPLC) were performed on Merck Kieselgel silica (Art. 9385) or Merck Lichroprep RP-18 (Art. 9303) reversed-phase silica obtained from E. Merck, Darmstadt, Germany;

(iv) yields are given for illustration only and are not necessarily the maximum attainable;

(v) melting points are uncorrected and were determined using a Mettler SP62 automatic melting point apparatus, an oil-bath apparatus or a Koffler hot plate apparatus.

(vi) the structures of the end-products of the formula I were confirmed by nuclear (generally proton) magnetic resonance (NMR) and mass spectral techniques; proton magnetic resonance chemical shift values were measured on the delta scale and peak multiplicities are shown as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; q, quartet;

(vii) intermediates were not generally fully characterised and purity was assessed by thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), infra-red (IR) or NMR analysis;

(viii) the following abbreviations have been used:-

DMF N,N-dimethylformamide

DMSO dimethylsulphoxide

THF tetrahydrofuran

NMP 1-methyl-2-pyrrolidinone

TFA trifluoroacetic acid.]

Example 1

A solution of 4-chloro-7-(2-methoxyethoxy)quinazoline hydrochloride (624mg, 2.27mmol) and 4-chloro-2-fluoroaniline (305µl, 2.6mmol) in isopropanol (20ml) was heated at reflux for 30 minutes. The solvent was removed by evaporation and the residue partitioned between ethyl acetate and water. The organic layer was separated, washed with aqueous sodium hydrogen carbonate solution, then with water, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was triturated with ether to give **4-(4-chloro-2-fluoroanilino)-7-(2-methoxyethoxy)quinazoline** (662mg, 84%) as a white solid.

m.p. 140-141°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.35(s, 3H); 3.74(t, 2H); 4.29(t, 2H); 7.21(s, 1H); 7.28(d, 1H); 7.35(d, 1H); 7.6(m, 2H); 8.36(d, 1H); 8.43(s, 1H); 9.75(s, 1H)

MS - ESI: 347 $[MH]^+$

Elemental analysis:	Found	C 58.49	H 4.41	N 12.08
$C_{17}H_{15}N_3O_2FCl$	Requires	C 58.70	H 4.31	N 12.08%

The starting material was prepared as follows:

A solution of 2-amino-4-fluorobenzoic acid (3g, 19.3mmol) in formamide (30ml) was heated at 150°C for 6 hours. The reaction mixture was poured onto ice/water (1/1) (250ml). The precipitated solid was collected by filtration, washed with water and dried to give 7-fluoro-3,4-dihydroquinazolin-4-one (2.6g, 82%).

Sodium (400mg, 17mmol) was added carefully to 2-methoxyethanol (10ml) and the mixture heated at reflux for 30 minutes. 7-Fluoro-3,4-dihydroquinazolin-4-one (750mg, 4.57mmol) was added to the resulting solution and the mixture heated at reflux for 15 hours. The mixture was cooled and poured into water (250ml). The mixture was acidified to pH4 with concentrated hydrochloric acid. The resulting solid product was collected by filtration, washed with water and then with ether, and dried under vacuum to give 7-(2-methoxyethoxy)-3,4-dihydroquinazolin-4-one (580mg, 58%).

A solution of 7-(2-methoxyethoxy)-3,4-dihydroquinazolin-4-one (500mg, 2.2mmol) in thionyl chloride (15ml) and DMF (0.1ml) was heated at reflux for 3 hours. The volatiles were removed by evaporation to give 4-chloro-7-(2-methoxyethoxy)quinazoline hydrochloride as a cream solid (520mg, 83%).

Example 2

4-Chloro-6,7-dimethoxyquinazoline hydrochloride (342mg, 1.3mmol), and 2-fluoro-5-hydroxyaniline (183mg, 1.4mmol) in isopropanol (10ml) were heated at reflux for 2 hours. The reaction mixture was allowed to cool, the precipitated product collected by filtration, washed with isopropanol and dried to give **6,7-dimethoxy-4-(2-fluoro-5-hydroxyanilino)quinazoline** hydrochloride (66mg, 15%) as a solid.

m.p. 219-220°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.99(s, 3H); 4.01(s, 3H); 6.81(dd, 1H); 6.90(dd, 1H); 7.20(t, 1H); 7.31(s, 1H); 8.15(s, 1H); 8.81(s, 1H); 9.72(s, 1H); 11.28(s, 1H)

MS - ESI: 316 $[MH]^+$

Elemental analysis:	Found	C 53.5	H 5.3	N 9.9
$C_{16}H_{14}N_3O_3F$ HCl $0.5H_2O$ $0.5C_3H_8O$	Requires	C 53.8	H 5.1	N 10.7%

The starting material was prepared as follows:

A mixture of 4,5-dimethoxyanthranilic acid (19.7g) and formamide (10ml) was stirred and heated at 190°C for 5 hours. The mixture was allowed to cool to approximately 80°C and water (50ml) was added. The mixture was stored at ambient temperature for 3 hours. The precipitate was isolated, washed with water and dried to give 6,7-dimethoxy-3,4-dihydroquinazolin-4-one (3.65g).

A mixture of a portion (2.06g) of the material so obtained, thionyl chloride (20ml) and DMF (1 drop) was stirred and heated at reflux for 2 hours. The volatiles were removed by evaporation to give 4-chloro-6,7-dimethoxyquinazoline hydrochloride.

4-Chloro-5-methoxycarbonyloxy-2-fluoronitrobenzene (1.2g, 4.8mmol), (as described in EP 61741 A2), and 10% palladium-on-charcoal catalyst (500mg) in ethanol (100ml) was stirred under hydrogen at 1 atmosphere pressure for 18 hours. A further batch of 10% palladium-on-charcoal catalyst (500mg) was added and the mixture stirred under hydrogen for a further 3 hours. The catalyst was removed by filtration through diatomaceous earth and the solvent removed from the filtrate by evaporation. The residue was purified by flash chromatography eluting with methylene chloride/hexane (1/4) to give 2-fluoro-5-methoxycarbonyloxyaniline (0.42g, 47%) as an oil.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.82(s, 3H); 5.33(s, 2H); 6.32(dt, 1H); 6.57(dd, 1H); 6.98(dd, 1H)

MS - ESI: 186 $[MH]^+$

Concentrated aqueous ammonia (15ml) was added to a solution of 2-fluoro-5-methoxycarbonyloxyaniline (400mg, 2.16mmol) in methanol (10ml). The mixture was stirred for 2 hours and most of the solvent was removed by evaporation. The resulting suspension was diluted with water, acidified to pH7 and extracted with ethyl acetate. The organic extracts were washed with water, dried ($MgSO_4$) and solvent removed by evaporation to give 2-fluoro-5-hydroxyaniline (200mg, 73%).

^{1}H NMR Spectrum: ($DMSOd_6$) 4.90(s, 2H); 5.84(dd, 1H); 6.17(dd, 1H); 6.65(ddd, 1H); 8.80(s, 1H)

Example 3

A mixture of 4-chloro-6,7-dimethoxyquinazoline hydrochloride (500mg, 1.916mmol), (prepared as described for the starting material in Example 2), and 4-chloro-3-

hydroxyaniline (300mg, 2.09mmol), (as described in UK patent 1427658), in isopropanol (10ml) was heated at reflux for 2 hours. The mixture was allowed to cool, the solid product collected by filtration, washed with isopropanol and dried to give **4-(4-chloro-3-hydroxyanilino)-6,7-dimethoxyquinazoline** hydrochloride (605mg, 86%).

m.p. >250°C

^{1}H NMR Spectrum: ($DMSOd_6$) 4.02(s, 3H); 4.04(s, 3H); 7.15(dd, 1H); 7.34-7.44(m, 3H); 8.28(s, 1H); 8.82(s, 1H); 10.52(s, 1H); 11.24(s, 1H)

MS: 332 $[MH]^+$

Elemental analysis:	Found	C 52.4	H 4.2	N 11.3
$C_{16}H_{14}N_3O_3Cl$ 1HCl	Requires	C 52.2	H 4.1	N 11.4%

Example 4

2-Bromoethyl methyl ether (712µl, 7.56mmol) was added dropwise to a solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (2.2g, 6.88mmol) and potassium carbonate (2.84g, 20.6mmol) in DMF (110ml). The mixture was stirred for 10 hours at 60°C then for 2 days at ambient temperature, the solvent was removed by evaporation and the crude product purified by flash chromatography eluting with ethyl acetate/petroleum ether (4/1). The resulting solid was dissolved in hot ethanol and ethanolic hydrogen chloride was added. After cooling, the resulting solid was collected by filtration, washed with ethanol and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline** hydrochloride (1.74g, 62%).

m.p. 255-257°C

^{1}H NMR Spectrum: ($DMSOd_6$; CD_3COOD) 3.36(s, 3H); 3.79(t, 2H); 4.02(s, 3H); 4.34(t, 2H); 7.33(s, 1H); 7.46(dd, 1H); 7.60-7.68(m, 2H); 8.15(s, 1H); 8.79(s, 1H)

MS - ESI: 378-380 $[MH]^+$

Elemental analysis:	Found	C 52.1	H 4.6	N 10.1
$C_{18}H_{17}N_3O_3ClF$ 1HCl	Requires	C 52.19	H 4.38	N 10.14%

The starting material was prepared as follows:

A mixture of 2-amino-4-benzyloxy-5-methoxybenzamide (J. Med. Chem. 1977, vol 20, 146-149, 10g, 0.04mol) and Gold's reagent (7.4g, 0.05mol) in dioxane (100ml) was stirred

and heated at reflux for 24 hours. Sodium acetate (3.02g, 0.037mol) and acetic acid (1.65ml, 0.029mol) were added to the reaction mixture and it was heated for a further 3 hours. The volatiles were removed by evaporation, water was added to the residue, the solid was collected by filtration, washed with water and dried. Recrystallisation from acetic acid gave 7-benzyloxy-6-methoxy-3,4-dihydroquinazolin-4-one (8.7g, 84%).

A mixture of 7-benzyloxy-6-methoxy-3,4-dihydroquinazolin-4-one (2.82g, 0.01mol), thionyl chloride (40ml) and DMF (0.28ml) was stirred and heated at reflux for 1 hour. The volatiles were removed by evaporation, the residue was azeotroped with toluene to give 7-benzyloxy-4-chloro-6-methoxyquinazoline hydrochloride (3.45g).

A solution of 7-benzyloxy-4-chloro-6-methoxyquinazoline hydrochloride (1.2g, 3.5mmol) and 4-chloro-2-fluoroaniline (444μl, 4mmol) in isopropanol (40ml) was refluxed for 1.5 hours. After cooling, the precipitate was collected by filtration, washed with isopropanol then ether and dried under vacuum to give 7-benzyloxy-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (1.13g, 71%).

m.p. 239-242°C

^{1}H NMR Spectrum: ($DMSOd_6$) 4.0(s, 3H); 5.36(s, 2H); 7.39-7.52(m, 9H); 8.1(s, 1H); 8.75(s, 1H)

MS - ESI: 410 $[MH]^+$

Elemental analysis:	Found	C 59.2	H 4.3	N 9.4
$C_{22}H_{17}N_3O_2ClF$ 1HCl	Requires	C 59.21	H 4.07	N 9.41%

A solution of 7-benzyloxy-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (892mg, 2mmol) in TFA (10ml) was refluxed for 50 minutes. After cooling, the mixture was poured onto ice. The precipitate was collected by filtration, dissolved in methanol (10ml) and basified to pH11 with aqueous ammonia. After concentration by evaporation, the solid product was collected by filtration, washed with water then ether and dried under vacuum to give 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline as a yellow solid (460mg, 72%).

m.p. 141-143°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.95(s, 3H); 7.05(s, 1H); 7.35(d, 1H); 7.54-7.59(m, 2H); 7.78(s, 1H); 8.29(s, 1H)

MS - ESI: 320-322 $[MH]^+$

Example 5

A mixture of 4-chloro-6,7-dimethoxyquinazoline hydrochloride (147mg, 0.56mmol), (prepared as described for the starting material in Example 2), and 4-chloro-2-fluoroaniline (82mg, 0.56mmol) in isopropanol (7ml) was heated at reflux for 2 hours. The mixture was allowed to cool, the solid product collected by filtration, washed with isopropanol and dried to give **4-(4-chloro-2-fluoroanilino)-6,7-dimethoxyquinazoline** hydrochloride (102mg, 49%).

^{1}H NMR Spectrum: ($DMSOd_6$) 4.00(s, 6H); 7.37(s, 1H); 7.42(d, 1H); 7.60(t, 1H); 7.67(dd, 1H); 8.27(s, 1H); 8.80 (s, 1H)

MS - ESI: 334 $[MH]^+$

Example 6

4-(3-Chloropropyl)morpholine (J. Am. Chem. Soc. 1945, 67, 736, 174mg, 1.06mmol) in DMF (0.5ml) was added to a stirred suspension of 4-(3-*t*-butyldiphenylsilyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline (471 mg, 0.88mmol) and potassium carbonate (200mg, 1.45mmol) in DMF (5ml). The mixture was then heated at 100°C for 2.5 hours. The solvent was removed by evaporation, and the residues partitioned between methylene chloride and water. The product was extracted with methylene chloride and the combined extracts passed through phase separating paper. The solvent was removed by evaporation to give a yellow oil. This oil was dissolved in THF (4ml) and tetra-*n*-butylammonium fluoride (2ml of a 1M solution in THF, 2mmol) added. The mixture was stirred at ambient temperature for 72 hours, the solvent was removed by evaporation and the residue partitioned between methylene chloride and saturated aqueous sodium hydrogen carbonate solution. The aqueous phase was extracted with methylene chloride (3x20ml), the combined extracts passed through phase separating paper and the solvent removed by evaporation. The residue was purified by flash chromatography eluting with methanol/methylene chloride (1/9) to give **4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline** as a pale yellow solid (225mg, 60% over two steps).

^{1}H NMR Spectrum : ($DMSOd_6$) 2.0(m, 2H); 2.15(s, 3H); 2.4(m, 4H); 3.6(t, 4H); 3.95(s, 3H); 4.20(t, 2H); 7.05(s, 2H); 7.15(s, 1H); 7.35(s, 1H); 7.85(s, 1H); 8.40(s, 1H); 9.25(s, 2H)

MS - ESI: 425 $[MH]^+$

The starting material was prepared as follows:

A mixture of 7-benzyloxy-6-methoxy-3,4-dihydroquinazolin-4-one (5.18g, 18.4mmol), (prepared as described for the starting material in Example 4), DMF (1ml) and thionyl chloride (70ml) was heated at reflux under argon for 2 hours. The mixture was allowed to cool, excess thionyl chloride was removed by evaporation and the residue azeotroped to dryness with toluene. The resulting crude 7-benzyloxy-4-chloro-6-methoxyquinazoline hydrochloride was suspended in isopropanol (50ml) and 3-hydroxy-4-methylaniline (2.60g, 21.1mmol) added. The mixture was heated at reflux for 3 hours and then allowed to cool. The precipitated product was collected by filtration, washed with isopropanol and dried to give 7-benzyloxy-4-(3-hydroxy-4-methylanilino)-6-methoxyquinazoline (4.7g, 60%).

^{1}H NMR Spectrum : ($DMSOd_6$) 2.15 (s, 3H); 4.0(s, 3H); 5.35(s, 2H); 6.95(dd, 1H); 7.15(m, 2H); 7.35-7.55(m, 5H); 8.25(s, 1H); 8.75(s, 1H); 9.6(s, 1H); 11.2(s, 1H)

Imidazole (1.45g, 21.6mmol) was added to 7-benzyloxy-4-(3-hydroxy-4-methylanilino)-6-methoxyquinazoline (4.11g, 9.69mmol) in DMF (50ml) and the mixture was stirred at ambient temperature until complete dissolution was achieved. *t*-Butyldiphenylsilyl chloride (2.5ml, 9.6mmol) was added dropwise and the reaction mixture stirred at ambient temperature for 72 hours. Saturated aqueous sodium hydrogen carbonate solution was added, and the product was extracted with methylene chloride. The solvent was removed by evaporation to give a damp solid which was dissolved in a mixture of DMF (40ml), methanol (40ml), and ethyl acetate (40ml). 10% Palladium-on-charcoal catalyst (500mg) was added and the mixture stirred under hydrogen at 1 atmosphere pressure for 36 hours. The catalyst was removed by filtration through diatomaceous earth, and the solvent removed from the filtrate by evaporation. The crude product was purified by flash chromatography eluting with methanol/methylene chloride (1/9) to give 4-(3-*t*-butyldiphenylsilyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline (2.2g, 42% over two steps) as a yellow solid.

^{1}H NMR Spectrum : ($DMSOd_6$) 1.1 (s, 9H); 2.35(s, 3H); 3.90(s, 3H); 6.9(m, 2H); 7.1(d, 1H); 7.4(m, 6H); 7.5(d, 1H); 7.7(m, 5H); 7.85(s, 1H); 9.05(s, 1H); 10.2(s, 1H)

MS - ESI : 536 $[MH]^+$

Example 7

A mixture of 7-(3-benzyloxypropoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (180mg, 0.4mmol) and 5% palladium-on-charcoal catalyst (50mg) in methanol (5ml), trichloromethane (5ml) and DMF (1ml) was stirred under hydrogen at 1 atmosphere pressure for 2 hours. The catalyst was removed by filtration through diatomaceous earth and the solvent removed by evaporation. The residue was partitioned between ethyl acetate and aqueous sodium hydrogen carbonate solution, the organic layer separated and dried ($MgSO_4$) and the solvent removed by evaporation. The residue was recrystallised from ethyl acetate/hexane to give **4-(4-chloro-2-fluoroanilino)-7-(3-hydroxypropoxy)-6-methoxyquinazoline** (48mg, 33%).

m.p. 199-201 °C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.92(t, 2H); 3.60(t, 2H); 3.95(s, 3H); 4.20(t, 2H); 4.55(t, 1H); 7.18(s, 1H); 7.33(dd, 1H); 7.51(dd, 1H); 7.55(td, 1H); 7.78(s, 1H); 8.38(s, 1H); 8.32(s, 1H); 9.50(s, 1H)

MS - ESI: 378 $[MH]^+$

Elemental analysis:	Found	C 57.2	H 4.6	N 11.0
$C_{18}H_{17}N_3O_3FCl$	Requires	C 57.2	H 4.5	N 11.1%

The starting material was prepared as follows:

A solution of 3-benzyloxy-1-propanol (150µl, 0.9mmol) was added to tributylphosphine (376mg, 1.9mmol) and 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (200mg, 0.63mmol), (prepared as described for the starting material in Example 4), in methylene chloride (20ml) at 5°C. To the resulting mixture 1,1'-azodicarbonyldipiperidine (480mg, 1.9mmol) was added, the mixture stirred at 5°C for 1 hour, allowed to warm to ambient temperature and stirred overnight. Ether (10ml) was added, the mixture stirred for 15 minutes and the precipitated solids removed by filtration. The volatiles were removed from the filtrate by evaporation, and the residue was partitioned between ethyl acetate and water. The organic layer was separated, dried ($MgSO_4$) and the solvent removed by evaporation.The residue was dissolved in acetone, and hydrogen chloride in ether (0.6ml of a 1M solution, 0.6mmol) added. The resulting precipitated product was collected by filtration

and dried to give 7-(3-benzyloxypropoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (90mg, 31%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.22(t, 2H); 3.74(t, 2H); 4.10(s, 3H); 4.37(t, 2H); 4.60(s, 2H); 7.34-7.56(m, 7H); 7.68(t, 1H); 7.76(dd, 1H); 8.38(s, 1H); 8.90(s, 1H); 11.73(s, 1H)

Example 8

4-(2-Chloroethyl)morpholine hydrochloride (40mg, 2.1mmol) was added to 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (63mg, 0.2mmol), (prepared as described for the starting material in Example 4), and potassium carbonate (95mg, 0.69mmol) in DMF (3ml) and the mixture heated at 100°C for 3 hours. The mixture was allowed to cool, the volatiles were removed by evaporation and the residues partitioned between water and methylene chloride. The organic phase was separated, passed through phase separating paper and the solvent was removed by evaporation. The residue was dissolved in acetone, and hydrogen chloride in ether (0.2ml of a 1M solution, 0.2mmol) was added. The precipitated product was collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline** hydrochloride (50mg, 50%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.6(m, 2H); 3.85(m, 4H); 3.95(s, 3H); 4.6(m, 2H); 7.35(m, 2H); 7.6(m, 2H); 8.05(s, 1H); 8.55(s, 1H)

MS - ESI: 433 $[MH]^+$

Elemental analysis:	Found	C 50.5	H 4.9	N 10.9
$C_{21}H_{22}N_4O_3FCl$ 2HCl	Requires	C 49.9	H 4.8	N 11.1%

Example 9

4-(3-Chloropropyl)morpholine (J. Am. Chem. Soc. 1945, 67, 736, 2.26g, 13.8mmol) was added to 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (2.21g, 6.9mmol), (prepared as described for the starting material in Example 4). and potassium carbonate (6.5g, 47mmol) in DMF (100ml). The mixture was heated at 110°C for 4 hours and then allowed to cool. The volatiles were removed by evaporation and the residue was partitioned between water and methylene chloride. The organic phase was separated, washed with brine, passed through phase separating paper and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene

chloride/methanol/ammonia (aq.) (100/8/1). The product was dissolved in acetone and isopropanol and hydrogen chloride in ether (4.8ml of a 1M solution, 4.8mmol) was added. The precipitated product was collected by filtration and washed with acetone and ether to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline** hydrochloride (2.16g, 65%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.25(m, 2H); 3.7-3.9(br s, 4H); 3.95(s, 3H); 4.25 (t, 2H); 7.2(s, 1H); 7.3(dt, 1H); 7.55(m, 2H); 7.95(s, 1H); 8.40(s, 1H); 9.85(br s, 1H); 11.0(br s, 1H)

MS - ESI: 447 $[MH]^+$

Elemental analysis:	Found	C 54.7	H 5.6	N 10.8
$C_{22}H_{24}N_4O_3FCl$ 1HCl $0.5C_3H_6O$	Requires	C 55.1	H 5.5	N 10.9%

Example 10

Concentrated aqueous ammonia (8ml) was added to a solution of 4-(3-acetoxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline (2.38g , 6mmol) in a mixture of trichloromethane (24ml) and methanol (24ml). The mixture was heated at reflux for 8 hours and the volatiles removed by evaporation. The residue was triturated with water, the resulting solid was collected by filtration and recrystallised from methylene chloride/ethanol. The product was redissolved in a mixture of methylene chloride/ethanol and a saturated solution of hydrogen chloride in ethanol was added. The solvent was partially removed by evaporation and the mixture cooled. The resulting precipitate was collected by filtration washed with ether and dried under vacuum to give **4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline** hydrochloride (1.68g, 80%).

m.p. 270°C (decomposition)

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.17(s, 3H); 3.35(s, 3H); 3.78(t. 2H); 4.00(s, 3H); 4.33(t, 2H); 6.96(d, 1H); 7.08(s, 1H); 7.16(d, 1H); 7.3(s, 1H); 8.09(s, 1H); 8.81(s, 1H)

MS - ESI: 378 $[MNa]^+$

Elemental analysis:	Found	C 58.0	H 5.9	N 10.7
$C_{19}H_{21}N_3O_4$ 1HCl	Requires	C 58.2	H 5.7	N 10.7%

The starting material was prepared as follows:

Acetic anhydride (1.9ml, 20.3mmol) was added to a mixture of 2-methyl-5-nitrophenol (2.5g, 16.3mmol) and 1M aqueous sodium hydroxide (24.5ml) at ambient temperature. The mixture was stirred for 40 minutes, the solid was removed by filtration and the filtrate extracted with ethyl acetate. The combined organic extracts were washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation to give 2-acetoxy-4-nitrotoluene (3.1g, 100%). A mixture of this material (3.1g, 15.9mmol) and 10% palladium-on-charcoal catalyst (0.12g) in ethyl acetate (50ml) was stirred at ambient temperature under hydrogen at 1 atmosphere pressure for 2 hours. The catalyst was removed by filtration through diatomaceous earth and the solvent removed from filtrate by evaporation to give 3-acetoxy-4-methylaniline (2.45g, 94%).

A mixture of 7-benzyloxy-4-chloro-6-methoxyquinazoline (2.18g, 7.25mmol), (prepared as described for the starting material in Example 4), 3-acetoxy-4-methylaniline (1.32g, 8mmol) and isopropanol (50ml) was stirred and heated at reflux for 1 hour. The mixture was cooled to ambient temperature. The precipitate was collected by filtration, washed with isopropanol and ether to give 4-(3-acetoxy-4-methylanilino)-7-benzyloxy-6-methoxyquinazoline.

A mixture of 4-(3-acetoxy-4-methylanilino)-7-benzyloxy-6-methoxyquinazoline (2.68g, 5.75mmol) and 10% palladium-on-charcoal catalyst (0.27g) in methanol (50ml), DMF (12ml) and trichloromethane (50ml) was stirred at ambient temperature under hydrogen at 1.5 atmospheres pressure for 30 minutes. The catalyst was removed by filtration through diatomaceous earth and the solvent removed from filtrate by evaporation. The residue was triturated with ether, collected by filtration and dried under vacuum at 50°C to give 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline (2.1g, 100%).

Potassium carbonate (2.2g, 16mmol) was added to a solution of 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline (1.51g, 4mmol) in DMF (30ml) and the mixture stirred for 15 minutes. 2-Bromoethyl methyl ether (667mg, 4.8mmol) was then added dropwise. The mixture was stirred for 1 hour at ambient temperature, then heated at 60°C for 17 hours and finally allowed to cool. The insoluble material was removed by filtration and the filter pad washed with DMF. The filtrate was partitioned between ethyl acetate and water, the organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting

with methylene chloride/methanol (95/5 followed by 93/7). The purified product was triturated with ether to give 4-(3-acetoxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline (1.34g, 84%) as a white powder.

m.p. 180-183°C

^{1}H NMR Spectrum: ($CDCl_3$) 2.16(s, 3H); 2.34(s, 3H); 3.47(s, 3H); 3.87(t, 2H); 3.99(s, 3H); 4.31(t, 2H); 6.98(s, 1H); 7.21(d, 1H); 7.24(d, 1H); 7.42(d, 1H); 7.50(s, 1H); 8.64(s, 1H)

MS - ESI: 420 $[MNa]^+$

Elemental analysis :	Found	C 63.1	H 6.1	N 10.4
$C_{21}H_{23}N_3O_5$	Requires	C 63.5	H 5.8	N 10.6%

Example 11

A mixture of 4-chloro-6,7-dimethoxyquinazoline hydrochloride (130mg, 0.5mmol), (prepared as described for the starting material in Example 2), and 4-bromo-2-fluoroaniline (95mg, 0.5mmol) in isopropanol (7ml) was heated at reflux for 2 hours. The mixture was allowed to cool to ambient temperature, the precipitated solid was collected by filtration, washed with isopropanol and ether and dried to give **4-(4-bromo-2-fluoroanilino)-6,7-dimethoxyquinazoline** hydrochloride (124mg, 60%) as an off-white solid.

HPLC characteristics:

Column:- 200 x 4.6 mm C18 ODS Hypersil (trade mark of Shandon) reversed phase 5μm

Solvent:- flow 1.5 ml/min.

0-3 minutes:- H_2O/CH_3CN (95/5) 0.03M triethylamine

3-17 minutes:- gradient H_2O/CH_3CN (95/5 to 5/95); constant 0.03M triethylamine

Retention time:- 13.01 minutes

Example 12

A mixture of 4-chloro-6,7-dimethoxyquinazoline hydrochloride (130mg, 0.5mmol), (prepared as described for the starting material in Example 2), and 2-fluoro-4-methylaniline (63mg, 0.5mmol) in isopropanol (7ml) was heated at reflux for 2 hours. The mixture was allowed to cool to ambient temperature, the precipitated solid was collected by filtration, washed with isopropanol and ether and dried to give **4-(2-fluoro-4-methylanilino)-6,7-dimethoxyquinazoline** hydrochloride (87mg, 50%) as an off-white solid.

HPLC characteristics:

Column - 200 x 4.6 mm C18 ODS Hypersil (trade mark of Shandon) reversed phase 5μm

Solvent - flow 1.5 ml/min.

0-3 minutes:- H_2O/CH_3CN (95/5) 0.03M triethylamine

3-17 minutes:- gradient H_2O/CH_3CN (95/5 to 5/95); constant 0.03M triethylamine

Retention time - 12.32 minutes

Example 13

A mixture of 4-chloro-6,7-dimethoxyquinazoline hydrochloride (130mg, 0.5mmol), (prepared as described for the starting material in Example 2), and 3-hydroxy-4-methylaniline (62mg, 0.5mmol) in isopropanol (7ml) was heated at reflux for 2 hours. The mixture was allowed to cool to ambient temperature, the precipitated solid was collected by filtration, washed with isopropanol and ether and dried to give **6,7-dimethoxy-4-(3-hydroxy-4-methylanilino)quinazoline** hydrochloride (98mg, 56%) as an off-white solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 2.14(s, 3H); 3.98(s, 3H); 4.00(s, 3H); 6.97(d, 1H); 7.12(s, 1H); 7.14(d, 1H); 7.38(s, 1H); 8.27(s, 1H); 8.77(s, 1H); 9.65(br s, 1H)

MS - ESI: 312 $[MH]^+$

Elemental analysis:	Found	C 59.1	H 5.4	N 11.8
$C_{17}H_{17}N_3O_3$ 1HCl	Requires	C 58.6	H 5.2	N 12.1%

Example 14

A mixture of 4-chloro-6-methoxy-7-(2-methoxyethoxy)quinazoline (107mg, 0.4mmol) and 4-bromo-2-fluoroaniline (76mg, 0.4mmol) in isopropanol (7ml) was heated at reflux for 2 hours. The mixture was allowed to cool to ambient temperature, the precipitated solid was collected by filtration, washed with isopropanol and ether and dried to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline** hydrochloride (151mg, 82%) as an off-white solid.

m.p. 200-204°C

^{1}H NMR Spectrum: ($DMSOd_6$; TFA) 3.36(s, 3H); 3.79(t, 2H); 4.02(s, 3H); 4.39(t, 2H); 7.37(s, 1H); 7.54-7.61(m, 2H); 7.81(dd, 1H); 8.16(s, 1H); 8.86(s, 1H)

MS - ESI: 422 $[MH]^-$

Elemental analysis:	Found	C 47.56	H 4.01	N 9.29
$C_{20}H_{21}N_3O_4BrF$ 0.95HCl	Requires	C 47.32	H 3.96	N 9.20%

The starting material was prepared as follows:

A mixture of ethyl 4-hydroxy-3-methoxybenzoate (9.8g, 50mmol), 2-bromoethyl methyl ether (8.46ml, 90mmol) and potassium carbonate (12.42g, 90mmol) in acetone (60ml) was heated at reflux for 30 hours. The mixture was allowed to cool and the solids removed by filtration. The volatiles were removed from the filtrate by evaporation and the residue triturated with hexane to give ethyl 3-methoxy-4-(2-methoxyethoxy)benzoate (11.3g, 89%) as a white solid.

m.p. 57-60°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.31(t, 3H); 3.29(s, 3H); 3.32(s, 3H); 3.68(m, 2H); 4.16(m, 2H); 4.28(q, 2H); 7.06(d, 1H); 7.45(d, 1H); 7.56(dd, 1H)

MS - FAB: 255 $[MH]^+$

Ethyl 3-methoxy-4-(2-methoxyethoxy)benzoate (9.5g, 37mmol) was added portionwise to stirred concentrated nitric acid (75ml) at 0°C. The mixture was allowed to warm to ambient temperature and stirred for a further 90 minutes. The mixture was diluted with water and extracted with methylene chloride, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was triturated with hexane to give ethyl 5-methoxy-4-(2-methoxyethoxy)-2-nitrobenzoate (10.6g, 95%) as an orange solid.

m.p. 68-69°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.27(t, 3H); 3.30(s, 3H); 3.69(m, 2H); 3.92(s, 3H); 4.25(m, 2H); 4.29(q, 2H); 7.30(s, 1H); 7.65(s, 1H)

MS - CI: 300 $[MH]^-$

A mixture of ethyl 5-methoxy-4-(2-methoxyethoxy)-2-nitrobenzoate (10.24g, 34mmol), cyclohexene (30ml) and 10% palladium-on-charcoal catalyst (2.0g) in methanol (150ml) was heated at reflux for 5 hours. The reaction mixture was allowed to cool and diluted with methylene chloride. The catalyst was removed by filtration and the volatiles removed from the filtrate by evaporation. The residue was recrystallised from ethyl acetate/hexane to give ethyl 2-amino-5-methoxy-4-(2-methoxyethoxy) benzoate (8.0g) as a buff solid. Formamide (80ml) was added to this product and the mixture heated at 170°C for

18 hours. About half the solvent was removed by evaporation under high vacuum and the residue was left to stand overnight. The solid product was collected by filtration, washed with ether and dried to give 6-methoxy-7-(2-methoxyethoxy)-3,4-dihydroquinazolin-4-one (5.3g, 62% over two steps) as a grey solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.35(s, 3H); 3.74(m, 2H); 3.89(s, 3H); 4.26(m, 2H); 7.15(s, 1H); 7.47(s, 1H); 7.98(s, 1H); 12.03(br s, 1H)

MS - CI: 251 $[MH]^+$

DMF (0.5ml) was added to a mixture of 6-methoxy-7-(2-methoxyethoxy)-3,4-dihydroquinazolin-4-one (5.1g, 20mmol) in thionyl chloride (50ml). The mixture was stirred and heated at reflux for 3 hours, allowed to cool and the excess thionyl chloride removed by evaporation. The residue was suspended in methylene chloride and washed with aqueous sodium hydrogen carbonate solution. The aqueous phase was extracted with methylene chloride and the combined extracts dried ($MgSO_4$). The crude product was recrystallised from methylene chloride/hexane to give 4-chloro-6-methoxy-7-(2-methoxyethoxy)quinazoline (2.8g, 51%) as a fine white solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.37(s, 3H); 3.77(m, 2H); 4.01(s, 3H); 4.37(m, 2H); 7.40(s, 1H); 7.49(s, 1H); 8.88(s, 1H)

MS - CI: 269 $[MH]^+$

Example 15

A mixture of 4-chloro-6-methoxy-7-(2-methoxyethoxy)quinazoline (107mg, 0.4mmol), (prepared as described for the starting material in Example 14), and 2-fluoro-4-methylaniline (50mg, 0.4mmol) in isopropanol (7ml) was heated at reflux for 2 hours. The mixture was allowed to cool to ambient temperature, the precipitated solid was collected by filtration, washed with isopropanol and ether and dried to give **4-(2-fluoro-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline** hydrochloride (95mg, 60%) as an off-white solid.

HPLC characteristics:

Column - 200 x 4.6 mm C18 ODS Hypersil (trade mark of Shandon) reversed phase 5μm

Solvent - flow 1.5 ml/min.

0-3 minutes:- H_2O/CH_3CN (95/5) 0.001M triethylamine

3-17 minutes:- gradient H_2O/CH_3CN (95/5 to 5/95); constant 0.001M triethylamine
Retention time:- 10.46 minutes

Example 16

A mixture of 4-chloro-7-(2-methoxyethoxy)quinazoline hydrochloride (450mg, 1.6mmol), (prepared as described for the starting material in Example 1), and 3-hydroxy-4-methylaniline (280mg, 2.27mmol) in isopropanol (20ml) was heated at reflux for 30 minutes. The solvent was removed by evaporation and the residue was triturated with isopropanol. The resulting solid was collected by filtration, washed with isopropanol and dried under vacuum to give **4-(3-hydroxy-4-methylanilino)-7-(2-methoxyethoxy)quinazoline** hydrochloride (428mg, 74%).

^{1}H NMR Spectrum : ($DMSOd_6$) 2.18 (s, 3H); 3.38 (s, 3H); 3.8 (t, 2H); 4.35 (t, 2H); 7.05 (d, 1H); 7.15 (m, 2H); 7.35 (s, 1H); 7.52 (d, 1H); 8.75 (d, 1H); 8.85 (s, 1H); 9.7 (br s, 1H)
MS - ESI : 326 $[MH]^+$

Elemental analysis:	Found	C 59.6	H 5.8	N 11.7
$C_{18}H_{19}N_3O_3$ 1HCl	Requires	C 59.7	H 5.5	N 11.6%

Example 17

A solution of 1-(2-hydroxyethyl)-4-methylpiperazine (112mg, 0.78mmol) in methylene chloride (1ml) was added to a stirred suspension of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxy-quinazoline (225mg, 0.7mmol), (prepared as described for the starting material in Example 4), and tributylphosphine (420mg, 2.1mmol) in methylene chloride (10ml). 1,1'-(Azodicarbonyl)dipiperidine (525mg, 2.1mmol) was then added in portions to the mixture. The resulting clear, pale yellow solution was stirred for 3.5 hours, then allowed to stand overnight. The reaction mixture was quenched with ether (8ml) and the precipitate was removed by filtration. The solvent was removed from the filtrate by evaporation and the residue dissolved in acetone and treated with 1M ethereal hydrogen chloride until the hydrochloride salt precipitated. The precipitate was collected by filtration, dissolved in methanol and then basified with excess triethylamine. The volatiles were removed by evaporation and the residue purified by column chromatography eluting with methylene chloride/methanol/0.88 aqueous ammonia (100/8/1). The resulting purified oil was triturated

with ether, collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline** (79mg, 25%) as a white solid.

m.p. 173-175°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.10(s, 3H); 2.3(m, 4H); 2.5(m, 4H); 2.75(t, 2H); 3.95(s, 3H); 4.25(t, 2H); 7.21(s, 1H); 7.30(dd, 1H); 7.50(d, 1H); 7.55(dd, 1H); 7.75(s, 1H); 8.30(s, 1H); 9.50(s, 1H)

MS - ESI: 446 $[MH]^+$

Elemental analysis:	Found	C 59.1	H 5.8	N 15.5
$C_{22}H_{25}N_5O_2FCl$	Requires	C 59.3	H 5.7	N 15.7%

The starting material was prepared as follows:-

2-Bromoethanol (2.36g, 19mmol) was added dropwise to a mixture of 1-methylpiperazine (1.26g, 13mmol) and potassium carbonate (5.0g, 36mmol) in absolute ethanol (150ml) and the mixture heated at reflux for 18 hours. The mixture was allowed to cool and the precipitates were removed by filtration and the solvent volatiles were removed by evaporation. The residue was treated with acetone/methylene chloride, the insolubles were removed by filtration and the solvent was removed from the filtrate by evaporation to give 1-(2-hydroxyethyl)-4-methylpiperazine (870mg, 48%) as a light brown oil.

^{1}H NMR Spectrum: ($CDCl_3$) 2.18(s, 3H); 2.3-2.7(br m, 8H); 2.56(t, 2H); 3.61(t, 2H)

MS - ESI: 145 $[MH]^+$

Example 18

A solution of 4-chloro-6-methoxy-7-(3-morpholinopropoxy)quinazoline (2.5g, 7.41mmol) and 4-bromo-2-fluoroaniline (1.55g, 8.15mmol) in DMF (20ml) was heated at 150°C for 4 hours. The mixture was diluted with ether (200ml) and the resulting precipitate collected by filtration. The solid was partitioned between methylene chloride and water and the aqueous phase was adjusted to pH8.5 with 1M aqueous sodium hydroxide solution. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by flash chromatography eluting with methylene chloride/methanol (9/1). The purified solid was dissolved in methanol and methylene chloride and 2.2M ethereal hydrogen chloride (3ml) was added. The volatiles were removed

by evaporation, the residue was resuspended in ether, collected by filtration and dried under vacuum to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline** hydrochloride (1.61g, 26%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.3-2.4 (m, 2H); 3.15 (t, 2H); 3.34 (t, 2H); 3.54 (d, 2H); 3.76 (t, 2H); 4.02 (s, 3H); 4.04 (m, 2H); 4.33 (t, 2H); 7.41 (s, 1H); 7.5-7.65 (m, 2H); 7.80 (dd, 1H); 8.20 (s, 1H); 8.9 (s, 1H)

Elemental analysis:	Found	C 46.5	H 5.0	N 9.9
$C_{22}H_{24}N_4O_3BrF$ 0.4H_2O 1.9HCl	Requires	C 46.5	H 4.7	N 9.9%

The starting material was prepared as follows:-

A mixture of 4-hydroxy-3-methoxybenzoic acid (4.5g, 26.8mmol), 3-morpholinopropyl chloride (9.5g, 58.0mmol), (prepared according to J. Am. Chem. Soc. 1945, 67, 736), potassium carbonate (8.0g, 58mmol), potassium iodide (1.0g, 0.22mmol) and DMF (80ml) was stirred and heated at 100°C for 3 hours. The solid was removed by filtration and the filtrate evaporated. The residue was dissolved in ethanol (50ml), 2M sodium hydroxide (50ml) was added and the mixture heated at 90°C for 2 hours. After partial evaporation, the mixture was acidified with concentrated hydrochloric acid, washed with ether and then subjected to purification on a Diaion (trade mark of Mitsubishi) HP20SS resin column, eluting with water and then with a gradient of methanol (0 to 25%) in hydrochloric acid (pH2). Partial evaporation of the solvents and lyophilisation gave 3-methoxy-4-(3-morpholinopropoxy)benzoic acid (8.65g, 97%).

^{1}H NMR Spectrum: ($DMSOd_6$; TFA) 2.17-2.24(m, 2H); 3.10-3.16(m, 2H); 3.30(t, 2H); 3.52(d, 2H); 3.71(t, 2H); 3.82(s, 3H); 4.01(br d, 2H); 4.14(t, 2H); 7.08(d, 1H); 7.48(d, 1H); 7.59(dd, 1H)

MS - ESI: 296 $[MH]^+$

Fuming nitric acid (1.5ml, 36.2mmol) was added slowly at 0°C to a solution of 3-methoxy-4-(3-morpholinopropoxy)benzoic acid (7.78g, 23.5mmol) in TFA (25ml). The cooling bath was removed and the reaction mixture stirred at ambient temperature for 1 hour. The TFA was removed by evaporation and ice was added to the residue. The precipitate was collected by filtration, washed with a minimum of water followed by toluene and ether. The

solid was dried under vacuum over phosphorus pentoxide to give 5-methoxy-4-(3-morpholinopropoxy)-2-nitrobenzoic acid (7.54g) which was used without further purification.
^{1}H NMR Spectrum: ($DMSOd_6$; TFA) 2.16-2.23(m, 2H); 3.10-3.17(m, 2H); 3.30(t, 2H); 3.52(d, 2H); 3.66(t, 2H); 3.93(s, 3H); 4.02(br d, 2H); 4.23(t, 2H); 7.34(s, 1H); 7.61(s, 1H)
MS - EI: 340 $[M]^+$

Thionyl chloride (15ml) and DMF (0.05ml) were added to 5-methoxy-4-(3-morpholinopropoxy)-2-nitrobenzoic acid (7.54g). The mixture was heated at 50°C for 1 hour, the excess thionyl chloride was removed by evaporation and by azeotroping with toluene (x2). The resulting solid was suspended in THF (200ml) and ammonia was bubbled through the mixture for 30 minutes. The precipitate was removed by filtration and washed with THF. After concentration of the filtrate by evaporation, the product crystallised and was collected by filtration to give 5-methoxy-4-(3-morpholinopropoxy)-2-nitrobenzamide (5.25g) as light yellow crystals which were used without further purification.
^{1}H NMR Spectrum: ($DMSOd_6$; TFA) 2.17-2.24(m, 2H); 3.11-3.18(m, 2H); 3.31(t, 2H); 3.53(d, 2H); 3.67(t, 2H); 3.93(s, 3H); 4.03(br d, 2H); 4.21(t, 2H); 7.17(s, 1H); 7.62(s, 1H)
MS - EI: 339 $[M]^+$

Concentrated hydrochloric acid (30ml) was added to a suspension of 5-methoxy-4-(3-morpholinopropoxy)-2-nitrobenzamide (5.67g) in methanol (150ml) and the mixture was heated to 60°C. When the 5-methoxy-4-(3-morpholinopropoxy)-2-nitrobenzamide had dissolved, iron powder (5.6g, 100mmol) was added in portions to the reaction mixture which was then heated for 90 minutes. After cooling, the insolubles were removed by filtration through diatomaceous earth, the volatiles were removed from the filtrate by evaporation and the residue was purified on a Diaion (trade mark of Mitsubishi) HP20SS resin column, eluting with water and then with hydrochloric acid (pH2). Concentration of the fractions by evaporation gave a precipitate which was collected by filtration and dried under vacuum over phosphorus pentoxide to give 2-amino-5-methoxy-4-(3-morpholinopropoxy)benzamide as a hydrochloride salt (4.67g, 75%) as beige crystals.
^{1}H NMR Spectrum: ($DMSOd_6$; TFA) 2.22-2.28(m, 2H); 3.12(br t, 2H); 3.29(t, 2H); 3.51(d, 2H); 3.75(t, 2H); 3.87(s, 3H); 4.00(br d, 2H); 4.12(t, 2H); 7.06(s, 1H); 7.53(s, 1H)
MS - EI: 309 $[M]^+$

A mixture of 2-amino-5-methoxy-4-(3-morpholinopropoxy)benzamide (4.57g, 12.25mmol) and Gold's reagent (2.6g, 15.89mmol) in dioxane (35ml) was heated at reflux for 5 hours. Acetic acid (0.55ml) and sodium acetate (1.0g) were added to the reaction mixture which was heated for a further 3 hours. The mixture was cooled to ambient temperature and the volatiles removed by evaporation. The residue was adjusted to pH7 with 2M sodium hydroxide and then purified on a Diaion (trade mark of Mitsubishi) HP20SS resin column, eluting with methanol (gradient of 0 to 60%) in water. Concentration of the fractions by evaporation gave a precipitate which was collected by filtration and dried under vacuum over phosphorus pentoxide to give 6-methoxy-7-(3-morpholinopropoxy)-3,4-dihydroquinazolin-4-one (3.04g, 78%) as a white solid.

^{1}H NMR Spectrum: ($CDCl_3$) 2.10(q, 2H); 2.48(m, 4H); 2.56(t, 2H); 3.72(t, 4H); 4.00(s, 3H); 4.24(t, 2H); 7.18(s, 1H); 7.60(s, 1H); 8.00(s, 1H); 10.86(br s, 1H)

MS - EI: 319 $[M]^+$

A mixture of 6-methoxy-7-(3-morpholinopropoxy)-3,4-dihydroquinazolin-4-one (638mg, 2mmol) and thionyl chloride (8ml) was heated at reflux for 30 minutes. Excess thionyl chloride was removed by evaporation and by azeotroping with toluene (x2). The residue was suspended in methylene chloride and 10% aqueous solution of sodium hydrogen carbonate was added to the mixture. The organic layer was separated, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was triturated with ether, the solid was collected by filtration, washed with ether and dried under vacuum to give 4-chloro-6-methoxy-7-(3-morpholinopropoxy)quinazoline (590mg, 87%).

^{1}H NMR Spectrum: ($CDCl_3$) 2.10-2.16(m, 2H); 2.48(br s, 4H); 2.57(t, 2H); 3.73(t, 4H); 4.05(s, 3H); 4.29(t, 2H); 7.36(s, 1H); 7.39(s, 1H); 8.86(s, 1H)

MS - ESI: 337 $[MH]^+$

Example 19

A mixture of 4-chloro-7-(3-morpholinopropoxy)quinazoline hydrochloride (238mg, 0.69 mmol) and 4-chloro-2-fluoroaniline (145mg, 1mmol) in isopropanol (5ml) was heated at reflux for 1 hour. The solvent was removed by evaporation and the residue partitioned between water and ethyl acetate and the aqueous layer adjusted to pH8 with sodium hydrogen carbonate. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the

solvent removed by evaporation. The residue was purified by flash chromatography eluting with methylene chloride/acetonitrile/methanol (60/30/10 followed by 60/20/20). The resulting semi-purified solid was repurified by chromatography on neutral alumina eluting with methylene chloride/methanol (95/5). The resulting white solid was dissolved in methylene chloride and 4M ethereal hydrogen chloride (0.5ml) was added. The volatiles were removed by evaporation, and the residue triturated by the addition of methylene chloride followed by methanol and ether. The precipitated solid was collected by filtration and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-7-(3-morpholinopropoxy)quinazoline** hydrochloride (35mg, 10%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.3(m, 2H); 3.13(t, 2H); 3.34(t, 2H); 3.52(d, 2H); 3.68(t, 2H); 4.01(d, 2H); 4.34(t, 2H); 7.37(s, 1H); 7.46(d, 1H); 7.54(dd, 1H); 7.61(t, 1H); 7.71(d, 1H); 8.71(d, 1H); 8.93(s, 1H)

MS - ESI : 417 $[MH]^+$

Elemental analysis:	Found	C 50.9	H 5.2	N 11.5
$C_{21}H_{22}N_4O_2ClF$ 0.8H_2O 1.75HCl	Requires	C 50.9	H 5.2	N 11.3%

The starting material was prepared as follows:

Sodium metal (2.2g, 95mmol) was added carefully to benzyl alcohol (50ml) at ambient temperature. The mixture was stirred for 30 minutes at ambient temperature and then heated at 80°C for 1 hour. The mixture was allowed to cool to 40°C and 7-fluoro-3,4-dihydroquinazolin-4-one (3.9g, 24mmol), (prepared as described for the starting material in Example 1), was added. The reaction mixture was then stirred and heated at 130°C for 4 hours and left to cool to ambient temperature overnight. The mixture was quenched with water, the resulting precipitate was triturated by the addition of ether (150ml), collected by filtration and dried for 4 hours at 60°C under high vacuum to give 7-benzyloxy-3,4-dihydroquinazolin-4-one (5.33g, 89%).

7-Benzyloxy-3,4-dihydroquinazolin-4-one (5.3g, 21mmol) was suspended in dry DMF (50ml) and sodium hydride (1g of a 60% suspension in mineral oil, 25mmol) was added. After hydrogen evolution had ceased, the reaction was allowed to cool to ambient temperature and then chloromethyl pivalate (4.1g, 27mmol) was added dropwise over 10 minutes. The mixture was stirred for 30 minutes then poured into aqueous citric acid solution

(pH5) (250ml) and extracted with ether (3x300ml). The combined extracts were washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The resulting solid was triturated with isohexane, collected by filtration and dried under vacuum to give 7-benzyloxy-3-methylpivaloyl-3,4-dihydroquinazolin-4-one (6.9g, 90%).

5% Palladium-on-charcoal catalyst (0.7g, 50% in water) was added to a solution of 7-benzyloxy-3-methylpivaloyl-3,4-dihydroquinazolin-4-one (6.85g, 18.7mmol) in ethyl acetate (300ml), methanol (40ml), DMF (40ml), and acetic acid (0.7ml). The mixture was vigorously stirred under hydrogen at atmospheric pressure for 4.5 hours. The catalyst was removed by filtration through diatomaceous earth, the filtrate concentrated by evaporation to about 60ml, diluted with water (300ml) and extracted with ether (3x300ml). The combined extracts were washed with brine, dried ($MgSO_4$), and the volatiles removed by evaporation. The resulting crude solid was dissolved in acetone (200ml) and acetic acid (0.2ml) and cooled to 0°C. Potassium permanganate (1.8g) was added and the mixture stirred for 10 minutes. The reaction mixture was poured into water (250ml) and ethyl acetate (250ml) was added. The precipitate was removed by filtration, the organic phase separated and the aqueous phase re-extracted with ethyl acetate (2x100ml). The combined extracts were washed with water and brine, dried ($MgSO_4$) and the volatiles removed by evaporation to give 7-hydroxy-3-methylpivaloyl-3,4-dihydroquinazolin-4-one (4.05g, 78%) as a cream solid.

7-hydroxy-3-methylpivaloyl-3,4-dihydroquinazolin-4-one (750mg, 2.7mmol) was suspended in methylene chloride (40ml) and 1-(3-hydroxypropyl)morpholine (490mg, 3.4mmol) and triphenylphosphine (890mg, 3.4mmol) were added at 5 °C. The mixture was stirred for 5 minutes at 5°C and diethyl azodicarboxylate (590mg, 3.4mmol) was added over 5 minutes. The reaction mixture was stirred at 5°C for 30 minutes then at ambient temperature for 1 hour. The reaction mixture was purified directly by column chromatography eluting with methylene chloride, then ethyl acetate, then acetonitrile/ethyl acetate (1/5), and finally acetonitrile/ethyl acetate/aqueous ammonia (50/50/0.5). The purified product was triturated with ether/isohexane and collected by filtration to give 3-methylpivaloyl-7-(3-morpholinopropoxy)-3,4-dihydroquinazolin-4-one (745mg, 68%).

A solution of 3-methylpivaloyl-7-(3-morpholinopropoxy)-3,4-dihydroquinazolin-4-one (680mg, 1.6mmol) in saturated methanolic ammonia (20ml) was stirred at 40°C for 6 hours then for 18 hours at ambient temperature. The solvent was removed by evaporation and

the residue was triturated with ether/isohexane. The resulting solid was collected by filtration to give 7-(3-morpholinopropoxy)-3,4-dihydroquinazolin-4-one (450mg, 92%) as a white solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 1.9(m, 2H); 2.35(t, 4H); 2.4(t, 2H); 3.55(t, 4H); 4.15(t, 2H); 7.05(m, 2H); 7.97(d, 1H); 8.02(s, 1H)

MS - ESI: 290 $[MH]^+$

A mixture of 7-(3-morpholinopropoxy)-3,4-dihydroquinazolin-4-one (200mg, 0.69mmol) in thionyl chloride (5ml) and DMF (0.1ml) was heated at reflux for 1 hour. The solution was diluted with toluene and the volatiles removed by evaporation. The residue was dissolved in methylene chloride and ether was added. The resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give 4-chloro-7-(3-morpholinopropoxy)quinazoline hydrochloride (238mg, 100%).

The 1-(3-hydroxypropyl)morpholine was prepared as follows:

Morpholine (94g, 1.08mol) was added dropwise to a solution of 3-bromo-1-propanol (75g, 0.54mol) in toluene (750ml) and the reaction then heated at 80°C for 4 hours. The mixture was allowed to cool to ambient temperature and the precipitated solid was removed by filtration. The volatiles were removed from the filtrate and the resulting yellow oil was purified by distillation at 0.4-0.7 mmHg to give 1-(3-hydroxypropyl)morpholine (40g, 50%) as a colourless oil.

b.p. 68-70°C (~0.5mmHg)

^{1}H NMR Spectrum: ($DMSOd_6$) 1.65-1.78(m, 2H); 2.50(t, 4H); 2.60(t, 2H); 3.68(t, 4H); 3.78(t, 2H); 4.90(br d, 1H)

Example 20

5M Isopropanolic hydrogen chloride (1.5ml) was added to a solution of 4-chloro-6-methoxy-7-(3-morpholinopropoxy)quinazoline (202mg, 0.6mmol), (prepared as described for the starting material in Example 18), and 4-cyano-2-fluoroaniline (100mg, 0.72mmol), (US patent 4,120,693), in isopropanol (5ml) heated at 50°C. The mixture was then heated at 80°C for 2 hours, allowed to cool to ambient temperature and left standing overnight. The resulting precipitate was collected by filtration and the solid was then partitioned between methylene

chloride and water and 1M aqueous sodium hydroxide solution (0.8ml) was added. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by flash chromatography eluting with methylene chloride/methanol (94/6). The purified solid was dissolved in methylene chloride and 2.2M ethereal hydrogen chloride was added. The precipitated product was collected by filtration, washed with ether and dried under vacuum to give **4-(4-cyano-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline** hydrochloride (125mg, 39%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.3-2.4(m, 2H); 3.15(t, 2H); 3.36(t, 2H); 3.54(d, 2H); 3.75(t, 2H); 4.02(d, 2H); 4.04(s, 3H); 4.34(t, 2H); 7.44(s, 1H); 7.8-7.9(m, 2H); 8.11(d, 1H); 8.25(s, 1H); 8.94(s, 1H)

Elemental analysis:	Found	C 52.7	H 5.4	N 12.9
$C_{23}H_{24}N_5O_3F$ 0.5H_2O 1.9HCl	Requires	C 52.7	H 5.3	N 13.1%
0.07 ether 0.15 methylene chloride				

Example 21

Diethyl azodicarboxylate (123µl, 0.88mmol) was added portionwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (250mg, 0.8mmol), (prepared as described for the starting material in Example 4), triphenylphosphine (228mg, 0.96mmol) and 3-methoxy-1-propanol (71mg, 0.8mmol) in methylene chloride (20ml) cooled at 0°C. The mixture was then allowed to warm to ambient temperature and stirred for 18 hours. The resulting precipitate was removed by filtration and the solvent removed from the filtrate by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol/concentrated aqueous ammonia (100/8/1). The purified oil was treated with ethereal hydrogen chloride and the volatiles were then removed by evaporation. The residue was triturated with ether and the solid collected by filtration to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-methoxypropoxy)quinazoline** hydrochloride (100mg, 32%) as a yellow solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 2.10(m, 2H); 3.25(s, 3H); 3.5(t, 2H); 4.00(s, 3H); 4.25(t, 2H); 7.4(s, 1H); 7.45(dd, 1H); 7.60(m, 2H); 8.25(s, 1H); 8.8(s, 1H); 11.5(s, 1H)

MS - ESI: 392 $[MH]^+$

Elemental analysis:	Found	C 52.7	H 4.4	N 10.1
$C_{19}H_{19}N_3O_3FCl$ 0.1H_2O 1HCl	Requires	C 53.1	H 4.7	N 9.8%

Example 22

Diethyl azodicarboxylate (123µl, 0.88mmol) was added portionwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (250mg, 0.8mmol), (prepared as described for the starting material in Example 4), triphenylphosphine (228mg, 0.96mmol) and 2-ethoxyethanol (71µl, 0.8mmol) in methylene chloride (20ml) cooled at 0°C. The mixture was then allowed to warm to ambient temperature and stirred for 18 hours. The resulting precipitate was removed by filtration and the solvent removed from the filtrate by evaporation. The residue was purified by chromatography eluting with methylene chloride/methanol (96/4). The resulting purified oil was dissolved in acetone and treated with water (80µl) then ethereal hydrogen chloride. The resulting granular solid was collected by filtration to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-ethoxyethoxy)quinazoline** hydrochloride (96mg, 31%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.15(t, 3H); 3.50(q, 2H); 3.8(t, 2H); 4.00(s, 3H); 4.30(t, 2H); 7.35(s, 1H); 7.40(dd, 1H); 7.60(dd, 1H); 7.65(dd 1H); 8.25(s, 1H); 8.8(s, 1H); 11.53(s, 1H)

MS - ESI: 392 $[MH]^+$

Elemental analysis:	Found	C 53.2	H 4.6	N 10.1
$C_{19}H_{19}N_3O_3FCl$ 1HCl	Requires	C 53.28	H 4.71	N 9.81%

Example 23

Lithium borohydride (150µl of a 2M solution in THF, 0.15mmol) was added to a solution of 4-(4-chloro-2-fluoroanilino)-7-(ethoxycarbonylmethoxy)-6-methoxyquinazoline (150mg, 0.3mmol) in THF (1ml) and the mixture stirred for 1.5 hours. The reaction mixture was quenched with aqueous ammonium chloride solution and extracted with ethyl acetate. The combined extracts were washed with water, dried ($MgSO_4$) and concentrated by evaporation. Hexane was added, the mixture was cooled and the precipitated solid was collected by filtration to give **4-(4-chloro-2-fluoroanilino)-7-(2-hydroxyethoxy)-6-methoxyquinazoline** (30mg, 23%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.82(t, 2H); 3.98(s, 3H); 4.18(t, 2H); 4.92(t, 1H); 7.20(dd, 1H); 7.54-7.63(m, 2H); 7.72(s, 1H); 7.92(s, 1H); 8.60(s, 1H)

MS - ESI: 364 $[MH]^+$

The starting material was prepared as follows:

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (3.0g, 9mmol), (prepared as described for the starting material in Example 4), ethyl bromoacetate (1.11ml, 10mmol) and potassium carbonate (2.84g, 20.6mmol) in NMP (60ml) was heated at 90°C for 3 hours. The mixture was allowed to cool, diluted with water and extracted with ethyl acetate. The combined extracts were washed with water, dried ($MgSO_4$) and concentrated by evaporation. Hexane was added, the mixture was cooled and the precipitated solid was collected by filtration to give 4-(4-chloro-2-fluoroanilino)-7-(ethoxycarbonylmethoxy)-6-methoxyquinazoline (1.75g, 48%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.20(t, 3H); 3.95(s, 3H); 4.18(q, 2H); 4.98(s, 2H); 7.08(s, 1H); 7.30(dd, 1H); 7.48-7.58(m, 2H); 7.82(s, 1H); 8.34(s, 1H); 9.57(s, 1H)

Example 24

Diethyl azodicarboxylate (209mg, 1.2mmol) was added dropwise to a suspension of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (146mg, 0.4mmol), triphenylphosphine (314mg, 1.2mmol) and 1-(2-hydroxyethyl)-4-methylpiperazine (86mg, 0.6mmol), (prepared as described for the starting material in Example 17), in methylene chloride (5ml). The mixture was stirred for 1 hour at ambient temperature and the mixture was purified by column chromatography eluting with methylene chloride/methanol (90/10 followed by 80/20). The purified product was triturated with ether, collected by filtration and dried under vacuum. The solid was dissolved in methylene chloride and 3M ethereal hydrogen chloride (0.5ml) was added. The volatiles were removed by evaporation and the resulting oil was triturated with ether. The solid was collected by filtration, washed with ether and dried under vacuum to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline** hydrochloride (100mg, 41%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD; 60°C) 2.89(s, 3H); 3.55-3.7(m, 8H); 3.74(t, 2H); 4.04(s, 3H); 4.68(t, 2H); 7.49(s, 1H); 7.55(m, 1H); 7.56(s, 1H); 7.75(d, 1H); 8.29(s, 1H); 8.84(s, 1H)

MS - EI: 490 $[M]^+$

Elemental analysis:	Found	C 43.9	H 5.1 N 11.0
$C_{22}H_{25}N_5O_2BrF$ $1H_2O$ 2.7HCl 0.2ether	Requires	C 44.0	H 5.1 N 11.3%

The starting material was prepared as follows:

A solution of 7-benzyloxy-4-chloro-6-methoxyquinazoline hydrochloride (8.35g, 24.8mmol), (prepared as described for the starting material in Example 4), and 4-bromo-2-fluoroaniline (5.65g, 29.7mmol) in isopropanol (200ml) was heated at reflux for 4 hours. The resulting precipitated solid was collected by filtration, washed with isopropanol and then ether and dried under vacuum to give 7-benzyloxy-4-(4-bromo-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (9.46g, 78%).

^{1}H NMR Spectrum: ($DMSOd_6$; CD_3COOD) 4.0(s, 3H); 5.37(s, 2H); 7.35-7.5(m, 4H); 7.52-7.62(m, 4H); 7.8(d, 1H); 8.14(9s, 1H); 8.79(s, 1H)

MS - ESI: 456 $[MH]^+$

Elemental analysis:	Found	C 54.0	H 3.7	N 8.7
$C_{22}H_{17}O_2N_3BrF$ 0.9HCl	Requires	C 54.2	H 3.7	N 8.6%

A solution of 7-benzyloxy-4-(4-bromo-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (9.4g, 19.1mmol) in TFA (90ml) was heated at reflux for 50 minutes. The mixture was allowed to cool and was poured on to ice. The resulting precipitate was collected by filtration and dissolved in methanol (70ml). The solution was adjusted to pH9-10 with concentrated aqueous ammonia solution. The mixture was concentrated to half initial volume by evaporation. The resulting precipitate was collected by filtration, washed with water and then ether, and dried under vacuum to give 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (5.66g, 82%).

^{1}H NMR Spectrum: ($DMSOd_6$; CD_3COOD) 3.95(s, 3H); 7.09(s, 1H); 7.48(s, 1H); 7.54(t, 1H); 7.64(d, 1H); 7.79(s, 1H); 8.31(s, 1H)

MS - ESI: 366 $[MH]^+$

Elemental analysis:	Found	C 49.5	H 3.1	N 11.3
$C_{15}H_{11}O_2N_3BrF$	Requires	C 49.5	H 3.0	N 11.5%

Example 25

Diethyl azodicarboxylate (209mg, 1.2mmol) was added dropwise to a suspension of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (146mg, 0.4mmol), (prepared as described for the starting material in Example 24), triphenylphosphine (314mg, 1.2mmol)

and 4-(2-hydroxyethyl)morpholine (79mg, 0.6mmol) in methylene chloride (5ml). The mixture was stirred for 1 hour at ambient temperature and purified by column flash chromatography eluting with methylene chloride/methanol (95/5 followed by 90/10) to give a white solid. The solid was dissolved in methylene chloride/methanol and 2M ethereal hydrogen chloride (0.5ml) was added. The mixture was concentrated by evaporation and the resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline** hydrochloride (155mg, 70%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.3(t, 2H); 3.6(d, 2H); 3.75(m, 2H); 3.8(m, 2H); 4.0(m, 2H); 4.03(s, 3H); 4.7(t, 2H); 7.5(s, 1H); 7.55-7.65(m, 2H); 7.8(d, 1H); 8.26(s, 1H); 8.9(s, 1H)

MS - EI: 477 $[M.]^+$

Elemental analysis:	Found	C 45.3	H 4.5	N 9.8	
$C_{21}H_{22}N_4O_3BrF$ 0.4H_2O 2.0HCl	Requires	C 45.2	H 4.5	N 10.0%	

Example 26

A solution of 7-(4-chlorobutoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (3.64g, 8.87mmol) in morpholine (70ml) was heated at 110°C for 2 hours. The mixture was allowed to cool and was partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the volatiles removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride and methanol (92/8). The purified solid product was dissolved in methylene chloride and 3M ethereal hydrogen chloride was added. The volatiles were removed by evaporation, and the residue triturated with ether. The solid was collected by filtration, washed with ether and dried under vacuum at 60°C to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-morpholinobutoxy)quinazoline** hydrochloride (3.8g, 78%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.85-2.0(m, 4H); 3.09(t, 2H); 3.2-3.3(t, 2H); 3.46(d, 2H); 3.74(t, 2H); 4.0(d, 2H); 4.03(s, 3H); 4.27(s, 2H); 7.42(s, 1H); 7.46(d, 1H); 7.63(t, 1H); 7.68(d, 1H); 8.21(s, 1H); 8.88(s, 1H)

MS - ESI: 461 $[MH]^+$

Elemental analysis:	Found	C 50.8	H 5.3	N 10.0

$C_{23}H_{26}N_4O_3ClF$ 1.95HCl 0.6H_2O 0.08ether	Requires	C 51.0	H 5.5	N 10.2%

The starting material was prepared as follows:

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (3.6g, 11.3mmol), (prepared as described for the starting material in Example 4), 1-bromo-4-chlorobutane (1.95ml, 16.9mmol) and potassium carbonate (4.66g, 33.8mmol) in DMF (75ml) was heated at 40°C for 4 hours. The mixture was allowed to cool and was partitioned between methylene chloride and water. The aqueous layer was adjusted to pH7 with 2M hydrochloric acid. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the volatiles removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/ethyl acetate (1/1). The purified solid product was triturated with pentane, collected by filtration and dried under vacuum to give 7-(4-chlorobutoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (3.64g, 79%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.9-2.1(m, 4H); 3.76(t, 2H); 4.01(s, 3H); 4.28(t, 2H); 7.33(s, 1H); 7.46(d, 1H); 7.63(t, 1H); 7.70(d, 1H); 8.08(s, 1H); 8.88(s, 1H)

Example 27

A suspension of 4-(4-chloro-2-fluoroanilino)-7-(3-chloropropoxy)-6-methoxyquinazoline (150mg, 0.38mmol) in 1-methylpiperazine (2ml) was heated at 100°C for 3 hours. The mixture was allowed to cool and was partitioned between aqueous sodium carbonate solution (pH11.5) and ethyl acetate. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the volatiles removed by evaporation. The residue was dissolved in methylene chloride and ether was added. The resulting precipitate was collected by filtration, washed with ether and dried. The solid was dissolved in methylene chloride and 2.2M ethereal hydrogen chloride (1ml) was added. After concentrating to half of initial volume, the resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)quinazoline** hydrochloride (158mg, 75%).

^{1}HNMR Spectrum: ($DMSOd_6$; CF_3COOD; 60°C) 2.35(m, 2H); 2.95(s, 3H); 3.43(t, 2H); 3.52-3.7(m, 8H); 4.03(s, 3H); 4.34(t, 2H); 7.41(s, 1H); 7.45(d, 1H); 7.6-7.7(m, 2H); 8.11(s, 1H); 8.8(s, 1H)

MS - EI: 460 $[MH]^+$

Elemental analysis:	Found	C 48.6	H 5.6 N 11.9
$C_{23}H_{27}N_5O_2FCl$ 0.7H_2O 2.75HCl	Requires	C 48.2	H 5.5 N 12.2%

The starting material was prepared as follows:

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (957mg, 3mmol), (prepared as described for the starting material in Example 4), 1-bromo-3-chloropropane (2.36g, 15mmol) and potassium carbonate (2.1g, 15mmol) in DMF (20ml) was heated at 40°C for 1.5 hours. The mixture was allowed to cool, was diluted with water and extracted with ethyl acetate (3x50ml). The organic extracts were combined, washed with water and brine, dried ($MgSO_4$) and the volatiles were removed by evaporation. The residue was triturated with hexane/ethyl acetate, collected by filtration and dried under vacuum to give 4-(4-chloro-2-fluoroanilino)-7-(3-chloropropoxy)-6-methoxyquinazoline (650mg, 55%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.26(m, 2H); 3.82(t, 2H); 3.95(s, 3H); 4.26(t, 2H); 7.20(s, 1H); 7.32(dd, 1H); 7.48-7.60(m, 2H); 7.80(s, 1H); 8.35(s, 1H); 9.52(s, 1H)

MS - EI: 396 $[MH]^+$

Example 28

The following illustrate representative pharmaceutical dosage forms containing the compound of formula I, or a pharmaceutically acceptable salt thereof (hereafter compound X), for therapeutic or prophylactic use in humans:

(a) Tablet I

	mg/tablet
Compound X	100
Lactose Ph.Eur	182.75
Croscarmellose sodium	12.0
Maize starch paste (5% w/v paste)	2.25
Magnesium stearate	3.0

(b) Tablet II

mg/tablet

Compound X	50
Lactose Ph.Eur	223.75
Croscarmellose sodium	6.0
Maize starch	15.0
Polyvinylpyrrolidone (5% w/v paste)	2.25
Magnesium stearate	3.0

(c) Tablet III

	mg/tablet
Compound X	1.0
Lactose Ph.Eur	93.25
Croscarmellose sodium	4.0
Maize starch paste (5% w/v paste)	0.75
Magnesium stearate	1.0

(d) Capsule

	mg/capsule
Compound X	10
Lactose Ph.Eur	488.5
Magnesium stearate	1.5

(e) Injection I

	(50 mg/ml)
Compound X	5.0% w/v
1N Sodium hydroxide solution	15.0% v/v
0.1N Hydrochloric acid (to adjust pH to 7.6)	
Polyethylene glycol 400	4.5% w/v
Water for injection to 100%	

(f) Injection II

	10 mg/ml)
Compound X	1.0% w/v
Sodium phosphate BP	3.6% w/v
0.1N Sodium hydroxide solution	15.0% v/v

Water for injection to 100%

(g) Injection III (1mg/ml,buffered to pH6)

Compound X	0.1% w/v
Sodium phosphate BP	2.26% w/v
Citric acid	0.38% w/v
Polyethylene glycol 400	3.5% w/v

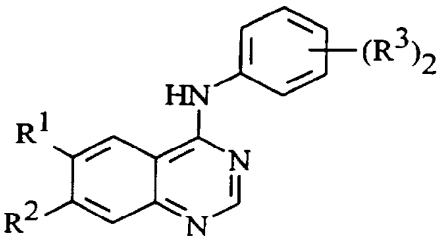
Water for injection to 100%

Note

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate.

CLAIMS

1. A quinazoline derivative of the formula I:



(I)

(wherein:

R^1 represents hydrogen or methoxy;

R^2 represents methoxy, ethoxy, 2-methoxyethoxy, 3-methoxypropoxy, 2-ethoxyethoxy, trifluoromethoxy, 2,2,2-trifluoroethoxy, 2-hydroxyethoxy, 3-hydroxypropoxy, 2-(N,N-dimethylamino)ethoxy, 3-(N,N-dimethylamino)propoxy, 2-morpholinoethoxy, 3-morpholinopropoxy, 4-morpholinobutoxy, 2-piperidinoethoxy, 3-piperidinopropoxy, 4-piperidinobutoxy, 2-(piperazin-1-yl)ethoxy, 3-(piperazin-1-yl)propoxy, 4-(piperazin-1-yl)butoxy, 2-(4-methylpiperazin-1-yl)ethoxy, 3-(4-methylpiperazin-1-yl)propoxy or 4-(4-methylpiperazin-1-yl)butoxy;

the phenyl group bearing $(R^3)_2$ is selected from: 2-fluoro-5-hydroxyphenyl, 4-bromo-2-fluorophenyl, 2,4-difluorophenyl, 4-chloro-2-fluorophenyl, 2-fluoro-4-methylphenyl, 2-fluoro-4-methoxyphenyl, 4-bromo-3-hydroxyphenyl, 4-fluoro-3-hydroxyphenyl, 4-chloro-3-hydroxyphenyl, 3-hydroxy-4-methylphenyl, 3-hydroxy-4-methoxyphenyl and 4-cyano-2-fluorophenyl);

and salts thereof.

2. A quinazoline derivative as claimed in claim 1 wherein R^1 is methoxy.

3. A quinazoline derivative as claimed in claim 1 or claim 2 wherein the phenyl group bearing $(R^3)_2$ is 4-chloro-2-fluorophenyl or 4-bromo-2-fluorophenyl.

4. A quinazoline derivative as claimed in any one of the preceding claims wherein R^2 is methoxy, ethoxy, 2-methoxyethoxy, 3-methoxypropoxy, trifluoromethoxy, 2,2,2-trifluoroethoxy, 2-hydroxyethoxy, 3-hydroxypropoxy, 2-(N,N-dimethylamino)ethoxy, 3-(N,N-dimethylamino)propoxy, 2-morpholinoethoxy, 3-morpholinopropoxy, 2-piperidinoethoxy, 3-piperidinopropoxy, 2-(piperazin-1-yl)ethoxy, 3-(piperazin-1-yl)propoxy, 2-(4-methylpiperazin-1-yl)ethoxy, or 3-(4-methylpiperazin-1-yl)propoxy.

5. A quinazoline derivative as claimed in claim 4 wherein R^2 is 2-methoxyethoxy, 2-morpholinoethoxy, 3-morpholinopropoxy or 2-(4-methylpiperazin-1-yl)ethoxy.

6. A quinazoline derivative as claimed in claim 5 wherein R^2 is 2-methoxyethoxy or 3-morpholinopropoxy.

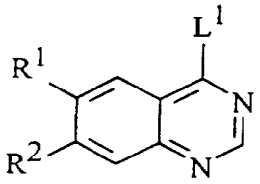
7. A quinazoline derivative as claimed in claim 1 selected from:
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropoxy)quinazoline
and salts thereof.

8. A quinazoline derivative as claimed in claim 1 selected from:
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-morpholinoethoxy)quinazoline
and salts thereof.

9. A quinazoline derivative as claimed in any one of the preceding claims in the form of a pharmaceutically acceptable salt.

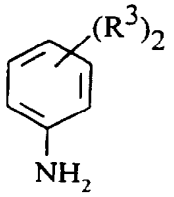
10. A process for the preparation of a quinazoline derivative of formula I or salt thereof (as defined in claim 1) which comprises:

(a) the reaction of a compound of the formula III:



(III)

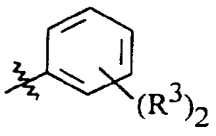
(wherein R^1 and R^2 are as defined in claim 1 and L^1 is a displaceable moiety), with a compound of the formula IV:



(IV)

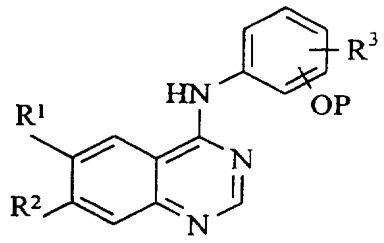
(wherein R^3 is as defined in claim 1) whereby to obtain compounds of the formula I and salts thereof;

(b) for the preparation of compounds of formula I and salts thereof in which the group of formula II:



(II)

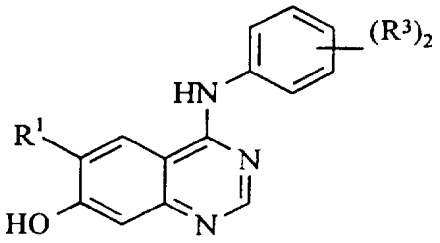
(wherein R^3 is as defined in claim 1) represents a phenyl group carrying a hydroxy group, the deprotection of a compound of formula V:



(V)

(wherein R^1, R^2 and R^3 are as defined in claim 1 and P represents a phenolic hydroxy protecting group);

(c) the reaction of a compound of the formula VI:



(VI)

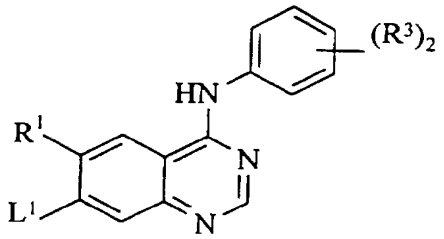
(wherein R^1 and R^3 are as defined in claim 1) with a compound of formula VII:

$$R^4\text{-}L^1 \qquad \text{(VII)}$$

(wherein L^1 is as defined herein and R^4 is methyl, ethyl, 2-methoxyethyl, 3-methoxypropyl, 2-ethoxyethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 2-hydroxyethyl, 3-hydroxypropyl, 2-(N,N-dimethylamino)ethyl, 3-(N,N-dimethylamino)propyl, 2-morpholinoethyl, 3-morpholinopropyl, 4-morpholinobutyl, 2-piperidinoethyl, 3-piperidinopropyl, 4-piperidinobutyl, 2-(piperazin-1-yl)ethyl, 3-(piperazin-1-yl)propyl, 4-(piperazin-1-yl)butyl, 2-

(4-methylpiperazin-1-yl)ethyl, 3-(4-methylpiperazin-1-yl)propyl or 4-(4-methylpiperazin-1-yl)butyl);

(d) the reaction of a compound of the formula VIII:



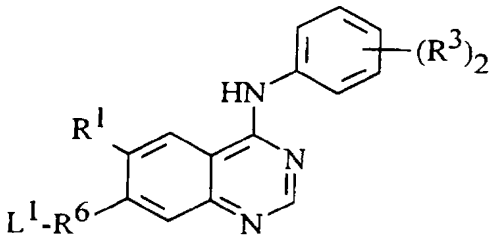
(VIII)

with a compound of the formula IX:

$$R^2\text{-}H \qquad \text{(IX)}$$

(wherein L^1 is as defined herein and R^1, R^2 and R^3 are as defined in claim 1):

(e) for the preparation of compounds of formula I and salts thereof wherein R^2 is R^5C_{1-4}alkoxy, (wherein R^5 is selected from methoxy, ethoxy, hydroxy, N,N-dimethylamino, morpholino, piperidino, piperazin-1-yl or 4-methylpiperazin-1-yl) reacting a compound of the formula X:



(X)

(wherein L^1 is as defined herein, R^1 and R^3 are as defined in claim 1 and R^6 is C_{1-4}alkoxy) with a compound of the formula XI:

R^5-H (XI)

(wherein R^5 is as defined herein);

and when a salt of a quinazoline derivative of formula I is required, reaction of the compound obtained with an acid or base whereby to obtain the desired salt.

11. A pharmaceutical composition which comprises as active ingredient a quinazoline derivative of formula I or a pharmaceutically acceptable salt thereof as claimed in any one of claims 1 to 9 in association with a pharmaceutically acceptable excipient or carrier.

12. A method for producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal in need of such treatment which comprises administering to said animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof as claimed in any one of claims 1 to 9.

13. A quinazoline derivative as claimed in any one of claims 1 to 9 for use as a medicament.

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/GB 97/00550

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D239/94 A61K31/505

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 566 226 A (ZENECA LTD) 20 October 1993 cited in the application see the whole document	1,11
Y	WO 95 15758 A (RHONE POULENC RORER PHARMA ;MYERS MICHAEL R (US); SPADA ALFRED P () 15 June 1995 see the whole document	1,11
Y	FR 2 435 248 A (SANKYO CO) 4 April 1980 see examples 11,22,28,30	1,11
A,P	WO 96 33977 A (ZENECA LTD ;BARKER ANDREW JOHN (GB)) 31 October 1996 see the whole document	1,11

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
29 May 1997	11.06.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Frelon, D

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 97/00550

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 12
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Intern al Application No
PCT/GB 97/00550

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07D 239/94, 405/12, 403/12, 401/12, A61K 31/505

A1

(11) International Publication Number: **WO 98/13354**

(43) International Publication Date: 2 April 1998 (02.04.98)

(21) International Application Number: PCT/GB97/02588

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(30) Priority Data:
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(34) Countries for which the regional or international application was filed: FR et al.
97401042.3 9 May 1997 (09.05.97) EP
(34) Countries for which the regional or international application was filed: FR et al.

(71) Applicants *(for all designated States except US)*: ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB). ZENECA PHARMA S.A. [FR/FR]; "Le Galien", 1, rue des Chauffours, Boîte postale 127, F–95022 Cergy Cedex (FR).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: THOMAS, Andrew, Peter [GB/GB]; Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). HENNEQUIN, Laurent, François, André [FR/FR]; Zeneca Pharma S.A., Zone Industrielle Sud–Est, Boîte postale 401, F–51064 Reims Cedex (FR). JOHNSTONE, Craig [GB/GB]; Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). STOKES, Elaine, Sophie, Elizabeth [GB/GB]; Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). LOHMANN, Jean–Jacques, Marcel [FR/FR]; Zeneca Pharma S.A., Zone Industrielle Sud–Est, Boîte postale 401, F–51064 Reims Cedex (FR). CLAYTON, Edward [GB/GB]; Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).

(74) Agent: MACK, John, Richard; Zeneca Pharmaceuticals, Intellectual Property Dept., Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).

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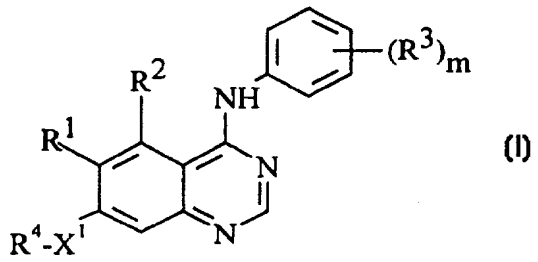
(57) Abstract

The invention relates to quinazoline derivatives of formula (I) wherein m is an integer from 1 to 2; R^1 represents hydrogen, hydroxy, halogeno, nitro, trifluoromethyl, cyano, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkylthio, or $-NR^5R^6$ (wherein R^5 and R^6, which may be the same or different, each represents hydrogen or C_{1-3}alkyl); R^2 represents hydrogen, hydroxy, halogeno, methoxy, amino or nitro; R^3 represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkanoyloxy, trifluoromethyl, cyano, amino or nitro; X^1 represents $-O-$, $-CH_2-$, $-S-$, $-SO-$, $-SO_2-$, $-NR^7CO-$, $-CONR^8-$, $-SO_2NR^9-$, $-NR^{10}SO_2-$ or $-NR^{11}-$ (wherein R^7, R^8, R^9, R^{10} and R^{11} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl); R^4 represents an optionally substituted 5 or 6 membered saturated carbocyclic or heterocyclic group or a group which is alkenyl, alkynyl or optionally substituted alkyl, which alkyl group may contain a heteroatom linking group, which alkenyl, alkynyl or alkyl group may carry a terminal optionally substituted group selected from alkyl and a 5 or 6 membered saturated carbocyclic or heterocyclic group, and salts thereof; processes for their preparation, pharmaceutical compositions containing a compound of formula (I) or a pharmaceutically acceptable salt thereof as active ingredient. The compounds of formula (I) and the pharmaceutically acceptable salts thereof inhibit the effects of VEGF, a property of value in the treatment of a number of disease states including cancer and rheumatoid arthritis.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QUINAZOLINE DERIVATIVES AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM.

The present invention relates to quinazoline derivatives, processes for their preparation, pharmaceutical compositions containing them as active ingredient, methods for the treatment of disease states associated with angiogenesis and/or increased vascular permeability, to their use as medicaments and to their use in the manufacture of medicaments for use in the production of antiangiogenic and/or vascular permeability reducing effects in warm-blooded animals such as humans.

Normal angiogenesis plays an important role in a variety of processes including embryonic development, wound healing and several components of female reproductive function. Undesirable or pathological angiogenesis has been associated with disease states including diabetic retinopathy, psoriasis, cancer, rheumatoid arthritis, atheroma, Kaposi's sarcoma and haemangioma (Fan et al, 1995, Trends Pharmacol. Sci. 16: 57-66; Folkman, 1995, Nature Medicine 1: 27-31). Alteration of vascular permeability is thought to play a role in both normal and pathological physiological processes (Cullinan-Bove et al, 1993, Endocrinology 133: 829-837; Senger et al, 1993, Cancer and Metastasis Reviews, 12: 303-324). Several polypeptides with in vitro endothelial cell growth promoting activity have been identified including, acidic and basic fibroblast growth factors (aFGF & bFGF) and vascular endothelial growth factor (VEGF). By virtue of the restricted expression of its receptors, the growth factor activity of VEGF, in contrast to that of the FGFs, is relatively specific towards endothelial cells. Recent evidence indicates that VEGF is an important stimulator of both normal and pathological angiogenesis (Jakeman et al, 1993, Endocrinology, 133: 848-859; Kolch et al, 1995, Breast Cancer Research and Treatment, 36:139-155) and vascular permeability (Connolly et al, 1989, J. Biol. Chem. 264: 20017-20024). Antagonism of VEGF action by sequestration of VEGF with antibody can result in inhibition of tumour growth (Kim et al, 1993, Nature 362: 841-844).

Receptor tyrosine kinases (RTKs) are important in the transmission of biochemical signals across the plasma membrane of cells. These transmembrane molecules characteristically consist of an extracellular ligand-binding domain connected through a segment in the plasma membrane to an intracellular tyrosine kinase domain. Binding of ligand to the receptor results in stimulation of the receptor-associated tyrosine kinase activity which

leads to phosphorylation of tyrosine residues on both the receptor and other intracellular molecules. These changes in tyrosine phosphorylation initiate a signalling cascade leading to a variety of cellular responses. To date, at least nineteen distinct RTK subfamilies, defined by amino acid sequence homology, have been identified. One of these subfamilies is presently comprised by the fms-like tyrosine kinase receptor, Flt or Flt1, the kinase insert domain-containing receptor, KDR (also referred to as Flk-1), and another fms-like tyrosine kinase receptor, Flt4. Two of these related RTKs, Flt and KDR, have been shown to bind VEGF with high affinity (De Vries et al, 1992, Science 255: 989-991; Terman et al, 1992, Biochem. Biophys. Res. Comm. 1992, 187: 1579-1586). Binding of VEGF to these receptors expressed in heterologous cells has been associated with changes in the tyrosine phosphorylation status of cellular proteins and calcium fluxes.

European Patent Publication No. 0326330 discloses certain quinoline, quinazoline and cinnoline plant fungicides. Certain of these plant fungicides are also stated to possess insecticidal and miticidal activity. There is however no disclosure or any suggestion that any of the compounds disclosed may be used for any purpose in animals such as humans. In particular, the European Patent Publication contains no teaching whatsoever concerning angiogenesis and/or increased vascular permeability mediated by growth factors such as VEGF.

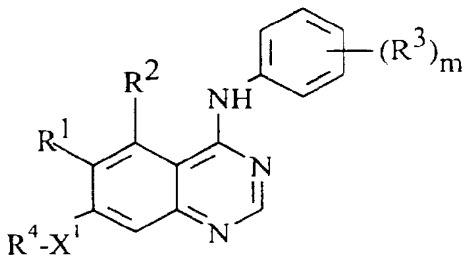
European Patent Publication No. 0566226 discloses anilinoquinazolines which have activity against epidermal growth factor (EGF) receptor tyrosine kinase. EP 0566226 contains no teaching whatsoever concerning angiogenesis and/or increased vascular permeability mediated by growth factors such as VEGF. Moreover compounds of EP 0566226 which have been tested do not show significant activity against VEGF receptor tyrosine kinase.

The present invention is based on the surprising discovery that certain quinazolines inhibit the effects of VEGF, a property of value in the treatment of disease states associated with angiogenesis and/or increased vascular permeability such as cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation and ocular diseases with retinal vessel proliferation. Compounds of the present invention possess good activity against VEGF receptor tyrosine kinase whilst possessing some activity against EGF receptor tyrosine kinase. Furthermore, some compounds of the present invention, possess substantially higher potency against VEGF receptor tyrosine kinase than against EGF receptor tyrosine

kinase or FGF R1 receptor tyrosine kinase. Thus certain compounds of the invention which have been tested possess activity against VEGF receptor tyrosine kinase such that they may be used in an amount sufficient to inhibit VEGF receptor tyrosine kinase whilst demonstrating no significant activity against EGF receptor tyrosine kinase or FGF R1 receptor tyrosine kinase. While we do not wish to be bound by theoretical considerations such compounds may for example be of interest in treating tumours which are associated with VEGF, especially those tumours which are dependent on VEGF for their growth.

Other compounds of the invention possess good activity against both VEGF and EGF receptor tyrosine kinases. Indeed certain compounds possess substantially equivalent activities against VEGF and EGF receptor tyrosine kinases. It is believed that these compounds may be of interest in treating tumour states associated with both VEGF and EGF, especially where a patient is suffering from a condition in which tumours are present which are dependent on both VEGF and EGF for their growth.

According to one aspect of the present invention there is provided a quinazoline derivative of the formula I:



(I)

[wherein:

m is an integer from 1 to 2;

R^1 represents hydrogen, hydroxy, halogeno, nitro, trifluoromethyl, cyano, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkylthio, or $-NR^5R^6$ (wherein R^5 and R^6, which may be the same or different, each represents hydrogen or C_{1-3}alkyl);

R^2 represents hydrogen, hydroxy, halogeno, methoxy, amino or nitro;

R^3 represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkanoyloxy, trifluoromethyl, cyano, amino or nitro;

X^1 represents -O-, -CH_2-, -S-, -SO-, -SO_2-, -NR^7CO-, -$CONR^8$-, -SO_2NR^9-, -$NR^{10}SO_2$- or -NR^{11}- (wherein R^7, R^8, R^9, R^{10} and R^{11} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

R^4 is selected from one of the following eight groups:

1) C_{1-5}alkylR^{12} (wherein R^{12} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to C_{1-5}alkyl through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy, and additional possible substituents are carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl) or C_{1-5}alkylR^{13} (wherein R^{13} is a group selected from pyrrolidin-1-yl, imidazolidin-1-yl and thiomorpholino, which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy, and additional possible substituents are carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

2) C_{2-5}alkenylR^{14} (wherein R^{14} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy, and additional possible substituents are carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

3) C_{2-5}alkynylR^{15} (wherein R^{15} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy, and additional possible substituents are carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

4) C_{1-5}alkylX^2C_{1-5}alkylX^3R^{16} (wherein X^2 and X^3 which may be the same or different are each -O-, -S-, -SO-, -SO_2-, -$NR^{17}CO$-, -$CONR^{18}$-, -SO_2NR^{19}-, -$NR^{20}SO_2$- or -NR^{21}- (wherein R^{17}, R^{18}, R^{19}, R^{20} and R^{21} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be -CH_2- when R^4 is C_{1-5}alkylX^2C_{1-5}alkylX^3R^{16};

5) C_{1-5}alkylX^4COR^{22} (wherein X^4 represents -O- or -NR^{23}- (wherein R^{23} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{22} represents -$NR^{24}R^{25}$ or -OR^{26} (wherein R^{24}, R^{25} and R^{26} which may be the same or different each represents hydrogen, C_{1-4}alkyl or C_{1-3}alkoxyC_{2-3}alkyl));

6) C_{1-5}alkylX^5R^{27} (wherein X^5 represents -O-, -S-, -SO-, -SO_2-, -OCO-, -NR^{28}CO-, -$CONR^{29}$-, -SO_2NR^{30}-, -$NR^{31}SO_2$- or -NR^{32}- (wherein R^{28}, R^{29}, R^{30}, R^{31} and R^{32} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) or X^5 is carbonyl, and R^{27} represents cyclopentyl, cyclohexyl or a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which cyclopentyl, cyclohexyl or heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy, and additional possible substituents are carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl or R^{27} is C_{1-3}alkyl with the proviso that when R^{27} is C_{1-3}alkyl, X^5 is -S-, -SO-, -SO_2-, -SO_2NR^{30}- or -$NR^{31}SO_2$- and X^1 is not -CH_2-);

7) C_{1-3}alkoxyC_{2-4}alkyl provided that X^1 is -S-, or X^1 is -SO- or -SO_2-; and

8) C_{1-3}alkoxyC_{2-4}alkyl or C_{1-4}alkyl provided that X^1 is -O-; and additionally R^4 may be selected from the following five groups:

9) C_{1-5}alkylX^6C_{1-5}alkylR^{33} (wherein X^6 represents -O-, -S-, -SO-, -SO_2-, -NR^{34}CO-, -$CONR^{35}$-, -SO_2NR^{36}-, -$NR^{37}SO_2$- or -NR^{38}- (wherein R^{34}, R^{35}, R^{36}, R^{37} and R^{38} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{33} represents cyclopentyl, cyclohexyl or a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which cyclopentyl, cyclohexyl or heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

10) R^{39} (wherein R^{39} is a group selected from pyrrolidin-3-yl, piperidin-3-yl and piperidin-4-yl which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

11) C_{1-5}alkylR^{40} (wherein R^{40} is piperazin-1-yl which bears at least one substituent selected from C_{1-4}alkanoyl, C_{1-4}alkoxycarbonyl, C_{1-4}hydroxyalkyl and -$CONR^{41}R^{42}$ (wherein R^{41} and R^{42} each independently represents hydrogen or C_{1-4}alkyl);

12) C_{1-5}alkylR^{43} (wherein R^{43} is morpholino which may bear one or two substituents selected from oxo, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl) with the proviso that when R^4 is C_{1-5}alkylR^{43}, X^1 is -S-, -SO-, $-SO_2-$, $-SO_2NR^9-$ or $-NR^{10}SO_2-$; and

13) C_{1-5}alkylR^{44} (wherein R^{44} is morpholino which bears at least one and optionally two substituents selected from oxo, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

with the further proviso that when R^4 is selected from group 8) R^1 and/or R^2 is/are nitro or at least one R^3 is C_{1-3}alkanoyloxy;]

and salts thereof.

Preferably m is 2.

R^1 is advantageously hydrogen, hydroxy, cyano, nitro, trifluoromethyl, C_{1-3}alkyl, C_{1-3}alkoxy or amino.

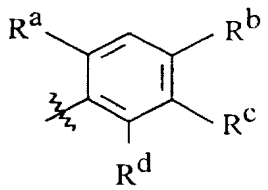
R^1 is preferably hydrogen, hydroxy, cyano, nitro, trifluoromethyl, methyl, ethyl, methoxy or ethoxy, more preferably hydrogen, methyl or methoxy, most preferably hydrogen or methoxy, but especially methoxy.

R^2 is preferably hydrogen, fluoro, amino or nitro, but especially hydrogen.

In one embodiment of the present invention R^3 represents hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}alkoxy, trifluoromethyl, cyano, amino or nitro.

Advantageously in another embodiment of the present invention one R^3 substituent is meta-hydroxy and the other one is selected from halogeno and methyl.

In another embodiment of the invention the phenyl group bearing $(R^3)_m$ is preferably of the formula II:



(II)

wherein:

R^a represents hydrogen, methyl, fluoro or chloro, preferably hydrogen or fluoro;

R^b represents hydrogen, methyl, methoxy, bromo, fluoro or chloro, especially hydrogen, methyl or chloro;

R^c represents hydrogen or hydroxy;

R^d represents hydrogen, fluoro or chloro, especially hydrogen or fluoro.

In a particular aspect of the present invention, the phenyl group bearing $(R^3)_m$ is the 3-hydroxy-4-methylphenyl group, the 2-fluoro-5-hydroxyphenyl group or the 4-chloro-2-fluorophenyl group, or the 4-bromo-2-fluorophenyl group, especially the 4-chloro-2-fluorophenyl group or the 4-bromo-2-fluorophenyl group more especially the 4-chloro-2-fluorophenyl group.

Advantageously X^1 represents -O-, -S-, $-NR^7CO-$, $-NR^{10}SO_2-$ or $-NR^{11}-$ (wherein R^7, R^{10} and R^{11} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Preferably X^1 represents -O-, -S-, $-NR^7CO-$ or $-NR^{10}SO_2-$ (wherein R^7 and R^{10} each independently represents hydrogen or C_{1-2}alkyl).

More preferably X^1 represents -O-, -S-, $-NR^7CO-$ (wherein R^7 represents hydrogen or methyl).

Particularly X^1 represents -O-, or -NHCO-, or -S-, especially -O-, or -S-, more especially -O-.

Conveniently X^2 and X^3 which may be the same or different each represents -O-, -S-, -SO-, $-SO_2-$, $-NR^{17}CO-$, or $-NR^{21}-$ (wherein R^{17} and R^{21} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Advantageously X^2 and X^3 which may be the same or different each represents -O-, -S-, -SO-, $-SO_2-$ or $-NR^{21}-$ (wherein R^{21} represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Preferably X^2 and X^3 which may be the same or different each represents -O-, -S- or $-NR^{21}-$ (wherein R^{21} represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

In a particular aspect of the present invention X^3 is -O- and X^2 is $-NR^{17}CO-$ (wherein R^{17} represents hydrogen, or methyl).

Advantageously X^4 represents -O- or $-NR^{23}-$ (wherein R^{23} represents hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl).

Advantageously X^5 represents -O-, -S-, -SO-, $-SO_2-$, $-NR^{28}CO-$, $-NR^{31}SO_2-$ or $-NR^{32}-$ (wherein R^{28}, R^{31} and R^{32} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl) or X^5 is carbonyl.

Preferably X^5 represents -O-, -S-, -SO-, $-SO_2-$ or $-NR^{32}-$ (wherein R^{32} represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

More preferably X^5 represents -O- or $-NR^{32}-$ (wherein R^{32} represents hydrogen or C_{1-2}alkyl).

Advantageously X^6 represents -O-, -S-, -SO-, $-SO_2-$, $-NR^{34}CO-$, $-NR^{37}SO_2-$ or $-NR^{38}-$ (wherein R^{34}, R^{37} and R^{38} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Preferably X^6 represents -O-.

Conveniently R^4 is selected from one of the following eight groups:

1) C_{1-5}alkylR^{12} (wherein R^{12} is a group selected from 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, pyrrolidin-2-yl and pyrrolidin-3-yl, and piperidin-2-yl, piperidin-3-yl, piperidin-4-yl, morpholin-2-yl, morpholin-3-yl and piperazin-2-yl which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy, and additional possible substituents are carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl) or C_{2-5}alkylR^{45} (wherein R^{45} is a group selected from imidazolidin-1-yl, pyrrolidin-1-yl and thiomorpholino, which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy, and additional possible substituents are carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

2) C_{3-5}alkenylR^{46} (wherein R^{46} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to C_{3-5}alkenyl through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy, and additional possible substituents are carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl) or C_{4-5}alkenylR^{47} (wherein R^{47} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to C_{4-5}alkenyl through a nitrogen atom and which heterocyclic group may bear one or two

substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy, and additional possible substituents are carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

3) C_{3-5}alkynylR^{48} (wherein R^{48} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to C_{3-5}alkynyl through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy, and additional possible substituents are carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl) or C_{4-5}alkynylR^{49} (wherein R^{49} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to C_{4-5}alkynyl through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl and C_{1-4}alkoxy, and additional possible substituents are carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

4) C_{2-3}alkylX^2C_{2-3}alkylX^3R^{16} (wherein X^2 and X^3 are as defined hereinbefore and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be $-CH_2-$ when R^4 is C_{2-3}alkylX^2C_{2-3}alkylX^3R^{16};

5) C_{2-3}alkylX^4COR^{22} (wherein X^4 is as defined hereinbefore and R^{22} represents $-NR^{24}R^{25}$ or $-OR^{26}$ (wherein R^{24}, R^{25} and R^{26} which may be the same or different each represents hydrogen, C_{1-4}alkyl or C_{1-2}alkoxyethyl));

6) C_{2-4}alkylX^5R^{27} (wherein X^5 is as defined hereinbefore and R^{27} represents cyclopentyl, cyclohexyl or a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which cyclopentyl, cyclohexyl or heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl and C_{1-3}alkoxy, and additional possible substituents are carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl or R^{27} is C_{1-3}alkyl with the proviso that when R^{27} is C_{1-3}alkyl, X^5 is -S-, -SO-, $-SO_2-$, $-SO_2NR^{30}-$ or $-NR^{31}SO_2-$ and X^1 is not $-CH_2-$);

7) C_{1-3}alkoxyC_{2-4}alkyl provided that X^1 is -S-, or X^1 is -SO- or $-SO_2-$; and

8) C_{1-3}alkoxyC_{2-4}alkyl or C_{1-4}alkyl provided that X^1 is -O-; and additionally R^4 may conveniently be selected from the following four groups:

9) C_{2-4}alkylX^6C_{2-4}alkylR^{33} (wherein X^6 is as defined hereinbefore and R^{33} represents a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

10) C_{2-4}alkylR^{40} (wherein R^{40} is piperazin-1-yl which bears at least one substituent selected from C_{2-3}alkanoyl, C_{1-3}alkoxycarbonyl, C_{1-3}hydroxyalkyl and $CONR^{41}R^{42}$ (wherein R^{41} and R^{42} each independently represents hydrogen or C_{1-3}alkyl));

11) C_{2-4}alkylR^{43} (wherein R^{43} is morpholino which may bear one or two substituents selected from oxo, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) with the proviso that when R^4 is C_{2-4}alkylR^{43}, X^1 is -S-, -SO-, $-SO_2-$, $-SO_2NR^9-$ or $-NR^{10}SO_2-$; and

12) C_{2-4}alkylR^{44} (wherein R^{44} is morpholino which bears at least one and optionally two substituents selected from oxo, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

with the further proviso that when R^4 is selected from group 8) R^1 and/or R^2 is/are nitro or at least one R^3 is C_{1-3}alkanoyloxy.

An additional convenient value of R^4 is C_{2-3}alkylX^2methylX^3R^{16} (wherein X^2 and X^3 are as defined hereinbefore and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be $-CH_2-$ when R^4 is C_{2-3}alkylX^2methylX^3R^{16}.

Advantageously R^4 is selected from one of the following seven groups:

1) C_{1-4}alkylR^{12} (wherein R^{12} is a group selected from 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, pyrrolidin-2-yl and pyrrolidin-3-yl, and piperidin-2-yl, piperidin-3-yl, piperidin-4-yl, morpholin-2-yl, morpholin-3-yl and piperazin-2-yl which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl and C_{1-3}alkoxy, and additional possible substituents are carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or C_{2-4}alkylR^{45} (wherein R^{45} is a group selected from imidazolidin-1-yl, pyrrolidin-1-yl and thiomorpholino which group may bear one or two substituents selected from oxo, hydroxy,

halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl and C_{1-3}alkoxy, and additional possible substituents are carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

2) 1-R^{46}prop-1-en-3-yl, 1-R^{46}but-2-en-4-yl, 1-R^{46}but-1-en-3-yl, 1-R^{46}pent-2-en-4-yl or 2-R^{46}pent-3-en-5-yl (wherein R^{46} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to the alkenyl group through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl and C_{1-3}alkoxy, and additional possible substituents are carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or 1-R^{47}but-2-en-4-yl, 1-R^{47}pent-2-en-4-yl or 2-R^{47}pent-3-en-5-yl (wherein R^{47} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to the alkenyl group through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl and C_{1-3}alkoxy, and additional possible substituents are carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

3) 1-R^{48}prop-1-yn-3-yl, 1-R^{48}but-2-yn-4-yl, 1-R^{48}but-1-yn-3-yl, 1-R^{48}pent-2-yn-4-yl or 2-R^{48}pent-3-yn-5-yl (wherein R^{48} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to the alkynyl group through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl and C_{1-3}alkoxy, and additional possible substituents are carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or 1-R^{49}but-2-yn-4-yl, 1-R^{49}pent-2-yn-4-yl or 2-R^{49}pent-3-yn-5-yl (wherein R^{49} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to the alkynyl group through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl and C_{1-3}alkoxy, and additional possible substituents are carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

4) C_{2-3}alkylX^2C_{2-3}alkylX^3R^{16} (wherein X^2 and X^3 are as defined hereinbefore and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be $-CH_2-$ when R^4 is C_{2-3}alkylX^2C_{2-3}alkylX^3R^{16};

5) C_{2-3}alkylX^4COR^{22} (wherein X^4 is as defined hereinbefore and R^{22} represents $-NR^{24}R^{25}$ or $-OR^{26}$ (wherein R^{24}, R^{25} and R^{26} which may be the same or different each represents hydrogen, C_{1-4}alkyl or C_{1-2}alkoxyethyl));

6) C_{2-3}alkylX^5R^{27} (wherein X^5 is as defined hereinbefore and R^{27} represents a group selected from cyclopentyl, cyclohexyl, pyrrolidinyl and piperidinyl which group is linked to X^5 through a carbon atom and which group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy, and additional possible substituents are carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl or R^{27} is C_{1-3}alkyl with the proviso that when R^{27} is C_{1-3}alkyl, X^5 is -S-, -SO-, $-SO_2-$, $-SO_2NR^{30}-$ or $-NR^{31}SO_2-$ and X^1 is not $-CH_2-$); and

7) C_{1-2}alkoxyC_{2-3}alkyl provided that X^1 is -S-, or X^1 is -SO- or $-SO_2-$; and additionally R^4 may advantageously be selected from the following four groups:

8) C_{2-3}alkylX^6C_{2-3}alkylR^{33} (wherein X^6 is as defined hereinbefore and R^{33} represents a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl, and C_{1-3}alkoxycarbonyl);

9) C_{2-3}alkylR^{40} (wherein R^{40} is piperazin-1-yl which bears at least one substituent selected from acetyl, C_{1-2}alkoxycarbonyl, C_{1-2}hydroxyalkyl and $CONR^{41}R^{42}$ (wherein R^{41} and R^{42} each independently represents hydrogen or C_{1-2}alkyl);

10) C_{2-3}alkylR^{43} (wherein R^{43} is morpholino which may bear one or two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl) with the proviso that when R^4 is C_{2-3}alkylR^{43}, X^1 is -S-, -SO-, $-SO_2-$, $-SO_2NR^9-$ or $-NR^{10}SO_2-$; and

11) C_{2-3}alkylR^{44} (wherein R^{44} is morpholino which bears at least one and optionally two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl).

An additional advantageous value of R^4 is C_{2-3}alkylX^2methylX^3R^{16} (wherein X^2 and X^3 are as defined hereinbefore and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be $-CH_2-$ when R^4 is C_{2-3}alkylX^2methylX^3R^{16}.

Preferably R^4 is selected from one of the following seven groups:

1) C_{1-3}alkylR^{12} (wherein R^{12} is a group selected from 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, pyrrolidin-2-yl and pyrrolidin-3-yl and piperidin-2-yl, piperidin-3-yl, piperidin-4-yl, morpholin-2-yl, morpholin-3-yl and piperazin-2-yl which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl and C_{1-3}alkoxy, and additional possible substituents are carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, acetyl and C_{1-3}alkoxycarbonyl) or C_{2-3}alkylR^{45} (wherein R^{45} is a group selected from imidazolidin-1-yl, pyrrolidin-1-yl and thiomorpholino which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl and C_{1-3}alkoxy and additional possible substituents are carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, acetyl and C_{1-3}alkoxycarbonyl);

2) 1-R^{46}but-2-en-4-yl (wherein R^{46} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to the alkenyl group through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl and C_{1-3}alkoxy, and additional possible substituents are carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, acetyl and C_{1-3}alkoxycarbonyl) or 1-R^{47}but-2-en-4-yl (wherein R^{47} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to the alkenyl group through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl and C_{1-3}alkoxy, and additional possible substituents are carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, acetyl and C_{1-3}alkoxycarbonyl);

3) 1-R^{48}but-2-yn-4-yl (wherein R^{48} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to the alkynyl group through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl and C_{1-3}alkoxy, and additional possible substituents are carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}

$_3$alkyl)carbamoyl, acetyl and C_{1-3}alkoxycarbonyl) or 1-R^{49}but-2-yn-4-yl (wherein R^{49} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to the alkynyl group through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl and C_{1-3}alkoxy, and additional possible substituents are carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, acetyl and C_{1-3}alkoxycarbonyl);

4) C_{2-3}alkylX^2C_{2-3}alkylX^3R^{16} (wherein X^2 and X^3 are as defined hereinbefore and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be $-CH_2-$ when R^4 is C_{2-3}alkylX^2C_{2-3}alkylX^3R^{16};

5) 2-(3,3-dimethylureido)ethyl, 3-(3,3-dimethylureido)propyl, 2-(3-methylureido)ethyl, 3-(3-methylureido)propyl, 2-ureidoethyl, 3-ureidopropyl, 2-(N,N-dimethylcarbamoyloxy)ethyl, 3-(N,N-dimethylcarbamoyloxy)propyl, 2-(N-methylcarbamoyloxy)ethyl, 3-(N-methylcarbamoyloxy)propyl, 2-(carbamoyloxy)ethyl, 3-(carbamoyloxy)propyl or 2-(1,3,3-trimethylureido)ethyl, 3-(1,3,3-trimethylureido)propyl, 2-(isopropoxycarbonylamino)ethyl, 3-(isopropoxycarbonylamino)propyl, 2-(isobutoxycarbonylamino)ethyl, 3-(isobutoxycarbonylamino)propyl, 2-(t-butoxycarbonylamino)ethyl or 3-(t-butoxycarbonylamino)propyl;

6) C_{2-3}alkylX^5R^{27} (wherein X^5 is as defined hereinbefore and R^{27} is a group selected from cyclopentyl, cyclohexyl, pyrrolidinyl and piperidinyl which group is linked to X^5 through a carbon atom and which group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy and additional possible substituents are carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl or R^{27} is C_{1-2}alkyl with the proviso that when R^{27} is C_{1-2}alkyl, X^5 is -S-, -SO-, $-SO_2-$, $-SO_2NR^{30}-$ or $-NR^{31}SO_2-$ and X^1 is not $-CH_2-$) ; and

7) C_{1-2}alkoxyC_{2-3}alkyl provided that X^1 is -S-, or X^1 is -SO- or $-SO_2-$; and additionally R^4 may preferably be selected from the following three groups:

8) C_{2-3}alkylX^6C_{2-3}alkylR^{33} (wherein X^6 is as defined hereinbefore and R^{33} represents a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, of which at least one is N, which heterocyclic group is linked to C_{2-3}alkyl through a nitrogen atom and which heterocyclic group may bear one or two substituents

selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, C_{1-2}alkoxy, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl);

9) C_{2-3}alkylR^{43} (wherein R^{43} is morpholino which may bear one or two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl) with the proviso that when R^4 is C_{2-3}alkylR^{43}, X^1 is -S-, -SO-, $-SO_2-$, $-SO_2NR^9-$ or $-NR^{10}SO_2-$); and

10) C_{2-3}alkylR^{44} (wherein R^{44} is morpholino which bears at least one and optionally two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl).

An additional preferred value of R^4 is C_{2-3}alkylX^2methylX^3R^{16} (wherein X^2 and X^3 are as defined hereinbefore and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be $-CH_2-$ when R^4 is C_{2-3}alkylX^2methylX^3R^{16}.

More preferably R^4 is selected from one of the following five groups:

1) C_{1-3}alkylR^{12} (wherein R^{12} is a group selected from 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, pyrrolidin-2-yl and pyrrolidin-3-yl and piperidin-2-yl, piperidin-3-yl, piperidin-4-yl, morpholin-2-yl, morpholin-3-yl and piperazin-2-yl which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy, and additional possible substituents are carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl) or C_{2-3}alkylR^{45} (wherein R^{45} is a group selected from imidazolidin-1-yl, pyrrolidin-1-yl and thiomorpholino which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy, and additional possible substituents are carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl);

2) 1-R^{50}but-2-en-4-yl (wherein R^{50} is a group selected from imidazolidin-1-yl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, piperidin-4-yl, pyrrolidin-1-yl, pyrrolidin-3-yl, piperazin-1-yl, morpholino and thiomorpholino and piperidino which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy, and additional possible substituents are carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl);

3) 1-R^{51}but-2-yn-4-yl (wherein R^{51} is a group selected from imidazolidin-1-yl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, piperidin-4-yl, pyrrolidin-1-yl,

pyrrolidin-3-yl, piperazin-1-yl, morpholino and thiomorpholino and piperidino which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl and C_{1-2}alkoxy, and additional possible substituents are carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl);

4) C_{2-3}alkyl$X^2$$C_{2-3}$alkyl$X^3$$R^{16}$ (wherein X^2 and X^3 are as defined hereinbefore and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be $-CH_2-$ when R^4 is C_{2-3}alkyl$X^2$$C_{2-3}$alkyl$X^3$$R^{16}$; and

5) C_{1-2}alkoxyC_{2-3}alkyl provided that X^1 is -S-, or X^1 is -SO- or $-SO_2-$; and additionally R^4 may more preferably be selected from the following four groups:

6) 2-(3,3-dimethylureido)ethyl, 3-(3,3-dimethylureido)propyl, 2-(3-methylureido)ethyl, 3-(3-methylureido)propyl, 2-ureidoethyl, 3-ureidopropyl, 2-(N,N-dimethylcarbamoyloxy)ethyl, 3-(N,N-dimethylcarbamoyloxy)propyl, 2-(N-methylcarbamoyloxy)ethyl, 3-(N-methylcarbamoyloxy)propyl, 2-(carbamoyloxy)ethyl, 3-(carbamoyloxy)propyl, 2-(1,3,3-trimethylureido)ethyl, 3-(1,3,3-trimethylureido)propyl, 2-(isopropoxycarbonylamino)ethyl, 3-(isopropoxycarbonylamino)propyl, 2-(isobutoxycarbonylamino)ethyl, 3-(isobutoxycarbonylamino)propyl, 2-(t-butoxycarbonylamino)ethyl or 3-(t-butoxycarbonylamino)propyl;

7) C_{2-3}alkyl$X^5$$R^{27}$ (wherein R^{27} is C_{1-2}alkyl and X^5 is -S-, -SO-, $-SO_2-$, $-SO_2NR^{30}-$ or $-NR^{31}SO_2-$ and with the proviso that X^1 is not $-CH_2-$);

8) C_{2-3}alkyl$X^6$$C_{2-3}$alkyl$R^{33}$ (wherein X^6 is as defined hereinbefore and R^{33} represents a group selected from morpholino, 2-oxopyrrolidin-1-yl, pyrrolidin-1-yl, piperidino, piperazin-1-yl and 4-methylpiperazin-1-yl); and

9) C_{2-3}alkylR^{43} (wherein R^{43} is morpholino which may bear one or two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl) with the proviso that when R^4 is C_{2-3}alkylR^{43}, X^1 is -S-, -SO-, $-SO_2-$, $-SO_2NR^9-$ or $-NR^{10}SO_2-$.

An additional more preferred value of R^4 is C_{2-3}alkylX^2methyl$X^3$$R^{16}$ (wherein X^2 and X^3 are as defined hereinbefore and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be $-CH_2-$ when R^4 is C_{2-3}alkylX^2methyl$X^3$$R^{16}$.

Most preferably R^4 is selected from one of the following five groups:

1) C_{1-3}alkylR^{12} (wherein R^{12} is 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, pyrrolidin-2-yl or pyrrolidin-3-yl or piperidin-2-yl, piperidin-3-yl, piperidin-4-yl, 1-methylpiperidin-2-yl, 1-methylpiperidin-3-yl, 1-methylpiperidin-4-yl, 1-methylpyrrolidin-2-yl, 1-methylpyrrolidin-3-yl, piperazin-2-yl, 1-methylpiperazin-2-yl, 4-methylpiperazin-2-yl, 1,4-dimethylpiperazin-2-yl, morpholin-2-yl, morpholin-3-yl, 4-methylmorpholin-2-yl or 4-methylmorpholin-3-yl) or C_{2-3}alkylR^{45} (wherein R^{45} is pyrrolidin-1-yl or thiomorpholino or 1,1-dioxothiomorpholino, 2-oxopyrrolidin-1-yl, 2-(N-methylcarbamoyl)pyrrolidin-1-yl, 2-(N,N-dimethylcarbamoyl)pyrrolidin-1-yl, 2-carbamoylpyrrolidin-1-yl, 2-oxoimidazolidin-1-yl or 3-methyl-2-oxoimidazolidin-1-yl);

2) 1-R^{50}but-2-en-4-yl (wherein R^{50} is 2-oxoimidazolidin-1-yl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, piperidin-4-yl, 1-methylpiperidin-4-yl, pyrrolidin-1-yl, 1-methylpyrrolidin-3-yl, piperazin-1-yl, morpholino or thiomorpholino or 4-methylpiperazin-1-yl, piperidino or 3-methyl-2-oxoimidazolidin-1-yl);

3) 1-R^{51}but-2-yn-4-yl (wherein R^{51} is 2-oxoimidazolidin-1-yl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, piperidin-4-yl, 1-methylpiperidin-4-yl, pyrrolidin-1-yl, 1-methylpyrrolidin-3-yl, piperazin-1-yl, morpholino or thiomorpholino or 4-methylpiperazin-1-yl, piperidino or 3-methyl-2-oxoimidazolidin-1-yl);

4) C_{2-3}alkylX^2C_{2-3}alkylX^3R^{16} (wherein X^2 and X^3 are as defined hereinbefore and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be $-CH_2-$ when R^4 is C_{2-3}alkylX^2C_{2-3}alkylX^3R^{16}; and

5) C_{1-2}alkoxyC_{2-3}alkyl provided that X^1 is -S-, or X^1 is -SO- or $-SO_2-$; and additionally R^4 may most preferably be selected from the following three groups:

6) C_{2-3}alkylX^5R^{27} (wherein R^{27} is C_{1-2}alkyl and X^5 is -S-, -SO-, $-SO_2-$, $-SO_2NR^{30}-$ or $-NR^{31}SO_2-$ and with the proviso that X^1 is not $-CH_2-$);

7) C_{2-3}alkylX^6C_{2-3}alkylR^{33} (wherein X^6 is as defined hereinbefore and R^{33} represents a group selected from pyrrolidin-1-yl, 4-methylpiperazin-1-yl and morpholino); and

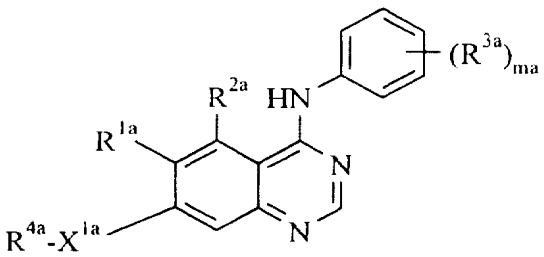
8) C_{2-3}alkylR^{43} (wherein R^{43} is morpholino which may bear one or two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl) with the proviso that when R^4 is C_{2-3}alkylR^{43}, X^1 is -S-, -SO-, $-SO_2-$, $-SO_2NR^9-$ or $-NR^{10}SO_2-$.

An additional most preferred value of R^4 is C_{2-3}alkylX^2methylX^3R^{16} (wherein X^2 and X^3 are as defined hereinbefore and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be $-CH_2-$ when R^4 is C_{2-3}alkylX^2methylX^3R^{16}.

Especially preferred values for the group R^4-X^1 are 3-(methylsulphonyl)propoxy, (1-methylpiperidin-3-yl)methoxy, 4-(pyrrolidin-1-yl)but-2-en-1-yloxy, 2-(2-methoxyethoxy)ethoxy, 3-(1,1-dioxothiomorpholino)propoxy, 2-(2-(pyrrolidin-1-yl)ethoxy)ethoxy, 2-(2-(4-methylpiperazin-1-yl)ethoxy)ethoxy, 3-morpholinopropylthio, 2-([N-methoxyacetyl-N-methyl]amino)ethoxy, 2-(2-oxopyrrolidin-1-yl)ethoxy, 2-thiomorpholinoethoxy, 3-(2-carbamoylpyrrolidin-1-yl)propoxy, 3-(2-oxopyrrolidin-1-yl)propoxy and 2-(2-morpholinoethoxy)ethoxy.

More especially preferred values for the group R^4-X^1 are 3-(methylsulphonyl)propoxy, (1-methylpiperidin-3-yl)methoxy and 4-(pyrrolidin-1-yl)but-2-en-1-yloxy.

In a particular aspect of the present invention there is provided a compound of the formula Ia:



(Ia)

[wherein:

R^{1a} is hydrogen or methoxy;

R^{2a} is hydrogen;

the phenyl group bearing $(R^{3a})_{ma}$ is the 4-chloro-2-fluorophenyl group or the 4-bromo-2-fluorophenyl group;

X^{1a} is -O-, -S-, $-NR^{5a}CO-$ or $-NR^{6a}SO_2-$ (wherein R^{5a} and R^{6a} each independently represents hydrogen or C_{1-2}alkyl);

R^{4a} is selected from one of the following eleven groups:

1) C_{1-4}alkylR^{7a} (wherein R^{7a} is a group selected from 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, pyrrolidin-2-yl and pyrrolidin-3-yl, and piperidin-2-yl, piperidin-3-yl, piperidin-4-yl, morpholin-2-yl, morpholin-3-yl and piperazin-2-yl which group

may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or C_{2-4}alkylR^{8a} (wherein R^{8a} is a group selected from imidazolidin-1-yl, pyrrolidin-1-yl and thiomorpholino which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

2) 1-R^{9a}prop-1-en-3-yl, 1-R^{9a}but-2-en-4-yl, 1-R^{9a}but-1-en-3-yl, 1-R^{9a}pent-2-en-4-yl or 2-R^{9a}pent-3-en-5-yl (wherein R^{9a} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to the alkenyl group through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or 1-R^{10a}but-2-en-4-yl, 1-R^{10a}pent-2-en-4-yl or 2-R^{10a}pent-3-en-5-yl (wherein R^{10a} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to the alkenyl group through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

3) 1-R^{11a}prop-1-yn-3-yl, 1-R^{11a}but-2-yn-4-yl, 1-R^{11a}but-1-yn-3-yl, 1-R^{11a}pent-2-yn-4-yl or 2-R^{11a}pent-3-yn-5-yl (wherein R^{11a} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to the alkynyl group through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or 1-R^{12a}but-2-yn-4-yl, 1-R^{12a}pent-2-yn-4-yl or 2-R^{12a}pent-3-yn-5-yl (wherein R^{12a} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to the alkynyl group through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}

$_3$hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

4) C_{2-3}alkyl$X^{2a}C_{2-3}$alkyl$X^{3a}R^{13a}$ (wherein X^{2a} and X^{3a} which may be the same or different each represents -O-, -S-, -SO-, $-SO_2-$, $-NR^{14a}CO-$, or $-NR^{15a}-$ (wherein R^{14a} and R^{15a} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl) and R^{13a} represents hydrogen or C_{1-3}alkyl);

5) C_{2-3}alkyl$X^{4a}COR^{16a}$ (wherein X^{4a} represents -O- or $-NR^{17a}-$ (wherein R^{17a} represents hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl) and R^{16a} represents $-NR^{18a}R^{19a}$ or $-OR^{20a}$ (wherein R^{18a}, R^{19a} and R^{20a} which may be the same or different each represents hydrogen, C_{1-4}alkyl or C_{1-2}alkoxyethyl));

6) C_{2-3}alkyl$X^{5a}R^{21a}$ (wherein X^{5a} represents carbonyl, -O-, -S-, -SO-, $-SO_2-$, $-NR^{22a}CO-$, $-NR^{23a}SO_2-$ or $-NR^{24a}-$ (wherein R^{22a}, R^{23a} and R^{24a} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl) and R^{21a} represents a group selected from cyclopentyl, cyclohexyl, pyrrolidinyl and piperidinyl which group is linked to X^{5a} through a carbon atom and which group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, C_{1-2}alkoxy, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl or R^{21a} is C_{1-3}alkyl with the proviso that when R^{21a} is C_{1-3}alkyl, X^{5a} is -S-, -SO-, $-SO_2-$ or $-NR^{23a}SO_2-$); and

7) C_{1-2}alkoxyC_{2-3}alkyl provided that X^{1a} is -S-;

8) C_{2-3}alkyl$X^{6a}C_{2-3}$alkylR^{25a} (wherein X^{6a} represents -O-, -S-, -SO-, $-SO_2-$, $-NR^{26a}CO-$, $-NR^{27a}SO_2-$ or $-NR^{28a}-$ (wherein R^{26a}, R^{27a} and R^{28a} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl) and R^{25a} represents a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl, and C_{1-3}alkoxycarbonyl);

9) C_{2-3}alkylR^{29a} (wherein R^{29a} is piperazin-1-yl which bears at least one substituent selected from acetyl, C_{1-2}alkoxycarbonyl, C_{1-2}hydroxyalkyl and $CONR^{30a}R^{31a}$ (wherein R^{30a} and R^{31a} each independently represents hydrogen or C_{1-2}alkyl);

10) C_{2-3}alkylR^{32a} (wherein R^{32a} is morpholino which may bear one or two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-}

$_2$alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl) with the proviso that when R^{4a} is C_{2-3}alkylR^{32a}, X^{1a} is -S- or -$NR^{6a}SO_2$- (wherein R^{6a} is as defined hereinbefore); and

11) C_{2-3}alkylR^{33a} (wherein R^{33a} is morpholino which bears at least one and optionally two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl);

and an additional value of R^{4a} is C_{2-3}alkylX^{2a}methyl$X^{3a}R^{13a}$ (wherein X^{2a} and X^{3a} are as defined hereinbefore and R^{13a} represents hydrogen or C_{1-3}alkyl);]

and salts thereof.

Preferred compounds of the present invention, by virtue of their substantially equivalent activity against VEGF and EGF receptor tyrosine kinases include:

4-(4-chloro-2-fluoroanilino)-7-(1,3-dioxolan-2-ylmethoxy)-6-methoxyquinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-morpholinobut-2-yn-1-yloxy)quinazoline,

(E)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-morpholinobut-2-en-1-yloxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-7-(3-(2,6-dimethylmorpholino)propoxy)-6-methoxyquinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-([N-methyl-N-methylsulphonyl]amino)propoxy)quinazoline,

7-(2-[N-tert-butoxycarbonylamino]ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline,

4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-([N-methyl-N-methylsulphonyl]amino)propoxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-oxoimidazolidin-1-yl)ethoxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(3-oxomorpholino)ethoxy)quinazoline and

4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(3-oxomorpholino)ethoxy)quinazoline

and salts thereof especially hydrochloride salts thereof.

More preferred compounds of the present invention, by virtue of their substantially equivalent activity against VEGF and EGF receptor tyrosine kinases include:

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-thiomorpholinoethoxy)quinazoline,

(S)-4-(4-bromo-2-fluoroanilino)-7-(3-(2-carbamoylpyrrolidin-1-yl)propoxy)-6-methoxyquinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(2-oxopyrrolidin-1-yl)propoxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-oxopyrrolidin-1-yl)ethoxy)quinazoline,

(S)-7-(3-(2-carbamoylpyrrolidin-1-yl)propoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-morpholinoethoxy)ethoxy)quinazoline and

4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-(2-oxopyrrolidin-1-yl)propoxy)quinazoline

and salts thereof especially hydrochloride salts thereof.

Particularly preferred compounds of the present invention, by virtue of their substantially equivalent activity against VEGF and EGF receptor tyrosine kinases include:

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-2-(2-methoxyethoxy)ethoxyquinazoline and

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-yl)methoxyquinazoline

and salts thereof especially hydrochloride salts thereof.

Additional particularly preferred compounds of the present invention, by virtue of their substantially equivalent activity against VEGF and EGF receptor tyrosine kinases include:

4-(4-bromo-2-fluoroanilino)-7-(3-(1,1-dioxothiomorpholino)propoxy)-6-methoxyquinazoline,

4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(2-methoxyethoxy)ethoxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-pyrrolidin-1-ylethoxy)ethoxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-[4-methylpiperazin-1-yl]ethoxy)ethoxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropylthio)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-([N-methyl-N-methoxyacetyl]amino)ethoxy)quinazoline and

4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(2-oxopyrrolidin-1-yl)ethoxy)quinazoline

and salts thereof especially hydrochloride salts thereof.

Especially preferred compounds of the present invention, by virtue of their substantially equivalent activity against VEGF and EGF receptor tyrosine kinases include:

(E)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-(pyrrolidin-1-yl)but-2-en-1-yloxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(methylsulphonyl)propoxy)quinazoline,

(S)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-yl)methoxyquinazoline

and

(R)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-yl)methoxyquinazoline

and salts thereof especially hydrochloride salts thereof, of which 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(methylsulphonyl)propoxy)quinazoline and salts thereof especially hydrochloride salts thereof, is more especially preferred.

In a particular aspect of the present invention preferred compounds are:

4-(3-acetoxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-7-(2-(1,3-dioxolan-2-yl)ethoxy)-6-methoxyquinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-7-(1,3-dioxolan-2-ylmethoxy)-6-methoxyquinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-thiomorpholinoethoxy)quinazoline

and salts thereof especially the hydrochloride salts thereof.

For the avoidance of doubt it is to be understood that where in this specification a group is qualified by 'hereinbefore defined' or 'defined hereinbefore' the said group encompasses the first occurring and broadest definition as well as each and all of the preferred definitions for that group.

In this specification unless stated otherwise the term "alkyl" includes both straight and branched chain alkyl groups but references to individual alkyl groups such as "propyl" are specific for the straight chain version only. An analogous convention applies to other generic terms. Unless otherwise stated the term "alkyl" advantageously refers to chains with 1-6 carbon atoms, preferably 1-4 carbon atoms. The term "alkoxy" as used herein, unless stated otherwise includes "alkyl"-O- groups in which "alkyl" is as hereinbefore defined. The term "aryl" as used herein unless stated otherwise includes reference to a C_{6-10} aryl group which may, if desired, carry one or more substituents selected from halogeno, alkyl, alkoxy, nitro, trifluoromethyl and cyano, (wherein alkyl and alkoxy are as hereinbefore defined). The term "aryloxy" as used herein unless otherwise stated includes "aryl"-O-groups in which "aryl" is as hereinbefore defined. The term "sulphonyloxy" as used herein refers to alkylsulphonyloxy and arylsulphonyloxy groups in which "alkyl" and "aryl" are as hereinbefore defined. The term "alkanoyl" as used herein unless otherwise stated includes formyl and alkylC=O groups in which "alkyl" is as defined hereinbefore, for example C_2alkanoyl is ethanoyl and refers to $CH_3C{=}O$, C_1alkanoyl is formyl and refers to CHO. In this specification unless stated otherwise the term "alkenyl" includes both straight and branched chain alkenyl groups but references to

individual alkenyl groups such as 2-butenyl are specific for the straight chain version only. Unless otherwise stated the term "alkenyl" advantageously refers to chains with 2-5 carbon atoms, preferably 3-5 carbon atoms. In this specification unless stated otherwise the term "alkynyl" includes both straight and branched chain alkynyl groups but references to individual alkynyl groups such as 2-butynyl are specific for the straight chain version only. Unless otherwise stated the term "alkynyl" advantageously refers to chains with 2-5 carbon atoms, preferably 3-5 carbon atoms.

In formula I, as hereinbefore defined, hydrogen will be present at positions 2 and 8 of the quinazoline group.

Within the present invention it is to be understood that a compound of the formula I or a salt thereof may exhibit the phenomenon of tautomerism and that the formulae drawings within this specification can represent only one of the possible tautomeric forms. It is to be understood that the invention encompasses any tautomeric form which inhibits VEGF receptor tyrosine kinase activity and is not to be limited merely to any one tautomeric form utilised within the formulae drawings.

It is also to be understood that certain compounds of the formula I and salts thereof can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms which inhibit VEGF receptor tyrosine kinase activity.

For the avoidance of any doubt, it is to be understood that when X^1 is, for example, a group of formula $-NR^7CO-$, it is the nitrogen atom bearing the R^7 group which is attached to the quinazoline ring and the carbonyl (CO) group is attached to R^4, whereas when X^1 is, for example, a group of formula $-CONR^8-$, it is the carbonyl group which is attached to the quinazoline ring and the nitrogen atom bearing the R^8 group is attached to R^4. A similar convention applies to the other two atom X^1 linking groups such as $-NR^{10}SO_2-$ and $-SO_2NR^9-$. When X^1 is $-NR^{11}-$ it is the nitrogen atom bearing the R^{11} group which is linked to the quinazoline ring and to R^4. An analogous convention applies to other groups, thus when R^4 is, for example, a group of the formula $C_{1-5}alkylX^5R^{27}$ and X^5 is a group $-NR^{28}CO-$, it is the nitrogen atom bearing the R^{28} group which is attached to the alkyl chain which is attached to the quinazoline ring and the carbonyl (CO) group is attached to R^{27}, whereas when X^5 is, for example, a group of formula $-CONR^{29}-$, it is the carbonyl group which is attached to the alkyl

chain which is attached to the quinazoline ring and the nitrogen atom bearing the R^{29} group is attached to R^{27}. It is further to be understood that when X^1 represents $-NR^{11}-$ and R^{11} is C_{1-3}alkoxyC_{2-3}alkyl it is the C_{2-3}alkyl moiety which is linked to the nitrogen atom of X^1 and an analogous convention applies to other groups.

For the avoidance of any doubt, it is to be understood that in a compound of the formula I when R^4 is, for example, a group of formula C_{1-5}alkylX^2C_{1-5}alkylX^3R^{16}, it is the terminal C_{1-5}alkyl moiety which is bound to X^1, similarly when R^4 is, for example, a group of formula C_{2-5}alkenylR^{14} it is the C_{2-5}alkenyl moiety which is bound to X^1 and an analgous convention applies to other groups. When R^4 is a group 1-R^{33}prop-1-en-3-yl it is the first carbon to which the group R^{33} is attached and it is the third carbon which is linked to X^1, similarly when R^4 is a group 2-R^{33}pent-3-en-5-yl it is the second carbon to which the group R^{33} is attached and it is the fifth carbon which is linked to X^1, and an analogous convention applies to other groups.

The present invention relates to the compounds of formula I as hereinbefore defined as well as to the salts thereof. Salts for use in pharmaceutical compositions will be pharmaceutically acceptable salts, but other salts may be useful in the production of the compounds of formula I and their pharmaceutically acceptable salts. Pharmaceutically acceptable salts of the invention may, for example, include acid addition salts of the compounds of formula I as hereinbefore defined which are sufficiently basic to form such salts. Such acid addition salts include for example salts with inorganic or organic acids affording pharmaceutically acceptable anions such as with hydrogen halides (especially hydrochloric or hydrobromic acid of which hydrochloric acid is particularly preferred) or with sulphuric or phosphoric acid, or with trifluoroacetic, citric or maleic acid. In addition where the compounds of formula I are sufficiently acidic, pharmaceutically acceptable salts may be formed with an inorganic or organic base which affords a pharmaceutically acceptable cation. Such salts with inorganic or organic bases include for example an alkali metal salt, such as a sodium or potassium salt, an alkaline earth metal salt such as a calcium or magnesium salt, an ammonium salt or for example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

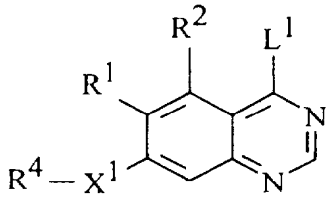
A compound of the formula I, or salt thereof, and other compounds of the invention (as hereinafter defined) may be prepared by any process known to be applicable to the

preparation of chemically-related compounds. Such processes include, for example, those illustrated in European Patent Applications, Publication Nos. 0520722, 0566226, 0602851 and 0635498. Such processes, are provided as a further feature of the invention and are as described hereinafter. Necessary starting materials may be obtained by standard procedures of organic chemistry. The preparation of such starting materials is described within the accompanying non-limiting Examples. Alternatively necessary starting materials are obtainable by analogous procedures to those illustrated which are within the ordinary skill of an organic chemist.

Thus the following processes (a) to (g) and (i) to (v) constitute further features of the present invention.

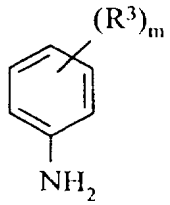
Synthesis of Compounds of Formula I

(a) Compounds of the formula I and salts thereof may be prepared by the reaction of a compound of the formula III:



(III)

(wherein R^1, R^2, X^1 and R^4 are as defined hereinbefore and L^1 is a displaceable moiety), with a compound of the formula IV:



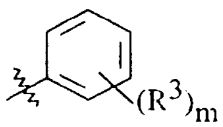
(IV)

(wherein R^3 and m are as defined hereinbefore) whereby to obtain compounds of the formula I and salts thereof. A convenient displaceable moiety L^1 is, for example, a halogeno, alkoxy (preferably C_{1-4}alkoxy), aryloxy or sulphonyloxy group, for example a chloro, bromo, methoxy, phenoxy, methanesulphonyloxy or toluene-4-sulphonyloxy group.

The reaction is advantageously effected in the presence of either an acid or a base. Such an acid is, for example, an anhydrous inorganic acid such as hydrogen chloride. Such a base is, for example, an organic amine base such as, for example, pyridine, 2,6-lutidine, collidine, 4-dimethylaminopyridine, triethylamine, morpholine, N-methylmorpholine or diazabicyclo[5.4.0]undec-7-ene, or for example, an alkali metal or alkaline earth metal carbonate or hydroxide, for example sodium carbonate, potassium carbonate, calcium carbonate, sodium hydroxide or potassium hydroxide. Alternatively such a base is, for example, an alkali metal hydride, for example sodium hydride, or an alkali metal or alkaline earth metal amide, for example sodium amide or sodium bis(trimethylsilyl)amide. The reaction is preferably effected in the presence of an inert solvent or diluent, for example an alkanol or ester such as methanol, ethanol, 2-propanol or ethyl acetate, a halogenated solvent such as methylene chloride, trichloromethane or carbon tetrachloride, an ether such as tetrahydrofuran or 1,4-dioxan, an aromatic hydrocarbon solvent such as toluene, or a dipolar aprotic solvent such as N,N-dimethylformamide, N,N-dimethylacetamide, N-methylpyrrolidin-2-one or dimethylsulphoxide. The reaction is conveniently effected at a temperature in the range, for example, 10 to 150°C, preferably in the range 20 to 80°C.

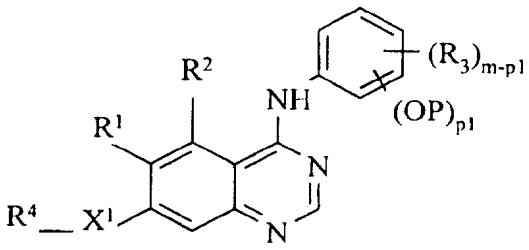
The compound of the invention may be obtained from this process in the form of the free base or alternatively it may be obtained in the form of a salt with the acid of the formula H-L^1 wherein L^1 has the meaning defined hereinbefore. When it is desired to obtain the free base from the salt, the salt may be treated with a base as defined hereinbefore using a conventional procedure.

(b) Where the group of formula IIa:



(IIa)

(wherein R^3 and m are as hereinbefore defined) represents a phenyl group carrying one or more hydroxy groups, a compound of the formula I and salts thereof can be prepared by the deprotection of a compound of formula V:

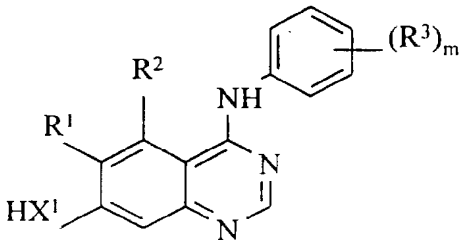


(V)

(wherein X^1, m, R^1, R^2, R^3 and R^4 are as hereinbefore defined, P represents a phenolic hydroxy protecting group and p1 is an integer from 1 to 5 equal to the number of protected hydroxy groups and such that m-p1 is equal to the number of R^3 substituents which are not protected hydroxy). The choice of phenolic hydroxy protecting group P is within the standard knowledge of an organic chemist, for example those included in standard texts such as "Protective Groups in Organic Synthesis" T.W. Greene and R.G.M.Wuts, 2nd Ed. Wiley 1991, including ethers (for example, methyl, methoxymethyl, allyl and benzyl and benzyl substituted with up to two substituents selected from C_{1-4}alkoxy and nitro), silyl ethers (for example, t-butyldiphenylsilyl and t-butyldimethylsilyl), esters (for example, acetate and benzoate) and carbonates (for example, methyl and benzyl and benzyl substituted with up to two substituents selected from C_{1-4}alkoxy and nitro). The removal of such a phenolic hydroxy protecting group may be effected by any of the procedures known for such a transformation, including those reaction conditions indicated in standard texts such as that indicated hereinbefore, or by a related procedure. The reaction conditions preferably being such that the hydroxy derivative is produced without unwanted reactions at other sites within the starting or product compounds. For example, where the protecting group P is acetate, the transformation may conveniently be effected by treatment of the quinazoline derivative with a base as defined hereinbefore and including ammonia, and its mono and di-alkylated derivatives, preferably in the presence of a protic solvent or co-solvent such as water or an alcohol, for example methanol or ethanol. Such

a reaction can be effected in the presence of an additional inert solvent or diluent as defined hereinbefore and at a temperature in the range 0 to 50°C, conveniently at about 20°C.

(c) Production of those compounds of formula I and salts thereof wherein the substituent X^1 is -O-, -S- or $-NR^{11}$- or $-SO_2$-, $-CONR^8$- or $-SO_2NR^9$- can be achieved by the reaction, conveniently in the presence of a base as defined hereinbefore, of a compound of the formula VI:



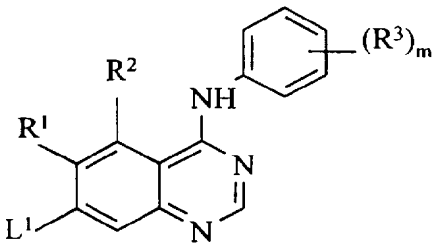
(VI)

(wherein m, X^1, R^1, R^2 and R^3 are as hereinbefore defined) with a compound of formula VII:

$$R^4\text{-}L^1 \qquad \text{(VII)}$$

(wherein R^4 and L^1 are as hereinbefore defined); L^1 is a displaceable moiety for example a halogeno or sulphonyloxy group such as a bromo or methanesulphonyloxy group. Conveniently L^1 is a group $O\text{-}^+P(R^{52})_3$ (wherein R^{52} is butyl or phenyl) and in such cases the compound of formula VII is conveniently formed in situ. The reaction is preferably effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), advantageously at a temperature in the range, for example 10 to 150°C, conveniently at about 50°C.

(d) Compounds of the formula I and salts thereof may be prepared by the reaction of a compound of the formula VIII:



(VIII)

with a compound of the formula IX:

$$R^4\text{-}X^1\text{-}H \qquad \text{(IX)}$$

(wherein L^1, R^1, R^2, R^3, R^4, m and X^1 are all as hereinbefore defined). The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), advantageously at a temperature in the range, for example 10 to 150°C, conveniently at about 100°C.

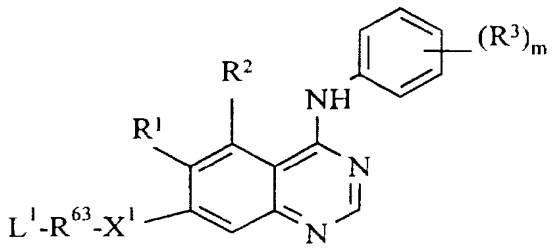
(e) Compounds of the formula I and salts thereof wherein R^4 is $C_{1-5}alkylR^{53}$, [wherein R^{53} is selected from one of the following three groups:

1) X^7R^{27} (wherein X^7 represents -O-, -S-, -SO_2-, -$NR^{54}CO$-, -$NR^{55}SO_2$- or -NR^{56}- (wherein R^{54}, R^{55} and R^{56} each independently represents hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$) and R^{27} is as defined hereinbefore); and

2) $X^8C_{1-5}alkylX^3R^{16}$ (wherein X^8 represents -O-, -S-, -SO_2-, -$NR^{57}CO$-, -$NR^{58}SO_2$- or -NR^{59}- (wherein R^{57}, R^{58} and R^{59} each independently represents hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$) and X^3 and R^{16} are as defined hereinbefore); and

3) $X^9C_{1-5}alkylR^{33}$ (wherein X^9 represents -O-, -S-, -SO_2-, -$NR^{60}CO$-, -$NR^{61}SO_2$- or -NR^{62}- (wherein R^{60}, R^{61} and R^{62} each independently represents hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$) and R^{33} is as defined hereinbefore);]

may be prepared by reacting a compound of the formula X:



(X)

(wherein L^1, X^1, R^1, R^2, R^3 and m are as hereinbefore defined and R^{63} is C_{1-5}alkyl) with a compound of the formula XI:

$$R^{53}\text{-}H \qquad \text{(XI)}$$

(wherein R^{53} is as defined hereinbefore) to give a compound of the formula I. The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), and at a temperature in the range, for example 0 to 150°C, conveniently at about 50°C.

Compounds of the formula I wherein R^4 is C_{2-5}alkylR^{45}, (wherein R^{45} is as defined hereinbefore), may be prepared by reacting a compound of formula X (wherein R^{63} is C_{2-5}alkyl) with a compound of the formula XIa:

$$R^{45}\text{-}H \qquad \text{(XIa)}$$

(wherein R^{45} is as defined hereinbefore) to give a compound of the formula I. The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), and at a temperature in the range, for example 0 to 150°C, conveniently at about 50°C.

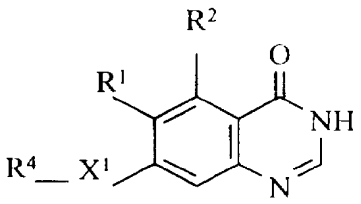
(f) The production of those compounds of the formula I and salts thereof wherein the substituent R^1 is represented by $-NR^5R^6$, where one or both of R^5 and R^6 are C_{1-3}alkyl, may be effected by the reaction of compounds of formula I wherein the substituent R^1 is an amino group and an alkylating agent, preferably in the presence of a base as defined hereinbefore.

Such alkylating agents are C_{1-3}alkyl moieties bearing a displaceable moiety as defined hereinbefore such as C_{1-3}alkyl halides for example C_{1-3}alkyl chloride, bromide or iodide. The reaction is preferably effected in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)) and at a temperature in the range, for example, 10 to 100°C, conveniently at about ambient temperature.

(g) The production of compounds of formula I and salts thereof wherein one or more of the substituents R^1, R^2 or R^3 is an amino group may be effected by the reduction of a corresponding compound of formula I wherein the substituent(s) at the corresponding position(s) of the quinazoline and/or aniline ring is/are a nitro group(s). The reduction may conveniently be effected as described in process (i) hereinafter. The production of a compound of formula I and salts thereof wherein the substituent(s) at the corresponding position(s) of the quinazoline and/or aniline ring is/are a nitro group(s) may be effected by the processes described hereinbefore and hereinafter in processes (a-e) and (i-v) using a quinazoline compound selected from the compounds of the formulae (I-XXVII) in which the substituent(s) at the corresponding position(s) of the quinazoline and/or aniline ring is/are a nitro group(s).

Synthesis of Intermediates

(i) The compounds of formula III and salts thereof, constitute a further feature of the present invention. Such compounds in which L^1 is halogeno may for example be prepared by halogenating a compound of the formula XII:



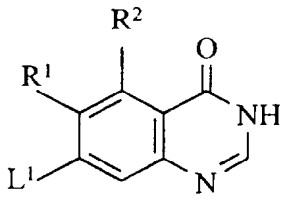
(XII)

(wherein R^1, R^2, R^4 and X^1 are as hereinbefore defined).

Convenient halogenating agents include inorganic acid halides, for example thionyl chloride, phosphorus(III)chloride, phosphorus(V)oxychloride and phosphorus(V)chloride. The halogenation reaction is conveniently effected in the presence of an inert solvent or diluent such

as for example a halogenated solvent such as methylene chloride, trichloromethane or carbon tetrachloride, or an aromatic hydrocarbon solvent such as benzene or toluene. The reaction is conveniently effected at a temperature in the range, for example 10 to 150°C, preferably in the range 40 to 100°C.

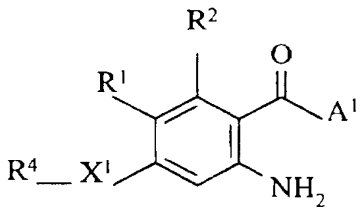
The compounds of formula XII and salts thereof which constitute a further feature of the present invention may for example be prepared by reacting a compound of the formula XIII:



(XIII)

(wherein R^1, R^2 and L^1 are as hereinbefore defined) with a compound of the formula IX as hereinbefore defined. The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), advantageously at a temperature in the range, for example 10 to 150°C, conveniently at about 100°C.

The compounds of formula XII and salts thereof may also be prepared by cyclising a compound of the formula XIV:

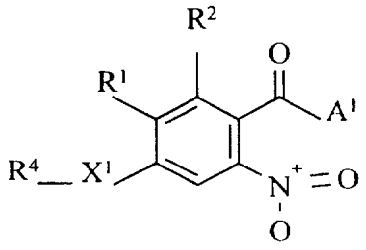


(XIV)

(wherein R^1, R^2, R^4 and X^1, are as hereinbefore defined, and A^1 is an hydroxy, alkoxy (preferably C_{1-4}alkoxy) or amino group) whereby to form a compound of formula XII or salt thereof. The cyclisation may be effected by reacting a compound of the formula XIV, where A^1

is an hydroxy or alkoxy group, with formamide or an equivalent thereof effective to cause cyclisation whereby a compound of formula XII or salt thereof is obtained, such as [3-(dimethylamino)-2-azaprop-2-enylidene]dimethylammonium chloride. The cyclisation is conveniently effected in the presence of formamide as solvent or in the presence of an inert solvent or diluent such as an ether for example 1,4-dioxan. The cyclisation is conveniently effected at an elevated temperature, preferably in the range 80 to 200°C. The compounds of formula XII may also be prepared by cyclising a compound of the formula XIV, where A^1 is an amino group, with formic acid or an equivalent thereof effective to cause cyclisation whereby a compound of formula XII or salt thereof is obtained. Equivalents of formic acid effective to cause cyclisation include for example a tri-C_{1-4}alkoxymethane, for example triethoxymethane and trimethoxymethane. The cyclisation is conveniently effected in the presence of a catalytic amount of an anhydrous acid, such as a sulphonic acid for example p-toluenesulphonic acid, and in the presence of an inert solvent or diluent such as for example a halogenated solvent such as methylene chloride, trichloromethane or carbon tetrachloride, an ether such as diethylether or tetrahydrofuran, or an aromatic hydrocarbon solvent such as toluene. The cyclisation is conveniently effected at a temperature in the range, for example 10 to 100°C, preferably in the range 20 to 50°C.

Compounds of formula XIV and salts thereof, which constitute a further feature of the present invention, may for example be prepared by the reduction of the nitro group in a compound of the formula XV:

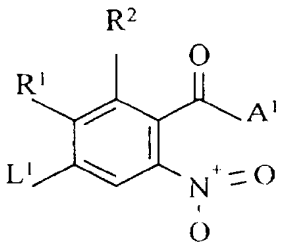


(XV)

(wherein R^1, R^2, R^4, X^1 and A^1 are as hereinbefore defined) to yield a compound of formula XIV as hereinbefore defined. The reduction of the nitro group may conveniently be effected by

any of the procedures known for such a transformation. The reduction may be carried out, for example, by the hydrogenation of a solution of the nitro compound in the presence of an inert solvent or diluent as defined hereinbefore in the presence of a metal effective to catalyse hydrogenation reactions such as palladium or platinum. A further reducing agent is, for example, an activated metal such as activated iron (produced for example by washing iron powder with a dilute solution of an acid such as hydrochloric acid). Thus, for example, the reduction may be effected by heating the nitro compound and the activated metal in the presence of a solvent or diluent such as a mixture of water and alcohol, for example methanol or ethanol, to a temperature in the range, for example 50 to 150°C, conveniently at about 70°C.

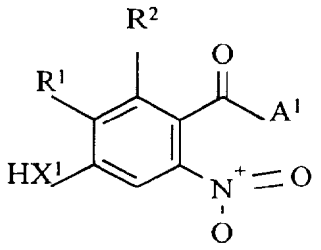
Compounds of the formula XV and salts thereof which constitute a further feature of the present invention, may for example be prepared by the reaction of a compound of the formula XVI:



(XVI)

(wherein R^1, R^2, L^1 and A^1 are as hereinbefore defined) with a compound of the formula IX as hereinbefore defined to give a compound of the formual XV. The reaction of the compounds of formulae XVI and IX is conveniently effected under conditions as described for process (d) hereinbefore.

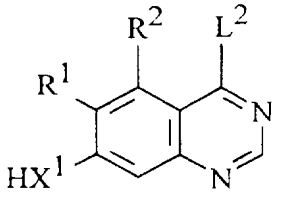
Compounds of formula XV and salts thereof, may for example also be prepared by the reaction of a compound of the formula XVII:



(XVII)

(wherein R^1, R^2, X^1 and A^1 are as hereinbefore defined with the proviso that X^1 is not $-CH_2-$) with a compound of the formula VII as hereinbefore defined to yield a compound of formula XV as hereinbefore defined. The reaction of the compounds of formulae XVII and VII is conveniently effected under conditions as described for process (c) hereinbefore.

The compounds of formula III and salts thereof may also be prepared for example by reacting a compound of the formula XVIII:

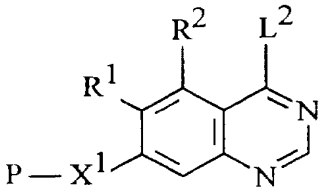


(XVIII)

(wherein R^1, R^2 and X^1 are as hereinbefore defined with the proviso that X^1 is not $-CH_2-$ and L^2 represents a displaceable protecting moiety) with a compound of the formula VII as hereinbefore defined, whereby to obtain a compound of formula III in which L^1 is represented by L^2.

A compound of formula XVIII is conveniently used in which L^2 represents a phenoxy group which may if desired carry up to 5 substituents, preferably up to 2 substituents, selected from halogeno, nitro and cyano. The reaction may be conveniently effected under conditions as described for process (c) hereinbefore.

The compounds of formula XVIII and salts thereof as hereinbefore defined may for example be prepared by deprotecting a compound of the formula XIX:

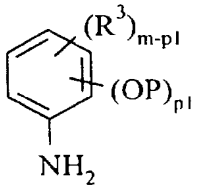


(XIX)

(wherein R^1, R^2, P, X^1 and L^2 are as hereinbefore defined with the proviso that X^1 is not $-CH_2-$). Deprotection may be effected by techniques well known in the literature, for example where P represents a benzyl group deprotection may be effected by hydrogenolysis or by treatment with trifluoroacetic acid.

One compound of formula III may if desired be converted into another compound of formula III in which the moiety L^1 is different. Thus for example a compound of formula III in which L^1 is other than halogeno, for example optionally substituted phenoxy, may be converted to a compound of formula III in which L^1 is halogeno by hydrolysis of a compound of formula III (in which L^1 is other than halogeno) to yield a compound of formula XII as hereinbefore defined, followed by introduction of halide to the compound of formula XII, thus obtained as hereinbefore defined, to yield a compound of formula III in which L^1 represents halogen.

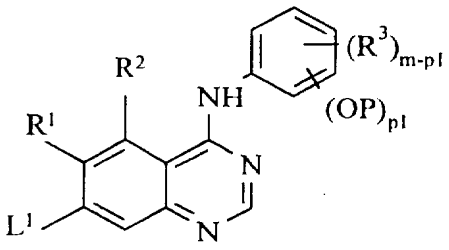
(ii) The compounds of formula V and salts thereof, constitute a further feature of the present invention, and may for example be prepared by the reaction of a compound of formula III as hereinbefore defined with a compound of the formula XX:



(XX)

(wherein R^3, m, p1 and P are as hereinbefore defined). The reaction may for example be effected as described for process (a) hereinbefore.

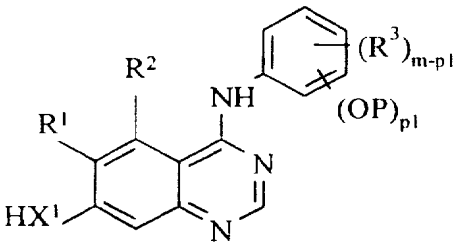
The compounds of formula V and salts thereof may also be prepared by reacting a compound of formula XXI:



(XXI)

(wherein R^1, R^2, L^1, R^3, m, p1 and P are as hereinbefore defined) with a compound of formula IX as hereinbefore defined. The reaction may for example be effected as described for process (d) above.

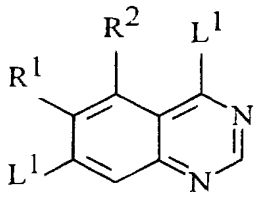
The compounds of formula V and salts thereof may also be prepared by reacting a compound of formula XXII:



(XXII)

(wherein R^1, R^2, R^3, X^1, P, p1 and m are as hereinbefore defined with the proviso that X^1 is not -CH_2-) with a compound of the formula VII as hereinbefore defined. The reaction may for example be effected as described for process (c) hereinbefore.

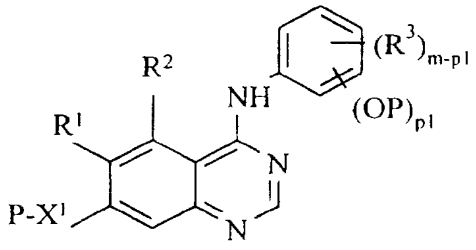
The compounds of formula XXI and salts thereof may for example be prepared by reaction of a compound of formula XXIII:



(XXIII)

(wherein R^1, R^2, and L^1 are as hereinbefore defined, and L^1 in the 4- and 7- positions may be the same or different) with a compound of the formula XX as hereinbefore defined. The reaction may be effected for example by a process as described in (a) above.

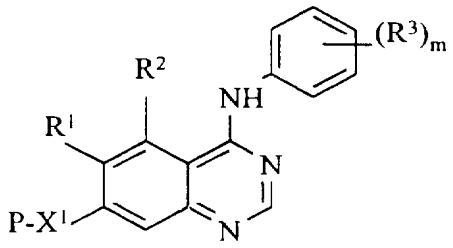
Compounds of the formula XXII and salts thereof may be made by reacting compounds of the formulae XIX and XX as hereinbefore defined, under conditions described in (a) hereinbefore, to give a compound of formula XXIV:



(XXIV)

(wherein R^1, R^2, R^3, P, X^1, p1 and m are as hereinbefore defined with the proviso that X^1 is not -CH_2-) and then deprotecting the compound of formula XXIV for example as described in (i) above.

(iii) Compounds of the formula VI as hereinbefore defined and salts thereof may be made by deprotecting the compound of formula XXV:



(XXV)

(wherein R^1, R^2, R^3, P, X^1 and m are as hereinbefore defined) by a process for example as described in (i) above.

Compounds of the formula XXV and salts thereof may be made by reacting compounds of the formulae XIX and IV as hereinbefore defined, under the conditions described in (a) hereinbefore, to give a compound of the formula XXV or salt thereof.

(iv) Compounds of the formula VIII and salts thereof as hereinbefore defined may be made by reacting compounds of the formulae XXIII and IV as hereinbefore defined, the reaction for example being effected by a process as described in (a) above.

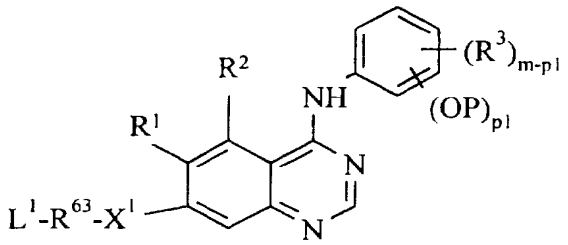
(v) Compounds of the formula X as defined hereinbefore and salts thereof may for example be made by the reaction of a compound of formula VI as defined hereinbefore with a compound of the formula XXVI:

$$L^1\text{-}R^{63}\text{-}L^1$$

(XXVI)

(wherein L^1 and R^{63} are as hereinbefore defined) to give a compound of the formula X. The reaction may be effected for example by a process as described in (c) above.

Compounds of the formula X and salts thereof may also be made for example by deprotecting a compound of the formula XXVII:



(XXVII)

(wherein L^1, R^{63}, X^1, R^1, R^2, R^3, P, m and p1 are as defined hereinbefore) by a process for example as described in (b) above.

Compounds of the formula XXVII and salts thereof may be made for example by reacting compounds of the formulae XXII and XXVI as defined hereinbefore, under the conditions described in (c) above.

When a pharmaceutically acceptable salt of a compound of the formula I is required, it may be obtained, for example, by reaction of said compound with, for example, an acid using a conventional procedure, the acid having a pharmaceutically acceptable anion.

Many of the intermediates defined herein are novel, for example, those of the formulae III, V, XII, XIV and XV, and these are provided as a further feature of the invention.

Intermediates of the formulae VIII, X, XXI, XXII, XXIV, XXV and XXVII are also provided as a further feature of the invention.

The identification of compounds which potently inhibit the tyrosine kinase activity associated with the VEGF receptors such as Flt and/or KDR and which inhibit angiogenesis and/or increased vascular permeability is desirable and is the subject of the present invention. These properties may be assessed, for example, using one or more of the procedures set out below:

(a) In Vitro Receptor Tyrosine Kinase Inhibition Test

This assay determines the ability of a test compound to inhibit tyrosine kinase activity. DNA encoding VEGF or epidermal growth factor (EGF) receptor cytoplasmic domains may be obtained by total gene synthesis (Edwards M, International Biotechnology Lab 5(3), 19-25, 1987) or by cloning. These may then be expressed in a suitable expression system to obtain polypeptide with tyrosine kinase activity. For example VEGF and EGF

receptor cytoplasmic domains, which were obtained by expression of recombinant protein in insect cells, were found to display intrinsic tyrosine kinase activity. In the case of the VEGF receptor Flt (Genbank accession number X51602), a 1.7kb DNA fragment encoding most of the cytoplasmic domain, commencing with methionine 783 and including the termination codon, described by Shibuya et al (Oncogene, 1990, 5: 519-524), was isolated from cDNA and cloned into a baculovirus transplacement vector (for example pAcYM1 (see The Baculovirus Expression System: A Laboratory Guide, L.A. King and R. D. Possee, Chapman and Hall, 1992) or pAc360 or pBlueBacHis (available from Invitrogen Corporation)). This recombinant construct was co-transfected into insect cells (for example Spodoptera frugiperda 21(Sf21)) with viral DNA (eg Pharmingen BaculoGold) to prepare recombinant baculovirus. (Details of the methods for the assembly of recombinant DNA molecules and the preparation and use of recombinant baculovirus can be found in standard texts for example Sambrook et al, 1989, Molecular cloning - A Laboratory Manual, 2nd edition, Cold Spring Harbour Laboratory Press and O'Reilly et al, 1992, Baculovirus Expression Vectors - A Laboratory Manual, W. H. Freeman and Co, New York). For other tyrosine kinases for use in assays, cytoplasmic fragments starting from methionine 806 (KDR, Genbank accession number L04947) and methionine 668 (EGF receptor, Genbank accession number X00588) may be cloned and expressed in a similar manner.

For expression of cFlt tyrosine kinase activity, Sf21 cells were infected with plaque-pure cFlt recombinant virus at a multiplicity of infection of 3 and harvested 48 hours later. Harvested cells were washed with ice cold phosphate buffered saline solution (PBS) (10mM sodium phosphate pH7.4, 138mM sodium chloride, 2.7mM potassium chloride) then resuspended in ice cold HNTG/PMSF (20mM Hepes pH7.5, 150mM sodium chloride, 10% v/v glycerol, 1% v/v Triton X100, 1.5mM magnesium chloride, 1mM ethylene glycol-bis(βaminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), 1mM PMSF (phenylmethylsulphonyl fluoride); the PMSF is added just before use from a freshly-prepared 100mM solution in methanol) using 1ml HNTG/PMSF per 10 million cells. The suspension was centrifuged for 10 minutes at 13,000 rpm at 4°C, the supernatant (enzyme stock) was removed and stored in aliquots at -70°C. Each new batch of stock enzyme was titrated in the assay by dilution with enzyme diluent (100mM Hepes pH 7.4, 0.2mM sodium orthovanadate,

0.1% v/v Triton X100, 0.2mM dithiothreitol). For a typical batch, stock enzyme is diluted 1 in 2000 with enzyme diluent and 50µl of dilute enzyme is used for each assay well.

A stock of substrate solution was prepared from a random copolymer containing tyrosine, for example Poly (Glu, Ala, Tyr) 6:3:1 (Sigma P3899), stored as 1 mg/ml stock in PBS at -20°C and diluted 1 in 500 with PBS for plate coating.

On the day before the assay 100µl of diluted substrate solution was dispensed into all wells of assay plates (Nunc maxisorp 96-well immunoplates) which were sealed and left overnight at 4°C.

On the day of the assay the substrate solution was discarded and the assay plate wells were washed once with PBST (PBS containing 0.05% v/v Tween 20) and once with 50mM Hepes pH7.4.

Test compounds were diluted with 10% dimethylsulphoxide (DMSO) and 25µl of diluted compound was transferred to wells in the washed assay plates. "Total" control wells contained 10% DMSO instead of compound. Twenty five microlitres of 40mM manganese(II)chloride containing 8µM adenosine-5'-triphosphate (ATP) was added to all test wells except "blank" control wells which contained manganese(II)chloride without ATP. To start the reactions 50µl of freshly diluted enzyme was added to each well and the plates were incubated at room temperature for 20 minutes. The liquid was then discarded and the wells were washed twice with PBST. One hundred microlitres of mouse IgG anti-phosphotyrosine antibody (Upstate Biotechnology Inc. product 05-321), diluted 1 in 6000 with PBST containing 0.5% w/v bovine serum albumin (BSA), was added to each well and the plates were incubated for 1 hour at room temperature before discarding the liquid and washing the wells twice with PBST. One hundred microlitres of horse radish peroxidase (HRP)-linked sheep anti-mouse Ig antibody (Amersham product NXA 931), diluted 1 in 500 with PBST containing 0.5% w/v BSA, was added and the plates were incubated for 1 hour at room temperature before discarding the liquid and washing the wells twice with PBST. One hundred microlitres of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) solution, freshly prepared using one 50mg ABTS tablet (Boehringer 1204 521) in 50ml freshly prepared 50mM phosphate-citrate buffer pH5.0 + 0.03% sodium perborate (made with 1 phosphate citrate buffer with sodium perborate (PCSB) capsule (Sigma P4922) per 100ml distilled water), was added to each well. Plates were then incubated for 20-60 minutes at

room temperature until the optical density value of the "total" control wells, measured at 405nm using a plate reading spectrophotometer, was approximately 1.0. "Blank" (no ATP) and "total" (no compound) control values were used to determine the dilution range of test compound which gave 50% inhibtion of enzyme activity.

(b) In Vitro HUVEC Proliferation Assay

This assay determines the ability of a test compound to inhibit the growth factor-stimulated proliferation of human umbilical vein endothelial cells (HUVEC).

HUVEC cells were isolated in MCDB 131 (Gibco BRL) + 7.5% v/v foetal calf serum (FCS) and were plated out (at passage 2 to 8), in MCDB 131 + 2% v/v FCS + 3μg/ml heparin + 1μg/ml hydrocortisone, at a concentration of 1000 cells/well in 96 well plates. After a minimum of 4 hours they were dosed with the appropriate growth factor (i.e. VEGF 3ng/ml, EGF 3ng/ml or b-FGF 0.3ng/ml) and compound. The cultures were then incubated for 4 days at 37°C with 7.5% carbon dioxide. On day 4 the cultures were pulsed with 1μCi/well of tritiated-thymidine (Amersham product TRA 61) and incubated for 4 hours. The cells were harvested using a 96-well plate harvester (Tomtek) and then assayed for incorporation of tritium with a Beta plate counter. Incorporation of radioactivity into cells, expressed as cpm, was used to measure inhibition of growth factor-stimulated cell proliferation by compounds.

(c) In Vivo Rat Uterine Oedema Assay

This test measures the capacity of compounds to reduce the acute increase in uterine weight in rats which occurs in the first 4-6 hours following oestrogen stimulation. This early increase in uterine weight has long been known to be due to oedema caused by increased permeability of the uterine vasculature and recently Cullinan-Bove and Koos (Endocrinology, 1993,133:829-837) demonstrated a close temporal relationship with increased expression of VEGF mRNA in the uterus. We have found that prior treatment of the rats with a neutralising monoclonal antibody to VEGF significantly reduces the acute increase in uterine weight, confirming that the increase in weight is substantially mediated by VEGF.

Groups of 20 to 22-day old rats were treated with a single subcutaneous dose of oestradiol benzoate (2.5μg/rat) in a solvent, or solvent only. The latter served as unstimulated controls. Test compounds were orally administered at various times prior to the administration of oestradiol benzoate. Five hours after the administration of oestradiol benzoate the rats were humanely sacrificed and their uteri were dissected, blotted and weighed. The increase in

uterine weight in groups treated with test compound and oestradiol benzoate and with oestradiol benzoate alone was compared using a Student T test. Inhibition of the effect of oestradiol benzoate was considered significant when $p<0.05$.

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises a compound of the formula I as defined hereinbefore or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable excipient or carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) for example as a sterile solution, suspension or emulsion, for topical administration for example as an ointment or cream or for rectal administration for example as a suppository. In general the above compositions may be prepared in a conventional manner using conventional excipients.

The compositions of the present invention are advantageously presented in unit dosage form. The compound will normally be administered to a warm-blooded animal at a unit dose within the range 5-5000mg per square metre body area of the animal, i.e. approximately 0.1-100mg/kg. A unit dose in the range, for example, 1-100mg/kg, preferably 1-50mg/kg is envisaged and this normally provides a therapeutically-effective dose. A unit dose form such as a tablet or capsule will usually contain, for example 1-250mg of active ingredient.

According to a further aspect of the present invention there is provided a compound of the formula I or a pharmaceutically acceptable salt thereof as defined hereinbefore for use in a method of treatment of the human or animal body by therapy.

We have found that compounds of the present invention inhibit VEGF receptor tyrosine kinase activity and are therefore of interest for their antiangiogenic effects and/or their ability to cause a reduction in vascular permeability.

A further feature of the present invention is a compound of formula I, or a pharmaceutically acceptable salt thereof, for use as a medicament, conveniently a compound of formula I, or a pharmaceutically acceptable salt thereof, for use as a medicament for producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal such as a human being.

Thus according to a further aspect of the invention there is provided the use of a compound of the formula I, or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for use in the production of an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal such as a human being.

According to a further feature of the invention there is provided a method for producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal, such as a human being, in need of such treatment which comprises administering to said animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof as defined hereinbefore.

As stated above the size of the dose required for the therapeutic or prophylactic treatment of a particular disease state will necessarily be varied depending on the host treated, the route of administration and the severity of the illness being treated. Preferably a daily dose in the range of 1-50mg/kg is employed. However the daily dose will necessarily be varied depending upon the host treated, the particular route of administration, and the severity of the illness being treated. Accordingly the optimum dosage may be determined by the practitioner who is treating any particular patient.

The antiangiogenic and/or vascular permeability reducing treatment defined hereinbefore may be applied as a sole therapy or may involve, in addition to a compound of the invention, one or more other substances and/or treatments. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate administration of the individual components of the treatment. In the field of medical oncology it is normal practice to use a combination of different forms of treatment to treat each patient with cancer. In medical oncology the other component(s) of such conjoint treatment in addition to the antiangiogenic and/or vascular permeability reducing treatment defined hereinbefore may be: surgery, radiotherapy or chemotherapy. Such chemotherapy may cover three main categories of therapeutic agent:

(i) other antiangiogenic agents that work by different mechanisms from those defined hereinbefore (for example linomide, inhibitors of integrin $\alpha v\beta 3$ function, angiostatin, razoxin, thalidomide);

(ii) cytostatic agents such as antioestrogens (for example tamoxifen,toremifene, raloxifene, droloxifene, iodoxyfene), progestogens (for example megestrol acetate), aromatase inhibitors

(for example anastrozole, letrazole, vorazole, exemestane), antiprogestogens, antiandrogens (for example flutamide, nilutamide, bicalutamide, cyproterone acetate), LHRH agonists and antagonists (for example goserelin acetate, luprolide), inhibitors of testosterone 5α-dihydroreductase (for example finasteride), anti-invasion agents (for example metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator receptor function) and inhibitors of growth factor function, (such growth factors include for example platelet derived growth factor and hepatocyte growth factor such inhibitors include growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors and serine/threonine kinase inhibitors); and

(iii) antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as antimetabolites (for example antifolates like methotrexate, fluoropyrimidines like 5-fluorouracil, purine and adenosine analogues, cytosine arabinoside); antitumour antibiotics (for example anthracyclines like doxorubicin, daunomycin, epirubicin and idarubicin, mitomycin-C, dactinomycin, mithramycin); platinum derivatives (for example cisplatin, carboplatin); alkylating agents (for example nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide, nitrosoureas, thiotepa); antimitotic agents (for example vinca alkaloids like vincristine and taxoids like taxol, taxotere); topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan).

As stated above the compounds defined in the present invention are of interest for their antiangiogenic and/or vascular permeability reducing effects. Such compounds of the invention are expected to be useful in a wide range of disease states including cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation and ocular diseases with retinal vessel proliferation. In particular such compounds of the invention are expected to slow advantageously the growth of primary and recurrent solid tumours of, for example, the colon, breast, prostate, lungs and skin. More particularly such compounds of the invention are expected to inhibit the growth of those primary and recurrent solid tumours which are associated with VEGF especially those tumours which are significantly dependent on VEGF for their growth and spread, including for example, certain tumours of the colon, breast, prostate, lung, vulva and skin.

In addition to their use in therapeutic medicine, the compounds of formula I and their pharmaceutically acceptable salts are also useful as pharmacological tools in the development and standardisation of in vitro and in vivo test systems for the evaluation of the effects of inhibitors of VEGF receptor tyrosine kinase activity in laboratory animals such as cats, dogs, rabbits, monkeys, rats and mice, as part of the search for new therapeutic agents.

It is to be understood that where the term "ether" is used anywhere in this specification it refers to diethyl ether.

The invention will now be illustrated in the following non-limiting Examples in which, unless otherwise stated:-

[(i) evaporations were carried out by rotary evaporation in vacuo and work-up procedures were carried out after removal of residual solids such as drying agents by filtration;

(ii) operations were carried out at ambient temperature, that is in the range 18-25°C and under an atmosphere of an inert gas such as argon;

(iii) column chromatography (by the flash procedure) and medium pressure liquid chromatography (MPLC) were performed on Merck Kieselgel silica (Art. 9385) or Merck Lichroprep RP-18 (Art. 9303) reversed-phase silica obtained from E. Merck, Darmstadt, Germany;

(iv) yields are given for illustration only and are not necessarily the maximum attainable;

(v) melting points are uncorrected and were determined using a Mettler SP62 automatic melting point apparatus, an oil-bath apparatus or a Koffler hot plate apparatus.

(vi) the structures of the end-products of the formula I were confirmed by nuclear (generally proton) magnetic resonance (NMR) and mass spectral techniques; proton magnetic resonance chemical shift values were measured on the delta scale and peak multiplicities are shown as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; q, quartet;

(vii) intermediates were not generally fully characterised and purity was assessed by thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), infra-red (IR) or NMR analysis;

(viii) the following abbreviations have been used:-

DMF N,N-dimethylformamide

DMSO dimethylsulphoxide

THF tetrahydrofuran

TFA trifluoroacetic acid

NMP 1-methyl-2-pyrrolidinone.]

Example 1

Potassium carbonate (2.2g, 16mmol) was added to a solution of 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline (1.51g, 4mmol) in DMF (30ml) and the mixture stirred for 15 minutes. 2-Bromoethyl methyl ether (667mg, 4.8mmol) was then added dropwise. The mixture was stirred for 1 hour at ambient temperature, then heated at 60°C for 17 hours and finally allowed to cool. The insoluble material was removed by filtration and the filter pad washed with DMF. The filtrate was partitioned between ethyl acetate and water, the organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (95/5 followed by 93/7). The purified product was triturated with ether to give **4-(3-acetoxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline** (1.34g, 84%) as a white powder.

m.p. 180-183°C

^{1}H NMR Spectrum: ($CDCl_3$) 2.16(s, 3H); 2.34(s, 3H); 3.47(s, 3H); 3.87(t, 2H); 3.99(s, 3H); 4.31(t, 2H); 6.98(s, 1H); 7.21(d, 1H); 7.24(d, 1H); 7.42(d, 1H); 7.50(s, 1H); 8.64(s, 1H)

MS - ESI: 420 $[MNa]^+$

Elemental analysis:	Found	C 63.1	H 6.1	N 10.4
$C_{21}H_{23}N_3O_5$	Requires	C 63.5	H 5.8	N 10.6%

The starting material was prepared as follows:

A mixture of 2-amino-4-benzyloxy-5-methoxybenzamide (J. Med. Chem. 1977, vol 20, 146-149, 10g, 0.04mol) and Gold's reagent (7.4g, 0.05mol) in dioxane (100ml) was stirred and heated at reflux for 24 hours. Sodium acetate (3.02g, 0.037mol) and acetic acid (1.65ml, 0.029mol) were added to the reaction mixture and it was heated for a further 3 hours. The mixture was evaporated, water was added to the residue, the solid was filtered off, washed with

water and dried ($MgSO_4$). Recrystallisation from acetic acid gave 7-benzyloxy-6-methoxy-3,4-dihydroquinazolin-4-one (8.7g, 84%).

A mixture of 7-benzyloxy-6-methoxy-3,4-dihydroquinazolin-4-one (2.82g, 0.01mol), thionyl chloride (40ml) and DMF (0.28ml) was stirred and heated to reflux for 1 hour. The mixture was evaporated, the residue was taken up in toluene and evaporated to dryness to give 7-benzyloxy-4-chloro-6-methoxyquinazoline (3.45g).

Acetic anhydride (1.9ml, 20.3mmol) was added to a mixture of 2-methyl-5-nitrophenol (2.5g, 16.3mmol) and 1M aqueous sodium hydroxide (24.5ml) at ambient temperature. The mixture was stirred for 40 minutes, the solid was removed by filtration and the filtrate extracted with ethyl acetate. The organic layers were combined, washed with an aqueous saturated sodium chloride solution, dried ($MgSO_4$) and evaporated to yield 2-acetoxy-4-nitrotoluene (3.1g, 100%). A mixture of this material (3.1g, 15.9mmol) and 10% palladium-on-charcoal catalyst (0.12g) in ethyl acetate (50ml) was stirred at ambient temperature under an atmosphere of hydrogen for 2 hours. The catalyst was removed by filtration and the filtrate evaporated to give 3-acetoxy-4-methylaniline (2.45g, 94%).

A mixture of 7-benzyloxy-4-chloro-6-methoxyquinazoline (2.18g, 7.25mmol), 3-acetoxy-4-methylaniline (1.32g, 8mmol) and 2-propanol (50ml) was stirred and heated to reflux for 1 hour. The mixture was cooled to ambient temperature. The precipitate was collected by filtration, washed with 2-propanol and ether to give 4-(3-acetoxy-4-methylanilino)-7-benzyloxy-6-methoxyquinazoline. A mixture of 4-(3-acetoxy-4-methylanilino)-7-benzyloxy-6-methoxyquinazoline (2.68g, 5.75mmol), 10% palladium-on-charcoal catalyst (0.27g) in methanol (50ml), DMF (12ml) and trichloromethane (50ml) was stirred at ambient temperature under 1.5 atmospheres of hydrogen for 30 minutes. The catalyst was removed by filtration and the filtrate evaporated. The residual solid was triturated in ether, collected by filtration and dried under vacuum at 50°C to give 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline (2.1g, 100%).

Example 2

A solution of 2-(2-bromoethyl)-1,3-dioxolane (258mg, 1.4mmol) in DMF (0.5ml) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (329mg, 1.02mmol) and potassium carbonate (264mg, 2mmol) in DMF (2ml). The mixture

was heated at 100°C for 3 hours and allowed to cool. The volatiles were removed by evaporation, and the residue partitioned between aqueous sodium hydrogen carbonate solution and methylene chloride. The organic phase was separated and passed through phase separating paper. The solvent was removed by evaporation, and the residue was purified by column chromatography eluting with methylene chloride/methanol (95/5) to give **4-(4-chloro-2-fluoroanilino)-7-(2-(1,3-dioxolan-2-yl)ethoxy)-6-methoxyquinazoline** (71mg, 17%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.1(m, 2H,); 3.8(m, 2H); 3.95(m, 5H); 4.25(t, 2H); 5.05(t, 1H); 7.18(s, 1H); 7.3(m, 1H); 7.55(m, 2H); 7.8(s, 1H); 8.35(s, 1H); 9.5(s, 1H)

MS - ESI: 420 $[MH]^+$

Elemental analysis:	Found	C 57.4	H 4.7	N 9.1
$C_{20}H_{19}N_3O_4ClF$	Requires	C 57.2	H 5.6	N 10.0%

The starting material was prepared as follows:

A solution of 7-benzyloxy-4-chloro-6-methoxyquinazoline (1.2g, 4mmol), (prepared as described for the starting material in Example 1), and 4-chloro-2-fluoroaniline (444µl, 4mmol) in 2-propanol (40ml) was refluxed for 1.5 hours. After cooling, the precipitate was collected by filtration, washed with 2-propanol then ether and dried under vacuum to give 7-benzyloxy-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (1.13g, 64%).

m.p. 239-242°C

^{1}H NMR Spectrum: ($DMSOd_6$) 4.0(s, 3H); 5.36(s, 2H); 7.39-7.52(m, 9H); 8.1(s, 1H); 8.75(s, 1H)

MS - ESI: 410 $[MH]^+$

Elemental analysis:	Found	C 59.2	H 4.3	N 9.4
$C_{22}H_{17}N_3O_2ClF$ 1HCl	Requires	C 59.2	H 4.1	N 9.41%

A solution of 7-benzyloxy-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (892mg, 2mmol) in TFA (10ml) was refluxed for 50 minutes. After cooling, the mixture was poured onto ice. The precipitate was collected by filtration, dissolved in methanol (10ml) and basified to pH11 with aqueous ammonia. After concentration by evaporation, the solid product was collected by filtration, washed with water then ether and dried under vacuum to give 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline as a yellow solid (460mg, 72%).

m.p. 141-143°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.95(s, 3H); 7.05(s, 1H); 7.35(d, 1H); 7.54-7.59(m, 2H); 7.78(s, 1H); 8.29(s, 1H)

MS - ESI: 320-322 $[MH]^+$

Example 3

1-(2-Chloroethyl)pyrrolidine hydrochloride (200mg, 1.2mmol) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (403mg, 1.26mmol), (prepared as described for the starting material in Example 2), and potassium carbonate (650mg, 4.7mmol) in DMF (4ml). The mixture was heated to 100°C and further portions of 1-(2-chloroethyl)pyrrolidine hydrochloride (800mg in total) were added periodically over 4 hours while the reaction mixture was maintained at 100°C. The reaction was then allowed to cool and volatiles were removed by evaporation. The residue was partitioned between methylene chloride and water, separated and the organic phase passed through phase separating paper. Column chromatography eluting with methylene chloride/methanol (95/5) gave **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)quinazoline** (50mg, 10%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.8-2.1(m, 4H); 3.1(m, 2H); 3.55-3.7(m, 4H); 4.05(s, 3H); 4.6(t, 2H); 7.4(m, 2H); 7.58(d, 1H); 7.65(dt, 1H); 8.5(s, 1H); 8.8(s, 1H)

MS - ESI: 417 $[MH]^+$

Elemental analysis:	Found	C 60.2	H 5.4	N 12.3
$C_{21}H_{22}N_4O_2ClF$	Requires	C 60.5	H 5.3	N 13.4%

Example 4

A solution of 1-(3-chloropropyl)pyrrolidine (230mg, 0.96mmol) was added to 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (295mg, 0.92mmol), (prepared as described for the starting material in Example 2), and potassium carbonate (130mg, 0.94mmol) in DMF (8ml). The mixture was heated at 100°C for 90 minutes and allowed to cool. The volatiles were removed by evaporation and the residues were partitioned between water and methylene chloride. The organic phase was separated and passed through phase separating paper, and the solvent was removed under reduced pressure. The residue was

dissolved in acetone and hydrogen chloride in ether (2ml of a 1M solution, 2mmol) was added. The mixture was stirred at ambient temperature for 30 minutes and the resulting precipitate was collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazoline** hydrochloride hydrate (320mg, 67%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.8-2.0(m, 6H); 3-3.6(m, 6H); 4.05(s, 3H); 4.3(t, 2H); 7.4(m, 2H); 7.55(d, 1H); 7.6(m, 1H); 8.4(s, 1H); 8.8(s, 1H)

MS - ESI: 431 $[MH]^+$

Elemental analysis:	Found	C 51.0	H 5.9	N 10.6	
$C_{22}H_{24}N_4O_2ClF\ 1.8H_2O\ 1.5HCl$	Requires	C 51.0	H 5.7	N 10.8%	

The starting material was prepared as follows:

Pyrrolidine (3g, 42mmol) was added to a solution of 1-bromo-3-chloropropane (3.2g, 20mmol) in toluene (20ml). The mixture was stirred at ambient temperature overnight and then heated at 60°C for 4 hours. The mixture was allowed to cool and the precipitate removed by filtration. The bulk of toluene was removed by evaporation to give an oil. ^{1}H NMR indicated the oil was a 1:1 mol:mol mixture of toluene and 1-(3-chloropropyl)pyrrolidine. This material was used without further purification.

^{1}H NMR Spectrum: ($CDCl_3$) 1.75(m, 4H); 2.0(q, 2H); 2.35(s, 3H, toluene); 2.45-2.6(m, 6H); 3.6(t, 2H); 7.15-7.3(m, 5H, toluene)

Example 5

A solution of 2-(2-methoxyethoxy)ethanol (90mg, 0.75mmol) in methylene chloride (1ml) was added to tributylphosphine (320mg, 1.58mmol) and 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (200mg, 0.63mmol), (prepared as described for the starting material in Example 2), in methylene chloride (6ml) at 0°C under argon. To the resulting mixture 1,1'-(azodicarbonyl)dipiperidine (400mg, 1.6mmol) was added in portions. The mixture was allowed to warm to ambient temperature and stirred under argon for 2 hours. Ether (5ml) was added, and the precipitated solids were removed by filtration. The volatiles were removed from the filtrate by evaporation, and the residue was purified by column chromatography eluting with methylene chloride/methanol (90/10). The resulting partially purified product was dissolved in acetone, and ethereal hydrogen chloride (0.6ml of a 1M

solution, 0.6mmol) added. The resulting precipitated product was collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-methoxyethoxy)ethoxy)quinazoline** hydrochloride (128mg, 44%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.25(s, 3H); 3.45(dd, 2H); 3.6(dd, 2H); 3.8(t, 2H); 4.0(s, 3H); 4.3(t, 2H); 7.4(s, 1H); 7.45(dd, 1H); 7.55-7.7(m, 2H); 8.3(s, 1H); 8.75(s, 1H); 11.5(br s, 1H)

MS - ESI: 422 $[MH]^+$

Elemental analysis :	Found	C 52.3	H 4.7	N 9.1
$C_{20}H_{21}N_3O_4ClF$ 1HCl	Requires	C 52.4	H 4.8	N 9.2%

Example 6

A solution of 2-(bromomethyl)-1,3-dioxolane (190mg, 1.1mmol) in DMF (1ml) was added to 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (258mg, 0.81mmol), (prepared as described for the starting material in Example 2), and potassium carbonate (200mg, 1.5mmol) in DMF (2ml). The mixture was heated at 100°C for 4 hours and then allowed to cool. The volatiles were removed by evaporation and the residue partitioned between water and methylene chloride. The organic phase was separated, passed through phase separating paper and purified by column chromatography eluting with methylene chloride/methanol (95/5) to give **4-(4-chloro-2-fluoroanilino)-7-(1,3-dioxolan-2-ylmethoxy)-6-methoxyquinazoline** (130mg, 38%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.8-4.1(m, 7H); 4.15(d, 2H); 5.30(t, 1H); 7.22(s, 1H); 7.30(m, 1H); 7.55(m, 2H); 7.80(s, 1H); 8.35(s, 1H); 9.55(s, 1H). Also contained 0.3 methanol.

MS - ESI: 406 $[MH]^+$

Elemental analysis:	Found	C 55.1	H 4.5	N 9.5
$C_{19}H_{17}N_3O_4ClF$ 0.3H_2O	Requires	C 55.1	H 4.5	N 10.0%

0.3Methanol

Example 7

A mixture of 6,7-dimethoxy-5-nitro-3,4-dihydroquinazolin-4-one (1.75g, 7.0mmol) and thionyl chloride (25ml) and DMF (3 drops) was heated at reflux for 2 hours. Excess thionyl chloride was removed by evaporation and the residue azeotroped with toluene. 3-Hydroxy-4-methylaniline (0.94g, 7.6mmol) in 2-propanol (40ml) was added to the residue

and the mixture heated at reflux for 2 hours. The mixture was allowed to cool, the precipitate collected by filtration, washed with 2-propanol and dried to give **6,7-dimethoxy-4-(3-hydroxy-4-methylanilino)-5-nitroquinazoline** hydrochloride (2.02g, 81%).

m.p. 206-208°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.90(s, 3H); 4.05(s, 1H); 6.50(d,1H); 6.65(s,1H); 6.97(d, 1H); 7.57(s, 1H); 8.15(s, 1H)

MS - ESI: 357 $[MH]^+$

Elemental analysis:	Found	C 52.0	H 4.3	N 13.9
$C_{17}H_{16}N_4O_5$ 1HCl	Requires	C 52.0	H 4.3	N 14.3%

The starting material was prepared as follows:

A mixture of 4,5-dimethoxyanthranilic acid (19.7g) and formamide (10ml) was stirred and heated at 190°C for 5 hours. The mixture was allowed to cool to approximately 80°C and water (50ml) was added. The mixture was stored at ambient temperature for 3 hours. The precipitate was isolated, washed with water and dried to give 6,7-dimethoxy-3,4-dihydroquinazolin-4-one (3.65g).

Fuming nitric acid (47ml) was added to 6,7-dimethoxy-3,4-dihydroquinazolin-4-one (10g, 48mmol), in water (40ml). The reaction mixture was heated at 120°C for 1 hour, then allowed to cool and diluted with water. The resulting precipitate was collected by filtration, washed with water and dried to give 6,7-dimethoxy-5-nitro-3,4-dihydroquinazolin-4-one (3.9g, 32%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.87(s, 3H); 4.05(s, 1H); 7.42(s, 1H); 8.13(s, 1H)

MS - ESI: 251 $[MH]^+$

Example 8

Sodium (148mg, 6.4mmol) was added to 2-methoxyethanol (10ml), the mixture stirred for 15 minutes to give a complete solution and the volatiles removed by evaporation. The residue was dissolved in DMSO (5ml) and 7-chloro-4-(4-chloro-2-fluoroanilino)-6-nitroquinazoline hydrochloride (500mg, 1.3mmol) was added. The mixture was stirred at ambient temperature for 18 hours then diluted with a solution of acetic acid (1ml) in water (20ml). The resulting precipitate was collected by filtration, washed with water, dried and

purified by column chromatography eluting with methylene chloride/methanol (96/4). The purified product was recrystallised from methylene chloride/isohexane to give **4-(4-chloro-2-fluoroanilino)-7-(2-methoxyethoxy)-6-nitroquinazoline** (304mg, 60%) as a yellow solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.15(s, 3H); 3.60(m, 2H); 4.31(m, 2H); 7.24(m, 1H); 7.4-7.5(m, 3H); 8.42(s, 1H); 9.03(s, 1H)

MS - ESI: 393 $[MH]^+$

Elemental analysis:	Found	C 51.8	H 3.7	N 14.1
$C_{17}H_{14}N_4O_4ClF$	Requires	C 52.0	H 3.6	N 14.3%

The starting material was prepared as follows:

A mixture of 7-chloro-6-nitro-3,4-dihydroquinazolin-4-one (40g, 0.18mol), (J. Org. Chem. 1975, 40, 356), phosphorus oxychloride (50ml) and DMF (1ml) in thionyl chloride (300ml) was heated at reflux for 4 hours. The reaction mixture was allowed to cool and the volatiles removed by evaporation and by azeotroping with toluene. The residue was basified with aqueous sodium hydrogen carbonate solution and extracted with methylene chloride (4x100ml). The extracts were combined, washed with brine and filtered through phase separating paper. The solvent was removed by evaporation and the residue triturated with ether/isohexane to give 4,7-dichloro-6-nitroquinazoline (35.2g, 81%) as a pale yellow solid.

A mixture of 4,7-dichloro-6-nitroquinazoline (24.4g, 0.1mol), 4-chloro-2-fluoroaniline and ethereal hydrogen chloride (100ml of a 1M solution) in 2-propanol (600ml) was heated at reflux for 1.5 hours. The mixture was allowed to cool and diluted with acetone. The solid product was collected by filtration, washed with acetone and dried to give 7-chloro-4-(4-chloro-2-fluoroanilino)-6-nitroquinazoline hydrochloride (35.0g, 90%) as a yellow powder.

MS - ESI: 353 $[MH]^+$

Example 9

Triphenylphosphine (410mg, 1.5mmol) and 1-methyl-3-pyrrolidinol (0.128ml, 1.5mmol) were added to a solution of 4-(chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (250mg, 0.78mmol), (prepared as described for the starting material in Example 2), in methylene chloride (4ml). Diethyl azodicarboxylate (0.246ml, 1.5mmol) was

added dropwise and the reaction mixture was stirred for 1 hour at ambient temperature. Additional triphenylphosphine (61mg, 0.23 mmol) followed by diethyl azodicarboxylate (37µl, 0.23mmol) was added and the mixture was stirred for 15 minutes at ambient temperature. The solvent was removed by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol (80/20) followed by methylene chloride/methanol/triethylamine (80/20/0.5). The purified product was dissolved in methylene chloride/methanol and the insolubles were removed by filtration. A solution of hydrogen chloride in 2-propanol (0.5ml of a 5M solution) was added to the filtrate and the volatiles were removed by evaporation. The residue was triturated with 2-propanol and ether, collected by filtration and dried to give **4-(4-chloro-2-fluoranilino)-6-methoxy-7-(1-methylpyrrolidin-3-yloxy)quinazoline** hydrochloride hydrate (149mg, 40%).

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD) 2.13-2.83(m, 2H); 2.92(s, 3H); 2.99(s, 3H); 3.20-3.32(m, 1H); 3.44-3.59(m, 1H); 3.72-3.81(m, 1H); 3.96-4.14(m, 2H); 4.01 (s, 3H); 5.35-5.43(m, 1H); 7.42-7.47(m, 2H); 7.58-7.63(m, 2H); 8.21(s, 1H); 8.88(s, 1H)

MS - ESI: 403 $[MH]^+$

Elemental analysis:	Found	C 48.8	H 5.2	N 11.0
$C_{20}H_{20}N_4O_2ClF$ $1H_2O$ 2HCl	Requires	C 48.7	H 4.9	N 11.4%

Example 10

4-(Chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (250mg, 0.78mmol), (prepared as described for the starting material in Example 2), and triphenylphosphine (512mg, 1.9mmol) were added to a stirred solution of 4-morpholino-2-butyn-1-ol (182mg, 1.1mmol), (J. Am. Chem. Soc. 1957, 79, 6184), in methylene chloride (4ml) under argon. Diethyl azodicarboxylate (0.307ml, 1.9mmol) was added dropwise and the reaction mixture was stirred for 30 minutes at ambient temperature. Additional 4-morpholino-2-butyn-1-ol (60mg, 0.39mmol), triphenylphosphine (102mg, 0.39mmol) and followed by diethyl azodicarboxylate (61µl, 0.39mmol) were added and the mixture was stirred for a further 15 minutes at ambient temperature. The solvent was removed by evaporation and the residue was purified by column chromatography eluting with methylene chloride/acetonitrile/methanol (60/37/3 followed by 60/35/5 and 55/37/8). The resulting purified oil was dissolved in a mixture of methylene chloride and methanol and ethereal

hydrogen chloride (1ml of a 2.9M solution) was added. The volatiles were removed by evaporation, the solid residue suspended in ether and collected by filtration. The product was recrystallised from 2-propanol/methanol/ether, collected by filtration, washed with 2-propanol and ether and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-morpholinobut-2-yn-1-yloxy)quinazoline** hydrochloride hydrate (75mg, 18%).

m.p. 175-178°C

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.10(m, 2H); 3.46(m, 2H); 3.72(m, 2H); 3.99(m, 2H); 4.03(s, 3H); 4.29(s, 2H); 5.28(s, 2H); 7.47(dd, 2H); 7.62(s, 1H); 7.62(t, 1H); 7.69(dd, 1H); 8.29(s, 1H); 8.89(s, 1H)

MS - ESI: 457 $[MH]^+$

Elemental analysis:	Found	C 50.8	H 4.9	N 10.3
$C_{23}H_{22}N_4O_3ClF\ 1H_2O\ 2HCl$	Requires	C 50.4	H 4.7	N 10.2%

Example 11

Tetrakis(triphenylphosphine)palladium(0) (23mg, 0.02mmol) followed by a solution of sodium triisopropylsilylthiolate (102mg, 0.48mmol), (Tetrahedron.Lett. 1994, 35, 3221), in THF (2ml) was added to a stirred solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(trifluoromethylsulphonyloxy)quinazoline (180mg, 0.4mmol) in THF (2ml) and benzene (2ml) under argon. The reaction mixture was heated at reflux for 2 hours and then allowed to cool. 2-Bromoethyl methyl ether (83mg, 0.6mmol) in DMF (1ml) and then a solution of tetrabutylammonium fluoride in THF (0.5ml of a 1M solution, 0.5 mmol) were added dropwise and the reaction mixture was stirred at ambient temperature for 30 minutes. The mixture was diluted with ethyl acetate, washed with water and brine, dried ($MgSO_4$) and the volatiles removed by evaporation. The residue was purified by column chromatography on neutral alumina eluting with methylene chloride/acetone (95/5). The purified product was triturated with ether, collected by filtration and dissolved in methylene chloride (4ml). Ethereal hydrogen chloride (0.4ml of 3M solution) was added, the solution was diluted with ether and the resulting precipitate was collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethylthio)quinazoline** hydrochloride (80mg, 46%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.33(t, 2H); 3.34(s, 3H); 3.71(t, 2H); 4.07(s, 3H); 7.48(dd, 1H); 7.64(t, 1H); 7.69(dd, 1H); 7.73(s, 1H); 8.10(s, 1H); 8.89(s, 1H).

MS - ESI: 394 $[MH]^+$

Elemental analysis:	Found	C 50.1	H 4.3	N 9.8	S 7.3	
$C_{18}H_{17}N_3O_2ClFS$ 1HCl	Requires	C 50.2	H 4.2	N 9.8	S 7.4%	

The starting material was prepared as follows:

Trifluoromethanesulphonic anhydride (0.55ml, 3.3mmol) was added to a stirred suspension of 4-(chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (959mg, 3mmol), (prepared as described for the starting material in Example 2), in methylene chloride (2.2ml) and pyridine (2.2ml) under argon at 0°C. The reaction mixture was stirred for 1 hour at 0°C, allowed to warm to ambient temperature and stirred for a further 1.5 hours. The volatiles were removed by evaporation, the residue was dissolved in ethyl acetate, washed with dilute hydrochloric acid and brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was triturated with ether/petroleum ether to give 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(trifluoromethylsulphonyloxy)quinazoline (270mg, 60%) as a beige solid.

^1H NMR Spectrum: ($DMSOd_6$) 4.07(s, 3H); 7.39(dd, 1H); 7.57-7.62(m, 2H); 7.92(s, 1H); 8.21(s, 1H); 8.49(s, 1H)

MS - ESI: 452 $[MH]^+$

Example 12

4-(2-Hydroxyethyl)thiomorpholine (114mg, 0.78mmol), (J. Am. Chem. Soc. 1934, 56, 1720), in methylene chloride (1ml) followed by 1,1'-(azodicarbonyl)dipiperidine (525mg, 2.08mmol) were added to a stirred solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (225mg, 0.70mmol), (prepared as described for the starting material in Example 2), and tributylphosphine (0.51ml, 2.08mmol) in methylene chloride (10ml) under nitrogen. The mixture was stirred for 3.5 hours and allowed to stand for a further 18 hours. Ether (8ml) was added, the precipitate removed by filtration and the solvent removed from the filtrate by evaporation. The residue was dissolved in acetone and ethereal hydrogen chloride (2.5ml of a 1M solution) added. The precipitated product was collected by filtration and purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (150/8/1). The purified product was triturated with ether to give **4-(4-chloro-2-**

fluoroanilino)-6-methoxy-7-(2-thiomorpholinoethoxy)quinazoline (70mg, 22%) as a pale yellow solid.

m.p. 181-182°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.56(t, 2H); 3.92(s, 3H); 4.59(t, 2H); 7.31(dd, 1H); 7.35(s, 1H); 7.46(d, 1H); 7.53(dd, 1H); 8.33(s, 1H); 8.68(s, 1H); 11.7(br s, 1H)

MS - ESI: 449 $[MH]^+$

Elemental analysis:	Found	C 56.4	H 5.1	N 12.3	
$C_{21}H_{22}N_4O_2ClFS$	Requires	C 56.2	H 4.9	N 12.5%	

Example 13

A solution of (R)-(1-methylpiperidin-3-yl)methanol (2.29g, 18mmol) in methylene chloride (10ml) was added to a stirred mixture of 4-(chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (4.0g, 12.5mmol), (prepared as described for the starting material in Example 2), and triphenylphosphine (9.81g, 37.5mmol) in methylene chloride (200ml). Diethyl azodicarboxylate (5.87ml, 37mmol) was added dropwise and the reaction mixture was stirred for 18 hours at ambient temperature. The volatiles were removed by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (a gradient from 100/0/0 to 85/15/0.1). The purified product was triturated with ethyl acetate, collected by filtration, washed with ethyl acetate and dried to give **(R)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-yl)methoxyquinazoline** (2.78g, 52%).

$[\alpha]_D$ +11.7°

^{1}H NMR Spectrum: ($DMSOd_6$) 1.08(m, 1H); 1.50(m, 1H); 1.64(m, 1H); 1.80(m, 3H); 2.07(m, 1H); 2.16(s, 3H); 2.62(d, 1H); 2.81(d, 1H); 3.92(s, 3H); 4.02(d, 2H); 7.18(s, 1H); 7.32(d, 1H); 7.55(m, 2H); 7.79(s, 1H); 8.34(s, 1H); 9.50(s, 1H)

MS - ESI: 431 $[MH]^+$

Elemental analysis:	Found	C 60.7	H 5.4	N 13.3	
$C_{22}H_{24}N_4O_2ClF$	Requires	C 61.3	H 5.6	N 13.0%	

The starting material was prepared as follows:

(R)-Ethyl nipecotate (5.7g 365mmol), (prepared by resolution of ethyl nipecotate by treatment with L(+)-tartaric acid as described in J. Org. Chem. 1991, (56), 1168), was dissolved in 38.5% aqueous formaldehyde solution (45ml) and formic acid (90ml) and the mixture heated at reflux for 18 hours. The mixture was allowed to cool and added dropwise to cooled saturated aqueous sodium hydrogen carbonate solution. The mixture was adjusted to pH12 by addition of sodium hydroxide and the mixture was extracted with methylene chloride. The organic extract was washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation to give (R)-ethyl 1-methylpiperidine-3-carboxylate (4.51g, 73%) as a colourless oil.

MS - ESI: 172 $[MH]^+$

A solution of (R)-ethyl 1-methylpiperidine-3-carboxylate (5.69g, 33mmol) in ether (20ml) was added dropwise to a stirred solution of lithium aluminium hydride (36.6ml of a 1M solution in THF, 36.6mmol) in ether (85ml) cooled to maintain a reaction temperature of 20°C. The mixture was stirred for 1.5 hours at ambient temperature and then water (1.4ml), 15% aqueous sodium hydroxide solution (1.4ml) and then water (4.3ml) were added. The insolubles were removed by filtration and the volatiles removed from the filtrate by evaporation to give (R)-(1-methylpiperidin-3-yl)methanol (4.02g, 94%) as a colourless oil.

^1H NMR Spectrum: ($DMSOd_6$) 1.06(q, 1H); 1.51-1.94(m, 5H); 2.04(s, 3H); 2.34(br s, 1H); 2.62(m, 1H); 2.78(d, 1H); 3.49(m, 1H); 3.59(m, 1H)

MS - ESI: 130 $[MH]^+$

Example 14

Using a method analogous to that in Example 13, (S)-(1-methylpiperidin-3-yl)methanol (185g, 1.1mmol), (prepared as described for the starting material in Example 13 but resolving with D(-)-tartaric acid), was treated with 4-(chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (319mg, 1mmol), (prepared as described for the starting material in Example 2), triphenylphosphine (785mg, 3mmol) and diethyl azodicarboxylate (0.475ml, 3mmol) to give, after work-up and purification, **(S)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-yl)methoxyquinazoline** (187mg, 44%).

Example 15

The final compounds in Examples 13 and 14 may be mixed, in any relative proportions, for example to give a racemic mixture.

Alternatively the racemate may be made as follows:

1,1'-(Azodicarbonyl)dipiperidine (560mg, 2.2mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (240mg, 0.75mmol), (prepared as described for the starting material in Example 2), 1-methyl-3-piperidinemethanol (115mg, 0.89mmol) and tributylphosphine (440mg, 2.2mmol) in methylene chloride (10ml). The mixture was stirred for 18 hours, diluted with ether and the resulting precipitate was removed by filtration. The volatiles were removed from the filtrate by evaporation, and the residue was dissolved in acetone and ethereal hydrogen chloride (1.5ml of a 1M solution, 1.5mmol) was added. The precipitated product was collected by filtration and purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (75/8/1). The purified solid product was triturated with ether collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-ylmethoxy)quinazoline** (105mg, 33%).

m.p. 211-212°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.08(m, 1H); 1.50(m, 1H); 1.78(m, 4H); 2.08(m, 1H); 2.16(m, 3H); 2.62(m, 1H); 2.82(m, 1H); 3.95(s, 3H); 4.00(d, 2H); 7.18(s, 1H); 7.32(m, 1H); 7.52(dd, 1H); 7.58(t, 1H); 7.79(s, 1H); 8.35(s, 1H); 9.52(s, 1H)

MS - ESI: 431 $[MH]^+$

Elemental analysis:	Found	C 59.9	H 5.5	N 12.9
$C_{22}H_{24}N_4O_2ClF\ 0.5H_2O$	Requires	C 60.0	H 5.7	N 12.7%

Example 16

3-(Methylsulphonyl)propan-1-ol (0.6g, 4.3mmol) followed by 1,1'-(azodicarbonyl)dipiperidine (4.2g, 16mmol) in portions were added to a stirred solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (1.5g, 4.7mmol), (prepared as described for the starting material in Example 2), and tributylphosphine (4.0ml, 16mmol) in methylene chloride (50ml) under nitrogen. The mixture was stirred for 18 hours, the resulting precipitate was collected by filtration and dried to give crude product (1.36g). The solvent was

removed from the filtrate by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol (a gradient from 100/0 to 90/10). The semi-purified product was triturated with acetone and the solid product collected by filtration and dried to give further crude product (0.53g). The filtrate from the trituration was repurified by column chromatography as before to give further crude product (0.23g). The crude products were combined and dissolved in acetone/methanol/methylene chloride and ethereal hydrogen chloride (6ml of a 1M solution) added. The precipitated product was collected by filtration and recrystallised from methanol/methylene chloride/hexane to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(methylsulphonyl)propoxy)quinazoline** hydrochloride (640mg, 29%).

m.p. >250°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.25(q, 2H); 3.02(s, 3H); 3.36(t, 2H); 4.00(s, 3H); 4.30(t, 2H); 7.35(s, 1H); 7.42(d, 1H); 7.60(t, 1H); 7.65(d, 1H); 8.25(s, 1H); 8.78(s, 1H); 11.5(br s, 1H)

MS - ESI: 440 $[MH]^+$

Elemental analysis:	Found	C 47.8 H 4.2 N 8.8 S 6.7
$C_{19}H_{19}N_3O_4ClFS$ 1HCl	Requires	C 47.4 H 4.2 N 9.0 S 6.8%

The starting material was prepared as follows:

A solution of 3-(methylthio)propan-1-ol (5.3g, 50mmol) in methanol (500ml) was added to a solution of OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc), (30g) in water (150ml) and the mixture stirred at ambient temperature for 24 hours. The precipitated solid was removed by filtration and the methanol removed from the filtrate by evaporation. The aqueous residue was saturated with sodium chloride and extracted with methylene chloride (4x25ml). The aqueous residue was then saturated with ammonium chloride and extracted with ethyl acetate (4x25ml). The extracts were combined, dried ($MgSO_4$) and the solvent removed by evaporation to give 3-(methylsulphonyl)propan-1-ol (610mg, 9%) as an oil.

^{1}H NMR Spectrum: ($CDCl_3$) 2.10(m, 2H); 2.96(s, 3H); 3.20(t, 2H); 3.80(t, 2H)

MS - ESI: 139 $[MH]^+$

Example 17

Diethyl azodicarboxylate (5.91ml, 37mmol) was added dropwise to a stirred mixture of (E)-4-(pyrrolidin-1-yl)but-2-en-1-ol (3.97g, 28mmol), 4-(chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (3.0g, 9mmol), (prepared as described for the starting material in Example 2), and triphenylphosphine (9.84g, 38mmol) in methylene chloride (300ml). The reaction mixture was stirred for 18 hours at ambient temperature. The volatiles were removed by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol (a gradient from 80/20 to 70/30). The purified product was dissolved in methylene chloride/methanol and 1M ethereal hydrogen chloride (25ml) was added. The precipitated product was collected by filtration, washed with ether and dried to give **(E)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-(pyrrolidin-1-yl)but-2-en-1-yloxy)quinazoline** hydrochloride (1.62g, 33%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.85-1.95(m, 2H); 2.0-2.15(m, 2H); 3.0-3.1(m, 2H); 3.5-3.6(m, 2H); 3.95(d, 2H); 4.1(s, 3H); 4.95(d, 2H); 6.1(td, 1H); 6.35(td, 1H); 7.4(s, 1H); 7.45(dd, 1H); 7.6-7.7(m, 2H); 8.15(s, 1H); 8.90(s, 1H)

MS - EI: 443 $[MH]^+$

Elemental analysis:	Found	C 52.7	H 5.3	N 10.8	
$C_{21}H_{24}N_4O_2ClF$ 0.6H_2O 1.85HCl	Requires	C 53.0	H 5.2	N 10.7%	

The starting material was prepared as follows:

Thionyl chloride (9.3ml, 128mmol) was added dropwise to a stirred solution of 2-butyne-1,4-diol (10g, 116mmol) in toluene (15ml) and pyridine (10.3ml) cooled at 0°C. The mixture was stirred for 3.5 hours at ambient temperature and then poured onto ice water. The mixture was extracted with ether, the organic layer was washed with saturated aqueous sodium hydrogen carbonate solution and then brine, dried ($MgSO_4$) and the volatiles removed by evaporation. The residue was purified by column chromatography eluting with petroleum ether/ether (7/3) to give 4-chlorobut-2-yn-1-ol (4.74g, 39%).

^{1}H NMR Spectrum: ($CDCl_3$) 1.68(t, 1H); 4.18(d, 2H); 4.33(d, 2H)

Pyrrolidine (7.8ml, 94mmol) was added dropwise to a solution of 4-chlorobut-2-yn-1-ol (4.74g, 45mmol) in toluene (40ml) and the mixture stirred and heated at 60°C for 1 hour. The volatiles were removed by evaporation and the residue was purified by chromatography

eluting with methylene chloride/methanol (96/4) to give 4-(pyrrolidin-1-yl)but-2-yn-1-ol (4.3g, 69%).

^{1}H NMR Spectrum: ($CDCl_3$) 1.82(t, 4H); 2.63(t, 4H); 3.44(t, 2H), 4.29(t, 2H)

A solution of 4-(pyrrolidin-1-yl)but-2-yn-1-ol (4.3g, 31mmol) in THF (20ml) was added dropwise to a suspension of lithium aluminium hydride (2.35g, 62mmol) in anhydrous THF (8ml) and the mixture stirred and heated at 60°C for 2 hours. The mixture was cooled to 5°C and 2M aqueous sodium hydroxide solution (28ml) was added dropwise. The resulting suspension was filtered and the volatiles removed from the filtrate by evaporation. The residue was dissolved in a mixture of methylene chloride/ethyl acetate, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography on aluminum oxide eluting with methylene chloride/methanol (97/3) to give (E)-4-(pyrrolidin-1-yl)but-2-en-1-ol (3.09g, 70%).

^{1}H NMR Spectrum: ($CDCl_3$) 1.82(m, 4H); 2.61(m, 4H); 3.17(m, 2H); 4.13(s, 2H); 5.84(m, 2H)

Example 18

A solution of 4-(4-bromo-2-fluoroanilino)-7-(3-chloropropoxy)-6-methoxyquinazoline (150mg, 0.34mmol) in 1-(2-hydroxyethyl)piperazine (5ml) was heated at 100°C for 30 minutes. The reaction mixture was allowed to cool and made basic with aqueous sodium hydrogen carbonate solution and extracted with ethyl acetate (3x50ml). The extracts were combined, washed twice with water, then brine and dried ($MgSO_4$). The volatiles were removed by evaporation and the residue dissolved in acetone/methanol (10/1) (50ml) and ethereal hydrogen chloride added. The resulting precipitate was collected by filtration, washed with ether and hexane and dried under vacuum to give **4-(bromo-2-fluoroanilino)-7-(3-[4-(2-hydroxyethyl)piperazinyl]propoxy)-6-methoxyquinazoline** hydrochloride (180mg, 80%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.35(br t, 2H); 3.2-3.8(br m, 12H); 3.80(t, 2H); 4.02(s, 3H); 4.35(t, 2H); 7.45(s, 1H); 7.30(s, 1H); 7.50-7.58(m, 2H); 7.76(dd, 1H); 8.42(s, 1H); 8.80(s, 1H); 11.82(br s, 1H)

MS - ESI: 534 $[MH]^+$

The starting material was prepared as follows:

A mixture of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (1.2g, 3.3mmol), (prepared as described for the starting material in Example 48), 1-bromo-3-chloropropane (1.6ml, 16mmol) and potassium carbonate (2.1g, 15mmol) in DMF (25ml) was heated at 45°C for 3 hours. The mixture was allowed to cool, was diluted with water and extracted with ethyl acetate (3x70ml). The organic extracts were combined, washed with water and brine, dried ($MgSO_4$) and the volatiles were removed by evaporation. The residue was triturated with hexane/ethyl acetate, collected by filtration and dried under vacuum to give 4-(4-bromo-2-fluoroanilino)-7-(3-chloropropoxy)-6-methoxyquinazoline (492mg, 34%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.24(m, 2H); 3.80(t, 2H); 3.95(s, 3H); 4.26(t, 2H); 7.20(s, 1H); 7.42-7.55(m, 2H); 7.63(dd, 1H); 7.80(s, 1H); 8.35(s, 1H); 9.52(s, 1H)

MS - ESI: 440 $[MH]^+$

Example 19

A solution of OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc), (390mg) in water (2ml) was added to a solution of 4-(chloro-2-fluoroanilino)-7-(3-(ethylthio)propoxy)-6-methoxyquinazoline (75mg, 0.18mmol) in methanol (10ml) and the mixture stirred for 18 hours at ambient temperature. The reaction mixture was basified with aqueous sodium hydrogen carbonate solution and extracted with ethyl acetate (3x25ml). The extracts were combined, washed twice with water and then with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The solid residue was recrystallised from ethyl acetate/hexane to give **4-(chloro-2-fluoroanilino)-7-(3-(ethylsulphonyl)propoxy)-6-methoxyquinazoline** (35mg, 43%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.24 (t, 3H); 2.22(m, 2H); 3.15(q, 2H); 3.95(s, 3H); 4.25(t, 2H); 7.20(s, 1H); 7.35(dd, 1H); 7.5-7.6(m, 2H); 7.80(s, 1H); 8.35(s, 1H); 9.54(s, 1H)

MS - ESI: 454 $[MH]^+$

Elemental analysis:	Found	C 51.7	H 4.6	N 9.2
$C_{20}H_{21}N_3O_4ClFS\ 0.5H_20$	Requires	C 51.9	H 4.8	N 9.1%

The starting material was prepared as follows:

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (957mg, 3mmol), (prepared as described for the starting material in Example 2), 1-bromo-3-

chloropropane (2.36g, 15mmol) and potassium carbonate (2.1g, 15mmol) in DMF (20ml) was heated at 40°C for 1.5 hours. The mixture was allowed to cool, was diluted with water and extracted with ethyl acetate (3x50ml). The organic extracts were combined, washed with water and brine, dried ($MgSO_4$) and the volatiles were removed by evaporation. The residue was triturated with hexane/ethyl acetate, collected by filtration and dried under vacuum to give 4-(4-chloro-2-fluoroanilino)-7-(3-chloropropoxy)-6-methoxyquinazoline (650mg, 55%).
^{1}H NMR Spectrum: ($DMSOd_6$) 2.26(m, 2H); 3.82(t, 2H); 3.95(s, 3H); 4.26(t, 2H); 7.20(s, 1H); 7.32(dd, 1H); 7.48-7.60(m, 2H); 7.80(s, 1H); 8.35(s, 1H); 9.52(s, 1H)
MS - EI: 396 $[MH]^+$

A mixture of sodium ethanethiolate (120mg, 1.5mmol) and 4-(4-chloro-2-fluoroanilino)-7-(3-chloropropoxy)-6-methoxyquinazoline (227mg, 0.57mmol) in DMF (10ml) was stirred and heated at 70°C for 3 hours. The reaction mixture was allowed to cool, was diluted with water and extracted with ethyl acetate (3x75ml). The extracts were combined, washed with water (x2), and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was recrystallised from ethyl acetate/hexane to give 4-(chloro-2-fluoroanilino)-7-(3-(ethylthio)propoxy)-6-methoxyquinazoline (86mg, 40%).
^{1}H NMR Spectrum: ($DMSOd_6$) 1.20(t, 3H); 2.03(m, 2H); 2.66(t, 2H); 3.95(s, 3H); 4.20(t, 2H); 7.18(s, 1H); 7.33(dd, 1H); 7.5-7.6(m, 2H); 7.78(s, 1H); 8.35(s, 1H); 9.52(s, 1H)
MS - ESI: 422 $[MH]^+$

Example 20

A mixture of 4-(chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (3.28g, 10mmol), (prepared as described for the starting material in Example 2), 1-bromo-3-tetrahydropyranyloxypropane (2.5g, 11mmol) and potassium carbonate (5.0g, 36mmol) in DMF (50ml) was stirred and heated at 90°C for 3 hours. The reaction mixture was allowed to cool, was diluted with water (500ml) and extracted with ethyl acetate (3x100ml). The extracts were combined, washed with water (x3), and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was purified by column chromatography eluting with ethyl acetate. The purified product was recrystallised from ethyl acetate/hexane to give **4-(chloro-2-fluoroanilino)-6-methoxy-7-(3-tetrahydropyran-2-yloxypropoxy)quinazoline** (2.25g, 49%).

m.p. 184-185°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.35-1.54(m, 4H); 1.55-1.75(m, 2H); 2.05(m, 2H); 3.35-3.45(m,1H); 3.66-3.84(m, 2H); 3.95(s, 3H); 4.23(t, 2H); 4.60(s, 1H); 7.18(s, 1H); 7.32(dd, 1H); 7.5-7.6(m, 2H); 7.78(s, 1H); 8.35(s, 1H); 9.53(s, 1H)

MS - ESI: 462 $[MH]^+$

Elemental analysis:	Found	C 59.6	H 5.3	N 9.1
$C_{23}H_{25}N_3O_4ClF$	Requires	C 59.9	H 5.4	N 9.4%

Example 21

A mixture of sodium methanethiolate (70mg, 1mmol) and 4-(4-chloro-2-fluoroanilino)-7-(3-chloropropoxy)-6-methoxyquinazoline (200mg, 0.5mmol), (prepared as described for the starting material in Example 19), in DMF (10ml) was stirred and heated at 70°C for 1 hour. The reaction mixture was allowed to cool, was diluted with water and extracted with ethyl acetate (3x25ml). The extracts were combined, washed with water (x2), and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was recrystallised from ethyl acetate/hexane to give **4-(chloro-2-fluoroanilino)-6-methoxy-7-(3-methylthiopropoxy)quinazoline** (143mg, 35%).

m.p. 169-170°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.0-2.12(m, 2H); 2.08(s, 3H); 2.64(t, 2H); 3.93(s, 3H); 4.21(t, 2H); 7.18(s, 1H); 7.33(d, 1H); 7.50-7.61(m, 2H); 7.78(s, 1H); 8.34(s, 1H); 9.53(s, 1H)

MS - ESI: 408 $[MH]^+$

Example 22

A mixture of 4-(bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (250mg, 0.7mmol), (prepared as described for the starting material in Example 48), 2-chloroethyl methyl sulphide (0.1ml, 1mmol) and potassium carbonate (1.0g, 7mmol) in DMF (10ml) was stirred and heated at 50°C for 4 hours. The reaction mixture was allowed to cool, was diluted with water and extracted with ethyl acetate (3x25ml). The extracts were combined, washed with water (x2), and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was purified by column chromatography eluting with ethyl acetate. The

purified product was recrystallised from ethyl acetate/hexane to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-methylthioethoxy)quinazoline** (100mg, 34%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.20(s, 3H); 2.90(t, 2H); 3.92(s, 3H); 4.30(t, 2H); 7.20(s, 1H); 7.42-7.54(m, 2H); 7.62(dd, 1H); 7.80(s, 1H); 8.36(s, 1H); 9.54(s, 1H)

MS - ESI: 438 $[MH]^+$

Elemental analysis:	Found	C 48.8	H 3.9	N 9.8	S 7.3	
$C_{18}H_{17}N_3O_2BrFS$	Requires	C 49.3	H 3.9	N 9.6	S 7.3%	

Example 23

A solution of 7-(2-bromoethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (130mg, 0.36mmol), (prepared as described for the starting material in Example 62), in 1-ethoxycarbonylpiperazine (1.5ml) was stirred and heated at 100°C for 2 hours. The mixture was allowed to cool, diluted with water and extracted with ethyl acetate (3x25ml). The extracts were combined, washed with water (x2) and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was dissolved in acetone and 1M ethereal hydrogen chloride (2ml) was added. The resulting precipitate was collected by filtration and then purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (94/5/1). The purified product was dissolved in acetone and 1M ethereal hydrogen chloride (2ml) was added. The resulting precipitate was collected by filtration, washed with ether and dried to give **4-(4-chloro-2-fluoroanilino)-7-(2-(4-ethoxycarbonylpiperazin-1-yl)ethoxy)-6-methoxyquinazoline** hydrochloride (85mg, 46%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.20(t, 3H); 3.1-3.6(m, 8H); 3.66(br s, 2H); 4.00(s, 3H); 4.08(q, 2H); 4.65(br s, 2H); 7.40(m, 2H); 7.90(t, 1H); 7.65(dd, 1H); 8.40(s, 1H); 8.80(s, 1H); 11.66(br s, 1H)

MS - ESI: 504 $[MH]^+$

Elemental analysis:	Found	C 48.6	H 5.0	N 12.2
$C_{24}H_{27}N_5O_4ClF\ 1H_2O\ 2HCl$	Requires	C 48.5	H 5.2	N 11.8%

Example 24

A mixture of 4-(bromo-2-fluoroanilino)-7-hydroxy -6-methoxyquinazoline (306mg, 0.84mmol), (prepared as described for the starting material in Example 48), 2-chloroethyl

ethyl sulphide (0.15ml, 1.3mmol) and potassium carbonate (0.5g, 3.6mmol) in DMF (10ml) was stirred and heated at 50°C for 1 hour. The reaction mixture was allowed to cool, was diluted with water and extracted with ethyl acetate (3x25ml). The extracts were combined, washed with water (x2), and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was purified by column chromatography eluting with ethyl acetate. The purified product was recrystallised from ethyl acetate/hexane to give **4-(4-bromo-2-fluoroanilino)-7-(2-ethylthioethoxy)-6-methoxyquinazoline** (221mg, 58%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.24(t, 3H); 2.66(q, 2H); 2.94(t, 2H); 3.95(s, 3H); 4.30(t, 2H); 7.20(s, 1H); 7.45(t, 1H); 7.52(d, 1H); 7.65(dd, 1H); 7.80(s, 1H); 9.55(s, 1H)

MS - ESI: 452 $[MH]^+$

Example 25

A solution of OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc), (150mg) in water (2ml) was added to a solution of 4-(4-bromo-2-fluoroanilino)-7-(2-ethylthioethoxy)-6-methoxyquinazoline (125mg, 0.28mmol), (prepared as described in Example 24), in methanol (10ml). The reaction mixture was stirred for 16 hours at ambient temperature, the methanol was removed by evaporation, the aqueous residue was basified with sodium hydrogen carbonate solution and then extracted with ethyl acetate (3x30ml). The extracts were combined, washed with water (x2), and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was purified by column chromatography eluting with ethyl acetate and then with methylene chloride/methanol (9/1) to give **4-(4-bromo-2-fluoroanilino)-7-(2-ethylsulphinylethoxy)-6-methoxyquinazoline** (32mg, 31%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.21(t, 3H); 2.72-2.84(m, 1H); 2.86-2.96(m, 1H); 3.04-3.12(m, 1H); 3.94(s, 3H); 4.42-4.58(m, 2H); 7.26(s, 1H); 7.42-7.55(m, 2H); 7.64(dd, 1H); 7.82(s, 1H); 8.35(s, 1H); 9.55(s, 1H)

MS - ESI: 468 $[MH]^+$

Example 26

Using a method analogous to that in Example 25, 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-methylthiopropoxy)quinazoline (250mg, 0.6mmol), (prepared as described in Example 21), was treated with OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc),

(84mg) and the product was purified and isolated to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-methylsulphinylpropoxy)quinazoline** (75mg, 29%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.18(t, 2H); 2.60(s, 3H); 2.78-2.98(m, 2H); 3.95(s, 3H); 4.25(t, 2H); 7.20(s, 1H); 7.35(dd, 1H); 7.50-7.61(m, 2H); 7.80(s, 1H); 8.53(s, 1H); 9.55(s, 1H)

MS - ESI: 424 $[MH]^+$

Elemental analysis:	Found	C 53.4	H 4.5	N 9.8
$C_{19}H_{19}N_3O_4ClFS$	Requires	C 53.9	H 4.5	N 9.8%

Example 27

A solution of OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc), (800mg) in water (3ml) was added to a solution of 4-(4-bromo-2-fluoroanilino)-7-(2-ethylthioethoxy)-6-methoxyquinazoline (320mg, 0.7mmol), (prepared as described in Example 24), in methanol (10ml). The reaction mixture was stirred for 20 hours at ambient temperature, the methanol was removed by evaporation, the aqueous residue was basified with sodium hydrogen carbonate solution, saturated with sodium chloride and then extracted with ethyl acetate (3x50ml). The extracts were combined, dried ($MgSO_4$) and the solvent was removed by evaporation. The residue was dissolved in acetone/methanol and 1M ethereal hydrogen chloride (2ml) was added. The volatiles were removed by evaporation, the residue was triturated with 2-propanol/hexane, collected by filtration and dried to give **4-(4-bromo-2-fluoroanilino)-7-(2-ethylsulphonylethoxy)-6-methoxyquinazoline** hydrochloride (200mg, 55%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.28(t, 3H); 3.25(q, 2H); 3.74(t, 2H); 4.00(s, 3H); 4.54(t, 2H); 7.43(s, 1H); 7.54(m, 1H); 7.56(s, 1H); 7.75(d, 1H); 8.36(s, 1H); 8.78(s, 1H); 11.61(br s, 1H)

MS - ESI: 484 $[MH]^+$

Example 28

A solution of OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc), (800mg) in water (3ml) was added to a solution of 4-(4-chloro-2-fluoroanilino)-7-(2-ethylthioethoxy)-6-methoxyquinazoline (220mg, 0.56mmol) in methanol (10ml). The reaction mixture was stirred for 20 hours at ambient temperature, the methanol was removed by evaporation, the aqueous residue was basified with sodium hydrogen carbonate solution, saturated with sodium

chloride and then extracted with ethyl acetate (3x50ml). The extracts were combined, dried ($MgSO_4$) and the solvent was removed by evaporation. The residue was dissolved in acetone/methanol and 1M ethereal hydrogen chloride (1.2ml) was added. The volatiles were removed by evaporation, the residue was triturated with 2-propanol, collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-7-(2-ethylsulphonylethoxy)-6-methoxyquinazoline** hydrochloride (24mg, 9%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.25(t, 3H); 3.30(q, 2H); 3.75(t, 2H); 4.00(s, 3H); 4.55(t, 2H); 7.36(s, 1H); 7.41(dd, 1H); 7.58(t, 1H); 7.64(dd, 1H); 8.22(s, 1H); 8.78(s, 1H)

MS - ESI: 440 $[MH]^+$

The starting material was prepared as follows:

A mixture of 4-(chloro-2-fluoroanilino)-7-hydroxy -6-methoxyquinazoline (450mg, 1.4mmol), (prepared as described for the starting material in Example 2), 2-chloroethyl ethyl sulphide (0.2ml, 1.7mmol) and potassium carbonate (1.5g, 11mmol) in DMF (10ml) was stirred and heated at 50°C for 2 hours. The reaction mixture was allowed to cool, was diluted with water and extracted with ethyl acetate (3x50ml). The extracts were combined, washed with 0.1M sodium hydroxide solution (x2), water and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation to give crude 4-(4-chloro-2-fluoroanilino)-7-(2-ethylthioethoxy)-6-methoxyquinazoline (230mg, 57%) which was used directly.

Example 29

A mixture of 4-(chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (400mg, 1.3mmol), (prepared as described for the starting material in Example 2), 2-chloroethyl methyl sulphide (0.168ml, 1.7mmol) and potassium carbonate (347mg, 2.5mmol) in NMP (10ml) was stirred and heated at 90°C for 1 hour, then allowed to cool and stirred for 16 hours at ambient temperature. The reaction mixture was diluted with water and extracted with ethyl acetate. The extracts were combined, washed with water, and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation, the residue was triturated with ethyl acetate/hexane and collected by filtration to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylthioethoxy)quinazoline** (220mg, 44%).

m.p. 174-176°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.20(s, 3H); 2.92(t, 2H); 3.94(s, 3H); 4.32(t, 2H); 7.20(s, 1H); 7.32(d, 1H); 7.49-7.6(m, 2H); 7.80(s, 1H); 8.36(s, 1H); 9.55(s, 1H)

MS - ESI: 452 $[MH]^+$

Example 30

A solution of OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc), (652mg) in water (1.6ml) was added to a solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylthioethoxy)quinazoline (200mg, 0.5mmol), (prepared as described in Example 29), in methanol (10ml) and the mixture was stirred for 18 hours at ambient temperature. The mixture was diluted with methylene chloride, was washed with aqueous sodium hydrogen carbonate solution, dried ($MgSO_4$) and the solvent was removed by evaporation. The residue was triturated with ethyl acetate/hexane, collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylsulphonylethoxy)quinazoline** (172mg, 80%).

m.p. 227-230°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.18(s, 3H); 3.70(t, 2H); 3.92(s, 3H); 4.50(t, 2H); 7.22-7.38(m, 2H); 7.42(s, 1H); 7.48-7.60(m, 2H); 8.37(s, 1H); 9.55(s, 1H)

MS - ESI: 426 $[MH]^-$

Elemental analysis:	Found	C 46.0	H 3.6	N 8.7
$C_{18}H_{17}N_3O_4ClFS\ 2.2H_2O$	Requires	C 46.4	H 4.1	N 9.0%

Example 31

1,1'-(Azodicarbonyl)dipiperidine (1.56g, 6.2mmol) followed by 3-(methylthio)-1-propanol (0.32ml, 3mmol) was added to a mixture of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (225mg, 7.0mmol), (prepared as described for the starting material in Example 48), and tributylphosphine (1.42ml, 6.1mmol) in methylene chloride (20ml) at 5°C. The mixture was stirred at 5°C for 1 hour and then for 18 hours at ambient temperature. The insolubles were removed by filtration and the volatiles were removed from the filtrate by evaporation. The residue was purified by column chromatography eluting with ethyl acetate/methanol (100/0 increasing to 95/5) to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-methylthiopropoxy)quinazoline** (400mg, 50%).

m.p. 250-252°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.08(t, 2H); 2.64(t, 2H); 4.00(s, 3H); 4.28(t, 2H); 7.40(s, 1H); 7.48-7.58(m, 2H); 7.78(d, 1H); 8.30(s, 1H); 8.80(s, 1H)

MS - ESI: 452 $[MH]^+$

Example 32

A solution of OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc), (800mg) in water (4.5ml) was added to a solution of 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-methylthiopropoxy)quinazoline (300mg, 0.66mmol), (prepared as described in Example 31), in methanol (15ml) and the mixture was stirred for 4 hours at ambient temperature. The mixture was diluted with methylene chloride, was washed with aqueous sodium hydrogen carbonate solution, dried ($MgSO_4$) and the solvent was removed by evaporation. The residue was triturated with ethyl acetate/hexane, collected by filtration and dried to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-methylsulphonylpropoxy)quinazoline** (235mg, 73%).

m.p. >250°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.30(t, 2H); 3.20(s, 3H); 3.30(t, 2H); 4.10(s, 3H); 4.30(t, 2H); 7.38(s, 1H); 7.5-7.6(m, 2H); 7.78(d, 1H); 8.30(s, 1H); 8.80(s, 1H)

MS - ESI: 484 $[MH]^+$

Elemental analysis:	Found	C 42.8	H 3.8	N 7.8
$C_{19}H_{19}N_3O_4BrFS\ 0.5H_2O$	Requires	C 43.1	H 3.9	N 7.9%

Example 33

1,1'-(Azodicarbonyl)dipiperidine (355mg, 1.4mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (150mg, 0.47mmol), (prepared as described for the starting material in Example 2), 2-(cyclopentyloxy)ethanol (91mg, 0.7mmol), (US Patent Number 4,515,814), and tributylphosphine (284mg, 1.4mmol) in methylene chloride (6ml) at 0°C. The mixture was then allowed to warm to ambient temperature and stirred for 3.5 hours. Ether (3ml) was added and the insolubles were removed by filtration and the volatiles were removed from the filtrate by evaporation. The residue was dissolved in acetone and 1M ethereal hydrogen chloride (0.6ml) was added. The mixture was left to stand for 60 hours and the precipitate was collected by filtration, washed with acetone and dried to give **4-(4-chloro-2-**

fluoroanilino)-7-(2-cyclopentyloxyethoxy)-6-methoxyquinazoline hydrochloride (130mg, 60%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.4-1.8(m, 8H); 3.75(t, 2H); 4.00(s, 4H); 4.30(t, 2H); 7.37(s, 1H); 7.42(dd, 1H); 7.60(t, 1H); 7.64(dd, 1H); 8.25(s, 1H); 8.78(s, 1H)

MS - ESI: 432 $[MH]^+$

Elemental analysis:	Found	C 55.8	H 5.0	N 8.8
$C_{22}H_{23}N_3O_3ClF$ $1H_2O$ 1HCl	Requires	C 56.0	H 5.2	N 8.9%

Example 34

Diethyl azodicarboxylate (0.94ml, 6mmol) was added dropwise to a mixture of triphenylphosphine (1.57g, 6mmol), 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (640mg, 2mmol), (prepared as described for the starting material in Example 2), and N-(tert-butoxycarbonyl)ethanolamine (0.354g, 2.2mmol) in methylene chloride (20ml) at 0°C. The reaction mixture was allowed to warm to ambient temperature and stirred for 4 hours. The reaction mixture was diluted with methylene chloride, washed with aqueous sodium hydrogen carbonate solution, water and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (100/8/1). The product was recrystallised from acetonitrile, collected by filtration, washed with ethyl acetate and dried to give **7-(2-[N-tert-butoxycarbonylamino]ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline** (235mg, 25%).

m.p. 190-191°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.36(s, 9H); 3.34(q, 2H); 3.91(s, 3H); 4.15(t, 2H); 6.98(t, 1H); 7.19(s, 1H); 7.33(dd, 1H); 7.56(m, 2H); 7.78(s, 1H); 8.34(s, 1H); 9.51(s, 1H)

MS - ESI: 463 $[MH]^+$

Elemental analysis:	Found	C 57.0	H 5.1	N 12.5
$C_{22}H_{24}N_4O_4ClF$	Requires	C 57.1	H 5.1	N 12.1%

Example 35

Sodium hydride (55mg of a 60% dispersion in mineral oil, 1.1mmol) was added to a solution of glutarimide (120mg, 1.06mmol) in DMF (5ml) at ambient temperature under

argon and the mixture stirred for 30 minutes. 7-(2-Bromoethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (428mg, 1mmol), (prepared as described for the starting material in Example 62), in DMF (2ml) was added and the resulting pale green solution was stirred for 18 hours and then quenched with water . The volatiles were removed by evaporation, and the residue was partitioned between water and ethyl acetate. The organic phase was separated and washed with water and then dried ($MgSO_4$). The solvent was removed by evaporation, and the residue was purified by column chromatography eluting with ethyl acetate then ethyl acetate/methanol (9/1). The purified product was recrystallised from ethyl acetate and hexane, collected by filtration and washed with ether to give **4-(4-chloro-2-fluoroanilino)-7-(2-(2,6-dioxopiperidino)ethoxy)-6-methoxyquinazoline** (252mg, 55%).

m.p. 202-203°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.84(m, 2H); 2.63(t, 4H); 3.91(s, 3H); 4.08(t, 2H); 4.17(t, 2H); 7.10(s, 1H); 7.34(dd, 1H); 7.55(m, 2H); 7.79(s, 1H); 8.34(s, 1H); 9.52(s, 1H)

MS - ESI: 459 $[MH]^+$

Elemental analysis:	Found	C 57.2	H 4.2	N 11.9
$C_{22}H_{20}N_4O_4ClF$	Requires	C 57.6	H 4.3	N 12.2%

Example 36

Isobutyl chloroformate (88mg, 5.9mmol) was added to a stirred solution of 7-(3-aminopropoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline trifluoroacetate (151mg, 0.4mmol) and triethylamine (0.2ml, 1.4mmol) in THF (15ml). The reaction mixture was stirred at ambient temperature for 30 minutes and the volatiles were removed by evaporation. The residue was dissolved in methylene chloride, the solution was washed with aqueous sodium hydrogen carbonate solution and then brine, dried ($MgSO_4$) and the solvent was removed by evaporation. The residue was recrystallised from acetonitrile to give **4-(4-chloro-2-fluoroanilino)-7-(3-[N-isobutoxycarbonylamino]propoxy)-6-methoxyquinazoline** (41.2mg, 20%) as a white solid.

m.p. 136-137°C

^{1}H NMR Spectrum: ($DMSOd_6$) 0.87(d, 6H); 1.80(m, 1H); 1.93(t, 2H); 3.16(q, 2H); 3.71(d, 2H); 3.94(s, 3H); 4.15(t, 2H); 7.16(s, 2H); 7.32(dd, 1H); 7.55(m, 2H); 7.79(s, 1H); 8.34(s, 1H); 9.50(s, 1H)

MS - ESI: 477 $[MH]^+$

Elemental analysis:	Found	C 57.1	H 4.9	N 11.6
$C_{23}H_{26}N_4O_4ClF$	Requires	C 57.1	H 5.5	N 11.6%

The starting material was prepared as follows:

A solution of di-tert-butyl dicarbonate (32g, 148mmol) in methylene chloride (70ml) was added dropwise to a stirred solution of 3-amino-1-propanol (10.1g, 134mmol) in methylene chloride (100ml). The reaction mixture was stirred overnight and was then washed with saturated aqueous sodium hydrogen carbonate solution, water and then brine. The organic layer was dried ($MgSO_4$) and the volatiles were removed by evaporation to give 3-(N-tert-butoxycarbonylamino)-1-propanol (23.3g, 100%) as a colourless oil.

^1H NMR Spectrum: ($CDCl_3$) 1.48(s, 9H); 1.68(m, 2H); 2.90(br s, 1H); 3.30(m, 2H); 3.65(m, 2H); 4.78(br s, 1H)

MS - ESI: 176 $[MH]^+$

Triphenylphosphine (2.46g, 9.3mmol) was added to a suspension of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (1.0g, 3.1mmol), (prepared as described for the starting material in Example 2), in methylene chloride (25ml) and the suspension stirred at 0°C for 30 minutes. A solution of 3-(N-tert-butoxycarbonylamino)-1-propanol (0.65g, 3.7mmol) in methylene chloride (3ml) was added and then diethyl azodicarboxylate (1.47ml, 7.6mmol) was added dropwise. The reaction mixture was allowed to warm to ambient temperature and stirred for 18 hours. The reaction mixture was diluted with methylene chloride and washed with aqueous sodium hydrogen carbonate solution, water and then brine. The resultant solution was dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol/triethylamine (100/0/0 and then 95/4/1) to give 7-(3-(N-tert-butoxycarbonylamino)propoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (620mg, 42%).

^1H NMR Spectrum: ($DMSOd_6$) 1.36(s, 9H); 1.89(t, 2H); 3.11(q, 2H); 3.91(s, 3H); 4.14(t, 2H); 6.89(m, 1H); 7.16(s, 1H); 7.31(dd, 1H); 7.56(m, 2H); 7.77(s, 1H); 8.32(s, 1H); 9.51(s, 1H)

MS - ESI: 477 $[MH]^+$

7-(3-(N-tert-butoxycarbonylamino)propoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (610mg, 1.28mmol) was added slowly to TFA (10ml). The reaction was stirred at ambient temperature for 2 hours and the volatiles were removed by evaporation and by azeotroping with toluene to give 7-(3-aminopropoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline trifluoroacetate (455mg, 94%) as an oil.

Example 37

A mixture of 7-(2-bromoethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (425mg, 1mmol), (prepared as described for the starting material in Example 62), and 1-methyl-4-(methylamino)piperidine (128mg, 1mmol) in N,N-dimethylacetamide (2ml) was stirred at 65°C for 3 hours. The volatiles were removed by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (75/8/1). The purified product was triturated with ether, collected by filtration, washed with ether and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-([N-methyl-N-(1-methylpiperidin-4-yl)]amino)ethoxy)quinazoline** (180mg, 38%) as a pale yellow powder.

m.p. 191-192°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.44(m, 2H); 1.70(m, 2H); 1.86(m, 2H); 2.15(s, 3H); 2.30(s, 3H); 2.78(m, 2H); 2.88(t, 2H); 3.94(s, 3H); 4.18(t, 2H); 7.19(s, 1H); 7.33(m, 1H); 7.52(m, 1H); 7.58(t, 1H); 7.78(s, 1H); 8.34(s, 1H); 9.48(s, 1H)

MS - ESI: 474 $[MH]^+$

Elemental analysis:	Found	C 60.9	H 6.3	N 14.7
$C_{24}H_{29}N_5O_2ClF$	Requires	C 60.8	H 6.2	N 14.8%

Example 38

1,1'-(Azodicarbonyl)dipiperidine (560mg, 2.2mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (240mg, 0.75mmol), (prepared as described for the starting material in Example 2), tetrahydro-3-furanmethanol (90mg, 0.88mmol) and tributylphosphine (440mg, 2.2mmol) in methylene chloride (12ml) and the mixture stirred for 18 hours. The mixture was diluted with ether, and the resulting precipitate was removed by filtration. The solvent was removed from the filtrate by

evaporation, and the residue was dissolved in acetone and ethereal hydrogen chloride (0.75ml of a 1M solution, 0.75mmol) was added. The mixture was diluted with ether and the resulting precipitate was collected by filtration. The solid was purified by column chromatography eluting with methylene chloride/acetonitrile/methanol (a gradient from 50/50/1 to 50/50/2). The purified product was triturated with ether, collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(tetrahydrofuran-3-ylmethoxy)quinazoline** (93mg, 31%).

m.p. 201-202°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.70(m, 1H); 2.05(m, 1H); 2.72(m, 1H); 3.56(m, 1H); 3.66(q, 1H); 3.79(m, 2H); 3.94(s, 3H); 4.08(m, 2H); 7.20(s, 1H); 7.32(m, 1H); 7.52(dd, 1H); 7.58(t, 1H); 7.78(t, 1H); 8.35(s, 1H); 9.52(s, 1H)

MS - ESI: 404 $[MH]^+$

Elemental analysis:	Found	C 59.2	H 4.6	N 10.6
$C_{20}H_{19}N_3O_3ClF$	Requires	C 59.5	H 4.7	N 10.4%

Example 39

1,1'-(Azodicarbonyl)dipiperidine (5.6g, 22mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (2.4g, 7.5mmol), (prepared as described for the starting material in Example 2), tributylphosphine (4.4g, 22mmol) and 1-(2-hydroxyethyl)-2-pyrrolidinone (1.1g, 8.5mmol) in methylene chloride (105ml). The mixture was stirred for 18 hours, diluted with ether (100ml) and the resulting precipitate was removed by filtration. The volatiles were removed from the filtrate by evaporation, and the residue was dissolved in acetone and ethereal hydrogen chloride (15ml of a 1M solution, 15mmol) was added. The solid was collected by filtration and was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (150/8/1). The purified product was dissolved in acetone and ethereal hydrogen chloride (15ml of a 1M solution, 15mmol) was added. The resulting precipitate was collected by filtration, washed with ether and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-oxopyrrolidin-1-yl)ethoxy)quinazoline** hydrochloride (2.1g, 60%).

m.p. 250-252°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.92(m, 2H); 2.22(t, 2H); 3.52(t, 2H); 3.68(t, 2H); 4.02(s, 3H); 4.30(t, 2H); 7.38(s, 1H); 7.42(m, 1H); 7.58(t, 1H); 7.66(dd, 1H); 8.35(s, 1H); 8.79(s, 1H); 11.69(br s, 1H)

MS - ESI: 431 $[MH]^+$

Elemental analysis:	Found	C 53.5	H 4.4	N 12.2	
$C_{21}H_{20}N_4O_3ClF$ 0.1H_2O 1HCl	Requires	C 53.8	H 4.6	N 11.9%	

Example 40

1,1'-(Azodicarbonyl)dipiperidine (525mg, 2.1mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (225mg, 7.0mmol), (prepared as described for the starting material in Example 2), tributylphosphine (420mg, 2.1mmol) and 1-(2-hydroxyethyl)-2-imidazolidinone (100mg, 7.7mmol) in methylene chloride (10ml). The mixture was stirred for 18 hours, diluted with ether and the resulting precipitate was removed by filtration. The volatiles were removed from the filtrate by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (150/8/1). The purified product was triturated with ether collected by filtration, washed with ether and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-oxoimidazolidin-1-yl)ethoxy)quinazoline** (19mg, 6%).

m.p. >250°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.27(t, 2H); 3.53(m, 4H); 3.97(s, 3H); 4.27(t, 2H); 6.39(s, 1H); 7.26(s, 1H); 7.35(m, 1H); 7.57(dd, 1H); 7.61(t, 1H); 7.82(s, 1H); 8.38(s, 1H); 9.55(s, 1H)

MS - ESI: 432 $[MH]^+$

Elemental analysis:	Found	C 53.7	H 4.4	N 15.4	
$C_{20}H_{19}N_5O_3ClF$ 1H_2O	Requires	C 53.4	H 4.7	N 15.6%	

Example 41

1,1'-(Azodicarbonyl)dipiperidine (525mg, 2.1mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (225mg, 7.0mmol), (prepared as described for the starting material in Example 2), tributylphosphine (420mg, 2.1mmol) and 4-(2-hydroxyethyl)-1,1-dioxothiomorpholine (140mg, 7.8mmol) in methylene chloride (10ml). The mixture was stirred for 18 hours, diluted with ether and the resulting

precipitate was removed by filtration. The volatiles were removed from the filtrate by evaporation, and the residue was dissolved in acetone and ethereal hydrogen chloride (14ml of a 1M solution, 14mmol) and the precipitate was collected by filtration. The residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (150/8/1). The purified product was triturated with ether/methylene chloride collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-7-(2-(1,1-dioxothiomorpholino)ethoxy)-6-methoxyquinazoline** (120mg, 36%).

m.p. 246-249°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.03(t, 2H); 3.10(br s, 8H); 3.95(s, 3H); 4.27(t, 2H); 7.24(s, 1H); 7.38(m, 1H); 7.53(dd, 1H); 7.58(t, 1H); 7.80(s, 1H); 8.35(s, 1H); 9.52(s, 1H)

MS - ESI: 481 $[MH]^+$

Elemental analysis:	Found	C 52.0	H 4.6	N 11.9	S 6.6
$C_{21}H_{22}N_4O_4ClFS$	Requires	C 52.4	H 4.6	N 11.6	S 6.7%

Example 42

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (94mg, 4.9mmol) was added to a mixture of 7-(3-carboxypropoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (164mg, 0.4mmol), morpholine (0.11g; 1.26mmol) and 4-dimethylaminopyridine (200mg, 1.64mmol) in DMF (5ml). The reaction mixture was stirred at ambient temperature for 24 hours and the volatiles were removed by evaporation. Water was added to the residue and the aqueous mixture was extracted with methylene chloride (3x30ml). The extracts were combined and the solvent removed by evaporation. The residue was triturated with ether and the precipitate was collected by filtration. The solid was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (100/8/1). The purified product was triturated with acetone, collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinocarbonylpropoxy)quinazoline** (88mg, 46%).

m.p. 216-217°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.02(m, 2H); 2.5(m, 2H); 3.45(m, 4H); 3.55(m, 4H); 3.92(s, 3H); 4.15(t, 2H); 7.18(s, 1H); 7.32(d, 1H); 7.55(m, 2H); 7.78(s, 1H); 8.34(s, 1H); 9.52(s, 1H)

MS - ESI: 475 $[MH]^+$

Elemental analysis:	Found	C 58.2	H 5.2	N 12.2
$C_{23}H_{24}N_4O_4ClF$	Requires	C 58.2	H 5.1	N 11.8%

The starting material was prepared as follows:

Ethyl 4-chlorobutyrate (0.154ml, 1.1mmol) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (319.5mg, 1mmol), (prepared as described for the starting material in Example 2), and anhydrous potassium carbonate (690mg, 5mmol) in DMF (10ml). The mixture was stirred and heated at 105°C for 4 hours then allowed to cool. The mixture was diluted with methylene chloride and the insolubles were removed by filtration. The solvent was removed from the filtrate by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (100/8/1). The purified product was triturated with ether collected by filtration and dried to give 4-(4-chloro-2-fluoroanilino)-7-(3-ethoxycarbonylpropoxy)-6-methoxyquinazoline (230mg, 53%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.18(t, 3H); 2.02(m, 2H); 2.48(m, 2H); 3.94(s, 3H); 4.06(q, 2H); 4.15(t, 2H); 7.18(s, 1H); 7.32(m, 1H); 7.54(m, 2H); 7.78(s, 1H); 8.34(s, 1H); 9.52(s, 1H)
MS - ESI: 434 $[MH]^+$

Elemental analysis:	Found	C 58.0	H 4.8	N 9.8
$C_{21}H_{21}N_3O_4ClF$	Requires	C 58.1	H 4.9	N 9.7%

A mixture of 4-(4-chloro-2-fluoroanilino)-7-(3-ethoxycarbonylpropoxy)-6-methoxyquinazoline (220mg, 0.5mmol) in aqueous sodium hydroxide solution (4ml of a 2M solution, 8mmol), water (2ml) and methanol (0.5ml) was stirred and heated at 40°C for 3 hours. The mixture was allowed to cool and was then acidified with 2M hydrochloric acid. The resulting white precipitate was collected by filtration and washed with acetone and water to give 7-(3-carboxypropoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (170mg, 83%).

Example 43

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (145mg, 0.75mmol) was added to a mixture of 7-(3-carboxypropoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (250mg, 0.62mmol), (prepared as described for the starting material in Example 42), 1-methylpiperazine (0.21ml, 2.32mmol) and 4-dimethylaminopyridine (300mg,

2.46mol) in DMF (7.5ml). The reaction mixture was stirred at ambient temperature for 24 hours and the volatiles were removed by evaporation. Water was added to the residue and the aqueous mixture was extracted with methylene chloride (3x30ml). The combined organic extracts were washed with brine and the solvent was removed by evaporation. The residue was triturated with ether and the precipitate was collected by filtration. The solid was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (100/8/1). The purified product was triturated with ether collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(4-methylpiperazin-1-ylcarbonyl)propoxy)quinazoline** (133mg, 44%).

m.p. 248-250°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.00(t, 2H); 2.15(s, 3H); 2.25(m, 4H); 2.45(m, 2H); 3.45(m, 4H); 3.92(s, 3H); 4.15(t, 2H); 7.18(s, 1H); 7.30(d, 1H); 7.55(m, 2H); 7.78(s, 1H); 8.34(s, 1H); 9.52(s, 1H)

MS - ESI: 488 $[MH]^+$

Elemental analysis:	Found	C 58.6	H 5.5	N 13.9
$C_{24}H_{27}N_5O_3ClF\ 0.2H_2O$	Requires	C 58.6	H 5.6	N 14.3%

Example 44

Oxalyl chloride (0.4ml, 2.2mmol) was added to a suspension of 7-(3-carboxypropoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (260mg, 0.64mmol), (prepared as described for the starting material in Example 42), in methylene chloride (25ml) followed by 1 drop of DMF. The mixture was stirred at ambient temperature for 2.5 hours and the volatiles were removed by evaporation. A solution of pyrrolidine (0.13ml, 2.1mmol) in N,N-dimethylacetamide (8ml) was added to the solid residue and the mixture was stirred at ambient temperature for 2 hours. The volatiles were removed by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (100/8/1). The purified product was triturated with acetone, collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-pyrrolidin-1-ylcarbonylpropoxy)quinazoline** (206mg, 70%).

m.p. 254-256°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.76(m, 2H); 1.85(m, 2H); 2.02(m, 2H); 2.41(t, 2H); 3.26(t, 2H); 3.38(t, 2H); 3.95(s, 3H); 4.15(t, 2H); 7.18(s, 1H); 7.32(d, 1H); 7.55(m, 2H); 7.78(s, 1H); 8.34(s, 1H); 9.52(s, 1H)

MS - ESI: 459 $[MH]^+$

Elemental analysis:	Found	C 59.9	H 5.3	N 12.0
$C_{23}H_{24}N_4O_3ClF$	Requires	C 60.2	H 5.3	N 12.2%

Example 45

A mixture of 4-(4-chloro-2-fluoroanilino)-7-(2,2-dimethoxyethoxy)-6-methoxyquinazoline (210mg, 0.52mmol), water (5ml) and TFA (5ml) was stirred at ambient temperature for 3 hours then heated at 60°C for 1 hour. The solution was allowed to cool, then diluted with water and the resulting precipitate was collected by filtration and dried. The solid was dissolved in methanol (10ml) and cyclopentylamine (0.057ml, 0.57mmol) and dried 3Å molecular sieves (2.5g) were added. The mixture was stirred for 30 minutes, glacial acetic acid (0.20ml, 3.2mmol) and sodium cyanoborohydride (150mg, 2.4mmol) were added and the reaction stirred for 4 hours then left to stand for 18 hours. The insolubles were removed by filtration and the solvent was removed from the filtrate by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (100/8/1). The purified product was triturated with ether/hexane, collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-7-(2-cyclopentylaminoethoxy)-6-methoxyquinazoline** (80mg, 36%).

m.p. 171-173°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.55(m, 8H); 2.94(t, 2H); 3.08(m, 1H); 3.94(s, 3H); 4.19(t, 2H); 7.19(s, 1H); 7.33(m, 1H); 7.52(dd, 1H); 7.59(t, 1H); 7.78(s, 1H); 8.34(s, 1H); 9.50(s, 1H)

MS - ESI: 431 $[MH]^+$

The starting material was prepared as follows:

Bromoacetaldehyde dimethyl acetal (0.74ml, 3.1mmol) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (1.0g, 3.13mmol), (prepared as described for the starting material in Example 2), and anhydrous potassium carbonate (2.16g, 15.6mmol) in DMF (30ml). The mixture was stirred and heated at 110°C for 4 hours, then

allowed to cool and the volatiles were removed by evaporation. Water was added to the residue and the aqueous mixture was extracted with methylene chloride (x4). The extracts were combined, washed with brine and dried by filtration through phase separating paper. The volatiles were removed by evaporation, the residue was triturated with ether, collected by filtration and dried to give 4-(4-chloro-2-fluoroanilino)-7-(2,2-dimethoxyethoxy)-6-methoxyquinazoline (440mg, 35%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.36(s, 6H); 3.94(s, 3H); 4.05(d, 2H); 4.75(t, 1H); 7.22(s, 1H); 7.32(m, 1H); 7.52(m, 1H); 7.58(t, 1H); 7.80(s, 1H); 8.35(s, 1H); 9.52(s, 1H)

MS - ESI: 408 $[MH]^+$

Example 46

Diethyl azodicarboxylate (1.55ml, 9.89mmol), 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (1.2g, 3.3mmol), (prepared as described for the starting material in Example 48), and a solution of (E)-4-(pyrrolidin-1-yl)but-2-en-1-ol (697mg, 4.9mmol), (prepared as described for the starting material in Example 17), in methylene chloride (5ml) were added successively to a solution of triphenylphosphine (2.59g, 9.89mmol) in methylene chloride (150ml) cooled at 5°C. The mixture was stirred at ambient temperature for 10 minutes then methylene chloride (100ml) was added followed successively by triphenylphosphine (432mg, 1.6mmol), (E)-4-(pyrrolidin-1-yl)but-2-en-1-ol (232mg, 1.6mmol) and diethyl azodicarboxylate (246µl, 1.6mmol). The mixture was stirred at ambient temperature for 30 minutes and then the solvent was removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (8/2 followed by 7/3 and 6/4). The semi-purified product was repurified by column chromatography eluting with methylene chloride/methanol (8/2 followed by 7.5/2.5). The purified product was dissolved in methylene chloride, 3.7M ethereal hydrogen chloride (3ml) was added and the volatiles were removed by evaporation. The residue was triturated with ether, collected by filtration and dried under vacuum to give **(E)-4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(4-pyrrolidin-1-ylbut-2-en-1-yloxy)quinazoline** hydrochloride (600mg, 32%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.8-1.9(m, 2H); 2.0-2.1(m, 2H); 3.0-3.1(m, 2H); 3.45-3.55(m, 2H); 3.88(d, 2H); 4.01(s, 3H); 4.9(d, 2H); 6.0(td, 1H); 6.3(td, 1H); 7.41(s, 1H); 7.5-7.65(m, 2H); 7.82(d, 1H); 8.13(s, 1H); 8.88(s, 1H)

MS - (EI): 487 $[M.]^+$

Elemental analysis: Found C 48.2 H 4.9 N 9.6

$C_{23}H_{24}N_4O_2BrF\ 0.5H_2O\ 2HCl$ Requires C 48.5 H 4.8 N 9.8%

Example 47

Diethyl azodicarboxylate (261mg, 1.5mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (160mg, 0.5mmol), (prepared as described for the starting material in Example 2), triphenylphosphine (393mg, 1.5mmol) and 1-(3-hydroxypropyl)-2-pyrrolidinone (107mg, 0.75mmol) in methylene chloride (5ml) under nitrogen. The mixture was stirred for 20 minutes at ambient temperature and then purified by pouring directly onto a column of silica eluting with methylene chloride/ethyl acetate/methanol (60/35/5 followed by 60/30/10). The purified product was triturated with ether and collected by filtration. The solid was dissolved in ethyl acetate and treated with 3M hydrogen chloride in ethyl acetate (0.4ml). The precipitate was collected by filtration, washed with ethyl acetate and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(2-oxopyrrolidin-1-yl)propoxy)quinazoline** hydrochloride (170mg, 70%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.9-2.0(m, 2H); 2.0-2.1(m, 2H); 2.21(t, 2H); 3.4-3.5(m, 4H); 4.02(s, 3H); 4.20(s, 3H); 4.20(t, 2H); 7.32(s, 1H); 7.46(dd, 1H); 7.63(t, 1H); 7.71(dd, 1H); 8.17(s, 1H); 8.87(s, 1H)

MS - ESI: 445 $[MH]^+$

Elemental analysis : Found C 54.9 H 4.7 N 11.6

$C_{22}H_{22}N_4O_3ClF\ 0.3H_2O\ 0.85HCl$ Requires C 54.9 H 4.9 N 11.6%

The starting material was prepared as follows:

A solution of γ-butyrolactone (8.6g, 0.1mol) and 3-amino-1-propanol (9g, 0.12mol) was heated at reflux for 18 hours. The crude product mixture was distilled under reduced pressure to give 1-(3-hydroxypropyl)-2-pyrrolidinone (2.5g, 17%).

b.p. ~ 130°C under ~0.05mmHg

^{1}H NMR Spectrum: ($CDCl_3$) 1.7-1.8(m, 3H); 2.0-2.15(m, 2H); 2.44(t, 2H); 3.4-3.5(m, 4H); 3.54(t, 2H)

MS - (EI): 143 $[M\cdot]^+$

Example 48

Using a method analogous to that in Example 47, 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (146mg, 0. mmol) in methylene chloride (5ml) was treated with 1-(3-hydroxypropyl)-2-pyrrolidinone (86mg, 0.6mmol), triphenylphosphine (314mg, 1.2mmol) and diethyl azodicarboxylate (209mg, 1.2mmol) and was purified and isolated to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-(2-oxopyrrolidin-1-yl)propoxy)quinazoline** hydrochloride (140mg, 67%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.9-2.0(m, 2H); 2.0-2.1(m, 2H); 2.21(t, 2H); 3.4-3.5(m, 4H); 4.02(s, 3H); 4.20(t, 2H); 7.32(s, 1H); 7.5-7.65(m, 2H); 7.82(d, 1H); 8.15(s, 1H); 8.87(s, 1H)

MS - ESI: 490 $[MH]^+$

Elemental analysis:	Found	C 49.9	H 4.4	N 10.5
$C_{22}H_{22}N_4O_3BrF$ 0.2H_2O 0.95HCl	Requires	C 50.1	H 4.5	N 10.6%

The starting material was prepared as follows:

A solution of 7-benzyloxy-4-chloro-6-methoxyquinazoline (8.35g, 27.8mmol), (prepared as described for the starting material in Example 1), and 4-bromo-2-fluoroaniline (5.65g, 29.7mmol) in 2-propanol (200ml) was heated at reflux for 4 hours. The resulting precipitate was collected by filtration, washed with 2-propanol and then ether and dried under vacuum to give 7-benzyloxy-4-(4-bromo-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (9.46g, 78%).

^{1}H NMR Spectrum: ($DMSOd_6$; CD_3COOD) 4.0(s, 3H); 5.37(s, 2H); 7.35-7.5(m, 4H); 7.52-7.62(m, 4H); 7.8(d, 1H); 8.14(9s, 1H); 8.79(s, 1H)

MS - ESI: 456 $[MH]^+$

Elemental analysis:	Found	C 54.0	H 3.7	N 8.7
$C_{22}H_{17}N_3O_2BrF$ 0.9HCl	Requires	C 54.2	H 3.7	N 8.6%

A solution of 7-benzyloxy-4-(4-bromo-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (9.4g, 19.1mmol) in TFA (90ml) was heated at reflux for 50 minutes. The mixture was allowed to cool and was poured on to ice. The resulting precipitate was collected by filtration and dissolved in methanol (70ml). The solution was adjusted to pH9-10 with concentrated aqueous ammonia solution. The mixture was concentrated to half initial volume by evaporation. The resulting precipitate was collected by filtration, washed with water and then ether, and dried under vacuum to give 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (5.66g, 82%).

^{1}H NMR Spectrum: ($DMSOd_6$; CD_3COOD) 3.95(s, 3H); 7.09(s, 1H); 7.48(s, 1H); 7.54(t, 1H); 7.64(d, 1H); 7.79(s, 1H); 8.31(s, 1H)

MS - ESI: 366 $[MH]^+$

Elemental analysis:	Found	C 49.5	H 3.1	N 11.3
$C_{15}H_{11}N_3O_2BrF$	Requires	C 49.5	H 3.0	N 11.5%

Example 49

Methanesulphonyl chloride (32mg, 0.275mmol) was added dropwise to a mixture of 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-methylaminopropoxy)quinazoline (109mg, 0.25mmol) and triethylamine (30mg, 0.3mmol) in methylene chloride (3ml) cooled at 0°C. The solution was stirred for 2 hours at 0°C and the volatiles were removed by evaporation. The residue was partitioned between ethyl acetate and water, the organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The solid was triturated with ether and collected by filtration. The solid was dissolved in methylene chloride containing methanol (0.5ml) and 3M hydrogen chloride in ethyl acetate (0.3ml) was added. The suspension was diluted with ethyl acetate and concentrated by evaporation. The resulting solid product was collected by filtration, washed with ether and dried under vacuum to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-([N-methyl-N-methylsulphonyl]amino)propoxy)quinazoline** hydrochloride (85mg, 61%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.1-2.2(m, 2H); 2.82(s, 3H); 2.89(s, 3H); 3.29(t, 2H); 4.02(s, 3H); 4.27(t, 2H); 7.35(s, 1H); 7.55-7.65(m, 2H); 7.79(d, 1H); 8.12(s, 1H); 8.88(s, 1H)

MS - (EI): 512 $[M.]^+$

Elemental analysis:	Found	C 43.5	H 4.2	N 10.0
$C_{20}H_{22}N_4O_4BrFS\ 0.6H_2O\ 0.75HCl$	Requires	C 43.5	H 4.4	N 10.2%

The starting material was prepared as follows:

Diethyl azodicarboxylate (522mg, 3mmol) was added dropwise to a suspension of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (364mg, 1mmol), (prepared as described for the starting material in Example 48), triphenylphosphine (786mg, 3mmol) and 3-methylamino-1-propanol (178mg, 2mmol), (J.Am.Chem.Soc., 1954, 76, 2789), in methylene chloride (4ml) under nitrogen. The mixture was stirred for 1 hour at ambient temperature, neutral alumina (~ 20g) was added to the reaction mixture and the solvent was removed by evaporation. The powder was poured onto a column of neutral alumina and was eluted with a mixture of methylene chloride/methanol (95/5 followed by 90/10 and 80/20). The purified product was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-methylaminopropoxy)quinazoline (220mg, 50%).

Example 50

A solution of diethyl azodicarboxylate (209mg, 1.2mmol) in methylene chloride (1ml) and then (S)-1-(3-hydroxypropyl)-pyrrolidine-2-carboxamide (97mg, 0.56mmol) was added dropwise to a suspension of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (146mg, 0.4mmol), (prepared as described for the starting material in Example 48), and triphenylphosphine (314mg, 1.2mmol) in methylene chloride (4ml) under nitrogen. The mixture was stirred for 1 hour at ambient temperature and further triphenylphosphine (109mg, 0.4mmol) and (S)-1-(3-hydroxypropyl)-pyrrolidine-2-carboxamide (40mg, 0.23mmol) were added followed by the dropwise addition of diethyl azodicarboxylate (70mg, 0.4mmol). The mixture was stirred for 30 minutes at ambient temperature and further (S)-1-(3-hydroxypropyl)-pyrrolidine-2-carboxamide (34mg, 0.2mmol) was added. The mixture was then stirred for 2 hours at ambient temperature and the mixture was purified by pouring directly onto a column of silica and eluting with methylene chloride/ethyl acetate/methanol (60/35/5). The purified product was triturated with ether, collected by filtration, washed with ether and dried under vacuum. The solid was

dissolved in methylene chloride and 3M hydrogen chloride in ethyl acetate (0.4ml) was added. The resulting precipitate was collected by filtration, washed with ethyl acetate and dried under vacuum to give **(S)-4-(4-bromo-2-fluoroanilino)-7-(3-(2-carbamoylpyrrolidin-1-yl)propoxy)-6-methoxyquinazoline** hydrochloride (110mg, 47%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD; 60°C) 1.9-2.0(m, 2H); 2.0-2.1(m, 2H); 2.15-2.25(m, 2H); 3.2-3.3(m, 1H); 3.3-3.5(m, 2H); 3.7-3.8(m, 1H); 4.02(s, 3H); 4.15-4.2(m, 1H); 4.3-4.4(m, 2H); 7.4(s, 1H); 7.5-7.6(m, 2H); 7.75(d, 1H); 8.2(s, 1H); 8.83(s, 1H)

MS - (EI): 518 $[M.]^+$

Elemental analysis:	Found	C 46.0	H 4.9	N 11.2
$C_{23}H_{25}N_5O_3BrF$ 0.8H_2O 1.9HCl	Requires	C 45.9	H 4.8	N 11.6%

The starting material was prepared as follows:

3-Bromo-1-propanol (584mg, 4.2mmol) was added to a mixture of (S)-pyrrolidine-2-carboxamide (399mg, 3.5mmol) and potassium carbonate (966mg, 7mmol) in acetonitrile (10ml). The mixture was heated at reflux for 5 hours and the mixture was stirred for 18 hours at ambient temperature. The insolubles were removed by filtration and the solvent was removed from the filtrate by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/methanol (9/1 followed by 8/2) to give (S)-1-(3-hydroxypropyl)-pyrrolidine-2-carboxamide (365mg, 60%).

MS - (EI): 173 $[M.]^+$

Example 51

Methoxyacetyl chloride (34mg, 0.31mmol) was added to a solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylaminoethoxy)quinazoline (113mg, 0.3mmol), (prepared as described for the starting material in Example 60), and triethylamine (33mg, 0.33mmol) in methylene chloride (3ml). The mixture was stirred for 18 hours at ambient temperature and was then partitioned between ethyl acetate and brine. The organic layer was separated, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by chromatography on silica eluting with methylene chloride/acetonitrile/methanol (6/3/1). The purified solid product was triturated with methylene chloride and ether, collected by filtration, washed with ether and dried under vacuum. The solid was dissolved in a mixture of

methylene chloride/methanol (1/1) and 2M hydrogen chloride in ethyl acetate (0.5ml) was added. The mixture was diluted with ether and and the resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-([N-methyl-N-methoxyacetyl]amino)ethoxy)quinazoline** hydrochloride (62mg, 42%).

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD; 80°C) 2.9-3.2(br s, 3H); 3.35(s, 3H); 3.8-3.9(br s, 2H); 4.05(s, 3H); 4.0-4.3(m, 2H); 4.4(t, 2H); 7.4(s, 1H); 7.45(d, 1H); 7.6-7.7(m, 2H); 8.1(s, 1H); 8.8(s, 1H)

MS - ESI: 449 $[MH]^+$

Elemental analysis:	Found	C 48.8	H 4.6	N 10.7
$C_{21}H_{22}N_4O_4ClF$ 0.9H_2O 1.35HCl	Requires	C 49.0	H 4.9	N 10.9%

Example 52

Diethyl azodicarboxylate (400mg, 2.3mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (250mg, 0.78mmol), (prepared as described for the starting material in Example 2), triphenylphosphine (615mg, 2.3mmol) and 4-(2-hydroxyethyl)-3-morpholinone (170mg, 1.17mmol), (EP 580402A2), in methylene chloride (5ml) under nitrogen. The mixture was stirred for 4 hours at ambient temperature, methylene chloride (5ml) was added and stirring was continued for a further 18 hours at ambient temperature. THF (5ml), 4-(2-hydroxyethyl)-3-morpholinone (113mg, 0.78mmol), triphenylphosphine (204mg, 0.78mmol) were added and diethyl azodicarboxylate (136mg, 0.78mmol) was then added dropwise. The mixture was stirred for 5 minutes at ambient temperature, and was purified by pouring directly onto a silica column, eluting with methylene chloride/ethyl acetate/methanol (5/4/1). The purified solid was dissolved in methylene chloride and 2M methanolic hydrogen chloride (0.5ml) was added. The mixture was concentrated by evaporation and then diluted with ether. The resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(3-oxomorpholino)ethoxy)quinazoline** hydrochloride (150mg, 39%).

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD) 3.6(t, 2H); 3.8-3.9(m, 4H); 4.05(s, 3H); 4.1(s, 3H); 4.4(t, 2H); 7.3(s, 1H); 7.45(d, 1H); 7.65(t, 1H); 7.7(d, 1H); 8.1(s, 1H); 8.9(s, 1H)

MS - ESI: 469 $[MNa]^+$

Elemental analysis:	Found	C 51.6	H 4.4	N 11.8
$C_{21}H_{20}N_4O_4ClF\ 0.35H_2O\ 0.95HCl$	Requires	C 51.7	H 4.5	N 11.5%

Example 53

Diethyl azodicarboxylate (209mg, 1.2mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (128mg, 0.4mmol), (prepared as described for the starting material in Example 2), triphenylphosphine (314mg, 1.2mmol) and 2-(2-morpholinoethoxy)ethanol (97mg, 0.56mmol) in methylene chloride (4ml) under nitrogen. The mixture was stirred for 1 hour at ambient temperature, triphenylphosphine (105mg, 0.4mmol), 2-(2-morpholinoethoxy)ethanol (49mg, 0.28mmol) and diethyl azodicarboxylate (70mg, 0.4mmol) were added. The mixture was stirred for 1 hour at ambient temperature and was purified by pouring directly onto a silica column eluting with methylene chloride/acetonitrile/methanol (6/3/1). The purified product was triturated with ether, collected by filtration and dissolved in methylene chloride. 2M Ethereal hydrogen chloride (0.5ml) was added and the resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-morpholinoethoxy)ethoxy)quinazoline** hydrochloride (100mg, 45%).

^1H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.1-3.2(m, 2H); 3.3-3.5(m, 5H); 3.7-3.8(m, 2H); 3.9-4.0(m, 5H); 4.02(s, 3H); 4.4(br s, 2H); 7.46(s, 1H); 7.48(d, 1H); 7.6(t, 1H); 7.7(d, 1H); 8.25(s, 1H); 8.89(s, 1H)

MS - ESI: 477 $[MH]^+$

Elemental analysis:	Found	C 48.8	H 5.6	N 9.9
$C_{23}H_{26}N_4O_4ClF\ 1H_2O\ 1.95HCl$	Requires	C 48.8	H 5.3	N 9.9%

The starting material was prepared as follows:

2-(2-Chloroethoxy)ethanol (1.25g, 10mmol) was added to a mixture of morpholine (2.58g, 30mmol) and potassium carbonate (5.5g, 40mmol) in acetonitrile (50ml). The mixture was heated at reflux for 6 hours and then stirred for 18 hours at ambient temperature. The insolubles were removed by filtration and the volatiles were removed from the filtrate by evaporation. The residue was purified by column chromatography eluting with methylene

chloride/methanol (95/5 followed by 90/10 and then 80/20) to give 2-(2-morpholinoethoxy)ethanol (600mg, 34%).

^{1}H NMR Spectrum: ($CDCl_3$) 2.5(br s, 4H); 2.59(t, 2H); 3.6-3.85(m, 10H)

MS - (EI): 175 $[M.]^+$

Example 54

Diethyl azodicarboxylate (209mg, 1.2mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (128mg, 0.4mmol), (prepared as described for the starting material in Example 2), triphenylphosphine (314mg, 1.2mmol) and (S)-1-(3-hydroxypropyl)-pyrrolidine-2-carboxamide (97mg, 0.56mmol), (prepared as described for the starting material in Example 50), in methylene chloride (4ml). The mixture was stirred for 2 hours at ambient temperature, and further triphenylphosphine (105mg, 0.4mmol) and (S)-1-(3-hydroxypropyl)-pyrrolidine-2-carboxamide (49mg, 0.28mmol) were added followed by the dropwise addition of diethyl azodicarboxylate (70mg, 0.4mmol). The mixture was stirred for 1 hour at ambient temperature, and was purified by pouring directly onto a silica column eluting with methylene chloride/acetonitrile/methanol (6/3/1 followed by 60/25/15). The purified oil was triturated with ether, collected by filtration, washed with ether and dried under vacuum. The solid was dissolved in methylene chloride and 2M ethereal hydrogen chloride (0.5ml) was added. The mixture was diluted with ether and the resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **(S)-7-(3-(2-carbamoylpyrrolidin-1-yl)propoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline** hydrochloride (70mg, 32%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.8-2.0(m, 2H); 2.05-2.15(m, 2H); 2.2-2.3(m, 2H); 3.1-3.5(m, 2H); 3.7-3.8(m, 1H); 4.02(s, 3H); 4.05-4.2(m, 2H); 4.3(m, 2H); 7.04(s, 1H); 7.45(d, 1H); 7.65(t, 1H); 7.7(d, 1H); 8.22(s, 1H); 8.88(s, 1H)

MS - ESI: 474 $[MH]^+$

Elemental analysis:	Found	C 49.4	H 12.4	N 5.3
$C_{23}H_{25}N_5O_3ClF$ 1.5H_2O 1.55HCl	Requires	C 49.5	H 12.6	N 5.3%

Example 55

Diethyl azodicarboxylate (209mg, 1.2mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (128mg, 0.4mmol), (prepared as described for the starting material in Example 2), cis-3-(2,6-dimethylmorpholino)-1-propanol (97mg, 0.56mmol) and triphenylphosphine (314mg, 1.2mmol) in methylene chloride (4ml) under nitrogen. The mixture was stirred for 1 hour at ambient temperature and the solvent was removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (95/5 followed by 90/10). The purified product was dissolved in methylene chloride and 2M ethereal hydrogen chloride (1ml) was added. The solution was diluted with ether and left to stand. The resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-7-(3-(2,6-dimethylmorpholino)propoxy)-6-methoxyquinazoline** hydrochloride (130mg, 59%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.17(d, 6H); 2.3-2.4(m, 2H); 2.7(t, 2H); 3.25-3.35(m, 2H); 3.55(d, 2H); 3.9-4.0(m, 2H); 4.03(s, 3H); 4.35(t, 2H); 7.43(s, 1H); 7.45(d, 1H); 7.63(t, 1H); 7.70(d, 1H); 8.25(s, 1H); 8.88(s, 1H)

MS - ESI: 475 $[MH]^+$

Elemental analysis:	Found	C 51.7	H 6.0	N 9.7
$C_{24}H_{28}N_4O_3ClF$ 0.6H_2O 1.95HCl	Requires	C 51.8	H 5.6	N 10.0%

The starting material was prepared as follows:

3-Chloro-1-propanol (1.04g, 11mmol) followed by potassium carbonate (2.07g, 15mmol) was added to a solution of 2,6-dimethylmorpholine (1.15g, 10mmol), (supplied by Aldrich Chemical Company Limited as a mixture of isomers), in acetonitrile (15ml). The mixture was heated at reflux overnight and allowed to cool, the insolubles were removed by filtration and the volatiles were removed from the filtrate by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/acetonitrile/methanol (60/35/5 followed by 60/30/10) to give cis-3-(2,6-dimethylmorpholino)-1-propanol (500mg).

^{1}H NMR Spectrum: ($CDCl_3$) 1.16(d, 6H); 1.7-1.8(m, 4H); 2.61(t, 2H); 2.91(d, 2H); 3.6-3.7(m, 2H); 3.81(t, 2H)

MS - ESI: 173 $[M.]^+$

Example 56

A solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(trifluoromethylsulphonyloxy)quinazoline (180mg, 0.4mmol), (prepared as described for the starting material in Example 11), in anhydrous THF (2ml) and benzene (2ml) was purged of oxygen and placed under nitrogen. Tetrakis(triphenylphosphine)palladium(0) (23mg, 0.02mmol) followed by a solution of sodium triisopropylsilylthiolate (102mg, 0.48mmol), (Tetrahedron Lett. 1994, 35, 3221), in THF (2ml) was added and the mixture was heated at reflux for 2 hours. The mixture was allowed to cool to ambient temperature and 4-(3-chloropropyl)morpholine (98mg, 0.6mmol), (J. Am. Chem. Soc. 1945, 67, 736), DMF (2ml) and tetrabutylammonium fluoride (0.5ml of a 1M solution in THF, 0.5mmol) were added sequentially. The mixture was stirred for 1 hour at ambient temperature, the volatiles were removed by evaporation and the residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified on neutral alumina eluting with methylene chloride/acetone (90/10 followed by 80/20). The purified product was triturated in a mixture of ether and hexane, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropylthio)quinazoline** hydrochloride (65mg, 30%).

^1H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.1-2.2(m, 2H); 3.1-3.2(m, 2H); 3.22(t, 2H); 3.3-3.4(m, 2H); 3.47(d, 2H); 3.74(t, 2H); 4.0(d, 2H); 4.08(s, 3H); 7.48(d, 1H); 7.64(t, 2H); 7.68(d, 1H); 7.86(s, 1H); 8.19(s, 1H); 8.91(s, 1H)

MS - ESI: 463 $[MH]^+$

Elemental analysis:	Found	C 47.6	H 5.16	N 47.6
$C_{22}H_{24}N_4O_2ClFS\ 1.2H_2O\ 1.85HCl$	Requires	C 47.8	H 5.16	N 47.8%

Example 57

3-Chloroperbenzoic acid (188mg, 1.05mmol) was added in portions to a solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethylthio)quinazoline (275mg, 0.7mmol), (prepared as described in Example 11), in methylene chloride (6ml). The mixture

was stirred for 30 minutes at ambient temperature, diluted with methylene chloride (20ml), washed with aqueous sodium hydrogen carbonate solution and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/acetone (8/2 followed by 7/3 and 6/4). The purified product was dissolved in methylene chloride and 3M ethereal hydrogen chloride (0.5ml) was added. The mixture was diluted with ether and the resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethylsulphinyl)quinazoline** hydrochloride (110mg 38%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.05(td, 1H); 3.24(s, 3H); 3.5-3.6(m, 1H); 3.7-3.8(m, 2H); 4.1(s, 3H); 7.5(d, 1H); 7.65(t, 1H); 7.75(d, 1H); 8.2(s, 1H); 8.4(s, 1H); 9.0(s, 1H)

MS - ESI: 410 $[MH]^+$

Elemental analysis:	Found	C 47.9	H 4.2	N 9.3
$C_{18}H_{17}N_3O_3ClFS$ 0.5H_2O 0.85HCl	Requires	C 48.0	H 4.2	N 9.3%

Example 58

Diethyl azodicarboxylate (218mg, 1.25mmol) was added dropwise to a solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (159mg, 0.5mmol), (prepared as described for the starting material in Example 2), 4-hydroxy-1-methylpiperidine (115mg, 1mmol) and triphenylphosphine (328mg, 1.25mmol) in methylene chloride (5ml) cooled at 5°C under nitrogen. The mixture was stirred for 1 hour at ambient temperature, the solvent was removed by evaporation and the residue was partitioned between 2M hydrochloric acid and ether. The aqueous layer was separated, adjusted to pH9 with aqueous sodium hydrogen carbonate solution and extracted with methylene chloride. The methylene chloride layer was washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified on neutral alumina eluting with methylene chloride/methanol (97/3). The purified product was triturated with ether, collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-yloxy)quinazoline** (180mg, 79%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.9-2.0(m, 1H); 2.05-2.15(m, 2H); 2.35-2.45(m, 1H); 2.85 and 2.90(2s, 3H); 3.05-3.25(m, 2H); 3.45(m, 1H); 3.6(d, 1H); 4.1 and 4.12(2s, 3H); 4.8-4.9(m, 0.5 H); 5-5.05(m, 0.5H); 7.4-7.7(m, 4H); 8.2(d, 1H); 8.9(s, 1H)

MS - ESI: 417 $[MH]^+$

Example 59

Methanesulphonyl chloride (35µl, 0.46mmol) was added dropwise to a solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-methylaminopropoxy)quinazoline (170mg, 0.43mmol) and triethylamine (67µl, 0.48mmol) in methylene chloride (3ml). The mixture was stirred for 5 hours at ambient temperature, the volatiles was removed by evaporation and the residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with water and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/acetonitrile/methanol (70/28/2). The purified product was dissolved in a mixture of methylene chloride/methanol (1/1) and 2M ethereal hydrogen chloride (1ml) was added. The volatiles were removed by evaporation and the residue was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-([N-methyl-N-methylsulphonyl]amino)propoxy)quinazoline** hydrochloride (133mg, 61%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.1-2.2(m, 2H); 2.82(s, 3H); 2.89(s, 3H); 3.3(t, 2H); 4.02(s, 3H); 4.27(t, 2H); 7.36(s, 1H); 7.46(d, 1H); 7.6-7.7(m, 2H); 8.14(s, 1H); 8.88(s, 1H)

MS - ESI: 469 $[MH]^+$

Elemental analysis:	Found	C 48.1	H 4.7	N 10.8
$C_{20}H_{22}N_4O_4ClFS$ 0.9HCl	Requires	C 47.9	H 4.6	N 11.2%

The starting material was prepared as follows:

A solution of di-tert-butyl dicarbonate (4.9g, 22mmol) in THF (12ml) was added dropwise to a solution of 3-methylamino-1-propanol (2g, 22mmol), (J.Am.Chem.Soc., 1954, 76, 2789), in a mixture of THF (12ml) and water (12ml). The mixture was stirred for 18 hours at ambient temperature, the THF was removed by evaporation. The aqueous residue was extracted with ether. The extracts were combined, washed with 0.1M hydrochloric acid, and then brine, dried ($MgSO_4$) and the solvent removed by evaporation to give 3-([N-(tert-butylcarbonyl)-N-methyl]amino)-1-propanol (3.95g, 95%).

^{1}H NMR Spectrum: ($CDCl_3$) 1.46(s, 9H); 1.6-1.8(m, 2H); 2.83(s, 3H); 3.3-3.4(br s, 2H); 3.5-3.6(br s, 2H)

MS - (EI): 190 $[MH]^+$

Diethyl azodicarboxylate (2.4ml, 15mmol) was added dropwise to a solution of 3-([N-(tert-butylcarbonyl)-N-methyl]amino)-1-propanol (1.77g, 9.4mmol), 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (2g, 6.26mmol), (prepared as described for the starting material in Example 2), and triphenylphosphine (4.1g, 15mmol) in methylene chloride (50ml) under nitrogen. The mixture was stirred for 1 hour at ambient temperature, and further 3-([N-(tert-butylcarbonyl)-N-methyl]amino)-1-propanol (236mg, 1.2mmol), triphenylphosphine (820mg, 3.1mmol) and diethyl azodicarboxylate (492µl, 3.1mmol) were added. The solution was stirred for 1 hour at ambient temperature and concentrated by evaporation. The residue was purified on column chromatography eluting with acetonitrile. The purified product was triturated with ether, collected by filtration and repurified by column chromatography eluting with methylene chloride/methanol (97/3) to give 7-(3-([N-(tert-butylcarbonyl)-N-methyl]amino)propoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (2.2g, 72%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.3(s, 9H); 2.0-2.1(m, 2H); 2.8-2.9(br s, 3H); 3.4-3.5(m, 2H); 4.0(s, 3H); 4.25(t, 2H); 7.3(s, 1H); 7.45(d, 1H); 7.6-7.7(m, 2H); 8.08(s, 1H); 8.88(s, 1H)

MS - (EI): 491 $[MH]^+$

Elemental analysis:	Found	C 58.6	H 5.8	N 11.3
$C_{24}H_{28}N_4O_4ClF$	Requires	C 58.7	H 5.7	N 11.4%

A solution of 7-(3-([N-(tert-butylcarbonyl)-N-methyl]amino)propoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (2.1g, 4.3mmol) in a mixture of methylene chloride (6ml) and TFA (5ml) was stirred at ambient temperature for 1 hour. Toluene was added and the volatiles were removed by evaporation. The residue was dissolved in water and the solution was adjusted to pH7-8 with saturated aqueous sodium hydrogen carbonate solution. The resulting precipitate was separated by centrifugation and decanting the filtrate and the solid product was thoroughly washed with water. The solid was recrystallised from methylene chloride/methanol, the product collected by filtration, washed with water, and then

ether and dried under vacuum over phosphorus pentoxide to give 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-methylaminopropoxy)quinazoline (1.4g, 83%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.2-2.3(m, 2H); 2.65(s, 3H); 3.1-3.2(m, 2H); 4.05(s, 3H); 4.32(t, 2H); 7.37(s, 1H); 7.48(d, 1H); 7.64(t, 1H); 7.67(d, 1H); 8.11(s, 1H); 8.9(s, 1H)

MS - (EI): 391 $[MH]^+$

Example 60

Triethylamine (44µl, 0.32mmol) and then a solution of 2-bromoethyl methyl ether (40mg, 0.29mmol) in acetone (0.5ml) was added dropwise to a solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylaminoethoxy)quinazoline (100mg, 0.26mmol) in acetone (2.5ml) heated at 50°C under nitrogen. The mixture was stirred for 7 hours at 50°C, the mixture was allowed to cool and partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the volatiles removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (92/8). The purified product was dissolved in methylene chloride, insolubles were removed by filtration and 2.2M ethereal hydrogen chloride (0.5ml) was added to the filtrate. The volatiles were removed by evaporation and the residue was triturated with ether, collected by filtration and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-([N-(2-methoxyethyl)-N-methyl]amino)ethoxy)quinazoline** hydrochloride (22mg, 16%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.0(s, 3H); 3.35(s, 3H); 3.4-3.6(m, 2H); 3.65-3.85(m, 4H); 4.03(s, 3H); 4.64(t, 2H); 7.45(s, 1H); 7.47(d, 1H); 7.63(t, 1H); 7.69(d, 1H); 8.23(s, 1H); 8.9(s, 1H)

MS - ESI: 435 $[MH]^+$

Elemental analysis:	Found	C 48.9	H 5.3	N 10.4
$C_{21}H_{24}N_4O_3ClF$ 0.8H_2O 1.85HCl	Requires	C 48.8	H 5.3	N 10.8%

The starting material was prepared as follows:

Diethyl azodicarboxylate (3.13g, 24mmol) was added dropwise to a suspension of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (2.56mg, 8mmol), (prepared as

described for the starting material in Example 2), 2-([N-(tert-butylcarbonyl)-N-methyl]amino)ethanol (2.1g, 1.2mmol), (Synth. Commun. 1993, 23, 2443), and triphenylphosphine (6.3g, 24mmol) in methylene chloride (50ml) under nitrogen. The mixture was stirred for 1.5 hours at ambient temperature and further 2-([N-(tert-butylcarbonyl)-N-methyl]amino)ethanol (0.21g, 1.2mmol), triphenylphosphine (630mg, 2.4mmol) and diethyl azodicarboxylate (0.31g, 2.4mmol) were added. The mixture was stirred for 1 hour; the mixture was purified by pouring it directly onto a silica column and eluting with methylene chloride/ether/methanol (60/30/10) to give 7-(2-([N-(tert-butylcarbonyl)-N-methyl]amino)ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (3.8g, 99%).

A solution of 7-(2-([N-(tert-butylcarbonyl)-N-methyl]amino)ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (2.38g, 5mmol) in methylene chloride (5ml) and TFA (10ml) was stirred at ambient temperature for 1 hour. Toluene was added and the volatiles were removed by evaporation. The residue was partitioned between 2M hydrochloric acid and ethyl acetate. The aqueous layer was adjusted to pH8 with sodium hydrogen carbonate and extracted with ethyl acetate. The organic extracts were combined, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation to give 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylaminoethoxy)quinazoline (700mg, 37%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.75(s, 3H); 3.5-3.6(m, 2H); 4.05(s, 3H); 4.5(t, 2H); 7.4(s, 1H); 7.4-7.5(m, 1H); 7.65(t, 1H); 7.7(d, 1H); 8.15(s, 1H); 8.8(s, 1H)

Example 61

Dimethylcarbamyl chloride (38µl, 0.42mmol) was added to a solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylaminoethoxy)quinazoline (150mg, 0.4mmol), (prepared as described for the starting material in Example 60), and triethylamine (61µl, 0.44mmol) in methylene chloride (4ml). The mixture was stirred for 2.5 hours at ambient temperature, the resulting precipitate was collected by filtration and washed with ether. The solid was purified by column chromatography, eluting with methylene chloride/methanol (92/8). The purified product was dissolved in methylene chloride/methanol (1/1), 2.9M ethereal hydrogen chloride (1ml) was added and the volatiles were removed by evaporation. The residue was triturated with ether and the solid product collected by filtration, washed with

ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(N',N',N-trimethylureido)ethoxy)quinazoline** hydrochloride (80mg, 41%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.73(s, 6H); 2.91(s, 3H); 3.59(t, 2H); 4.0(s, 3H); 4.34(t, 2H); 7.36(s, 1H); 7.5(d, 1H); 7.63(t, 1H); 7.68(d, 1H); 8.1(s, 1H); 8.9(s, 1H)

MS - (EI): 447 $[M.]^+$

Elemental analysis:	Found	C 51.2	H 5.1	N 13.9
$C_{21}H_{23}N_5O_3ClF$ $0.5H_2O$ 1HCl Requires		C 51.1	H 5.1	N 14.2%

Example 62

7-(2-Bromoethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (150mg, 0.35mmol) and 1-acetylpiperazine (135mg, 1mmol) were heated together at 140°C for 10 minutes. The mixture was allowed to cool and was dissolved in a mixture of methylene chloride/ethyl acetate. The solution was washed with water, and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (9/1). The purified product was dissolved in methylene chloride and 2.9M ethereal hydrogen chloride was added. The precipitate was collected by filtration, washed with ether and dried under vacuum to give **7-(2-(4-acetylpiperazin-1-yl)ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline** hydrochloride (152mg, 79%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD; 50°C) 2.07(s, 3H); 3.3-3.7(br s, 8H); 3.75(t, 2H); 4.05(s, 3H); 4.65(t, 2H); 7.45(br s, 2H); 7.6-7.7(m, 2H); 8.15(s, 1H); 8.9(s, 1H)

MS - (EI): 473 $[M.]^+$

Elemental analysis:	Found	C 49.8	H 5.0	N 12.5
$C_{23}H_{25}N_5O_3ClF$ $0.5H_2O$ 1.9HCl	Requires	C 50.0	H 5.1	N 12.7%

The starting material was prepared as follows:

1,2-Dibromoethane (5.4ml, 62mmol) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (5g, 15.6mmol), (prepared as described for the starting material in Example 2), and potassium carbonate (8.6g, 62mmol) in DMF (50ml) and the mixture stirred for 18 hours at ambient temperature. Water was added and the resulting precipitate was collected by filtration. The solid was purified by chromatography on

neutral alumina eluting with methylene chloride/methanol (95/5). The semi-purified product was repurified by chromatography on silica eluting with methylene chloride/methanol (97/3). The purified product was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give 7-(2-bromoethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (3.58g, 54%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.28(s, 3H); 3.96(s, 3H); 4.48(t, 2H); 4.85(t, 2H); 7.21(s, 1H); 7.34(d, 1H); 7.5-7.6(m, 2H); 7.80(s, 1H); 8.36(s, 1H); 9.55(s, 1H)

Example 63

A mixture of 7-(2-(2-bromoethoxy)ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (150mg, 0.32mmol) in 1-methylpiperazine (2ml) was heated at 100°C for 1 hour. The mixture was allowed to cool and was partitioned between ethyl acetate and water. The organic layer was separated and washed with water and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/methanol (85/15 followed by 80/20). The purified solid product was dissolved in methylene chloride/methanol (1/1) and 2.9M ethereal hydrogen chloride was added. The volatiles were removed by evaporation and the solid was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-[4-methylpiperazin-1-yl]ethoxy)ethoxy)quinazoline** hydrochloride (54mg, 28%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD; 50°C) 2.9(s, 3H); 3.5-3.8(m, 10H); 3.95(br s, 4H); 4.03(s, 3H); 4.4(m, 2H); 7.40-7.45(m, 1H); 7.42(s, 1H); 7.55-7.65(m, 2H); 8.15(s, 1H); 8.8(s, 1H)

MS - ESI: 490 $[MH]^+$

Elemental analysis:	Found	C 46.0	H 5.6	N 10.9
$C_{24}H_{29}N_5O_3ClF$ $1.5H_2O$ 2.9HCl	Requires	C 46.3	H 5.6	N 11.2%

The starting material was prepared as follows:

2-Bromoethyl ether (1.57ml, 12mmol) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (1g, 3.1mmol), (prepared as described for the starting material in Example 2), and potassium carbonate (1.73g, 12mmol) in DMF (10ml).

The mixture was stirred for 18 hours at ambient temperature and was partitioned between ethyl acetate and water. The organic layer was separated, washed with water and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/acetonitrile/methanol (60/38/2). The purified product was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give 7-(2-(2-bromoethoxy)ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (763mg, 52%).

^{1}H NMR Spectrum: ($CDCl_3$) 3.5(t, 2H); 3.9(t, 2H); 3.95(t, 2H); 4.03(s, 3H); 4.35(t, 2H); 7.03(s, 1H); 7.2-7.4(m, 4H); 8.55(t, 1H); 8.7(s, 1H)

MS - ESI: 472 $[MH]^+$

Example 64

A solution of 7-(2-(2-bromoethoxy)ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (150mg, 0.32mmol), (prepared as described for the starting material in Example 63), in pyrrolidine (2ml) was heated at 80°C for 5 hours. The mixture was allowed to cool and was partitioned between ethyl acetate and water. The organic layer was washed with water and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/methanol/triethylamine (80/20/0 followed by 80/20/1). The purified product was dissolved in methylene chloride and 2.9M ethereal hydrogen chloride (1ml) was added. The volatiles were removed by evaporation, the residue was triturated with ether, collected by filtration and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-pyrrolidin-1-ylethoxy)ethoxy)quinazoline** hydrochloride (35mg, 20%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.8-1.9(m, 2H); 1.95-2.1(m, 2H); 3.05-3.15(m, 2H); 3.45(t, 2H); 3.55-3.65(m, 2H); 3.8-3.85(m, 2H); 3.95-4.0(m, 2H); 4.01(s, 3H); 4.4(br s, 2H); 7.39(s, 1H); 7.48(d, 1H); 7.65(t, 1H); 7.7(d, 1H); 8.11(s, 1H); 8.89(s, 1H)

MS - ESI: 461 $[MH]^+$

Elemental analysis: Found C 49.4 H 5.4 N 10.1

$C_{23}H_{26}N_4O_3ClF$ $1.2H_2O$ 2HCl Requires C 49.7 H 5.5 N 10.1%

Example 65

1-(2-(2-Bromoethyl)ethoxy)-2-pyrrolidinone (272mg, 1.1mmol) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (250mg, 0.78mmol), (prepared as described for the starting material in Example 2), and potassium carbonate (324mg, 2.3mmol) in DMF (5ml) and the mixture stirred for 4 hours at ambient temperature. The mixture was partitioned between ethyl acetate and water, the organic layer was separated, washed with water and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography on alumina eluting with methylene chloride/acetonitrile/methanol (60/37/3). The semi-purified product was repurified by column chromatography on silica eluting with methylene chloride/methanol (95/5). The purified product was dissolved in methylene chloride and 2.9M ethereal hydrogen chloride (1ml) was added. The volatiles were removed by evaporation, the residue was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-[2-oxopyrrolidin-1-yl]ethoxy)ethoxy)quinazoline** hydrochloride (63mg, 16%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.85-1.95(m, 2H); 2.2(t, 2H); 3.35-3.45(m, 4H); 3.65(t, 2H); 3.9(br s, 2H); 4.02(s, 3H); 4.35(br s, 2H); 7.35(s, 1H); 7.45(d, 1H); 7.65(t, 1H); 7.7(d, 1H); 8.15(s, 1H); 8.88(s, 1H)

MS - ESI: 475 $[MH]^+$

Elemental analysis:	Found	C 53.2	H 5.0	N 11.1
$C_{23}H_{24}N_4O_4ClF$ 0.6H_2O 0.85HCl	Requires	C 53.5	H 5.1	N 10.8%

The starting material was prepared as follows:

A solution of 2-pyrrolidinone (1.5g, 17.6mmol) in anhydrous toluene (8ml) was added dropwise to a suspension of sodium hydride (741mg, 18mmol, prewashed with pentane) in anhydrous toluene (60ml) and the mixture was stirred at 100°C for 1.5 hours. The mixture was allowed to cool to ambient temperature and tetrabutylammonium bromide (57mg, 0.176mmol) was added followed by 2-bromoethyl ether (8ml, 35mmol). The mixture was stirred for 21 hours at ambient temperature, the insolubles were removed by filtration and the solid was washed with ether. The volatiles were removed from the filtrate by evaporation and the residue was purified by column chromatography on silica eluting with methylene

chloride/acetonitrile/methanol (60/38/2) to give 1-(2-(2-bromoethyl)ethoxy)2-pyrrolidinone (971mg, 23%).

^{1}H NMR Spectrum: ($CDCl_3$) 2.0-2.1(m, 2H); 2.4(t, 2H); 3.4-3.5(m, 4H); 3.52(t, 2H); 3.65(t, 1H); 3.78(t, 2H)

MS - (EI): 237 $[M]^+$

Example 66

Diethyl azodicarboxylate (325µl, 2mmol) was added dropwise to a mixture of (E)-4-morpholinobut-2-en-1-ol (151mg, 0.96mmol), (J. Med. Chem. 1972, 15, 110-112), 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (220mg, 0.688mol), (prepared as described for the starting material in Example 2), and triphenylphosphine (541mg, 2mmol) in methylene chloride (4ml). The mixture was stirred for 30 minutes at ambient temperature, and further (E)-4-morpholinobut-2-en-1-ol (10mg, 0.06mmol), triphenylphosphine (36mg, 0.137mol) and diethyl azodicarboxylate (22µl, 0.14mmol) were added. The mixture was stirred for 20 minutes and the volatiles were removed by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/methanol (92/8). The purified solid was dissolved in methylene chloride and 2M ethereal hydrogen chloride (3ml) was added. The volatiles were removed by evaporation and the solid was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **(E)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-morpholinobut-2-en-1-yloxy)quinazoline** hydrochloride (165mg, 45%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.1-3.15(m, 2H); 3.35-3.45(m, 2H); 3.75(t, 2H); 3.9(d, 2H); 4.0(d, 2H); 4.03(s, 3H); 4.95(d, 2H); 6.05(td, 1H); 6.3(td, 1H); 7.45(s, 1H); 7.47(d, 1H); 7.62(t, 1H); 7.7(d, 1H); 8.25(s, 1H); 8.88(s, 1H)

MS - ESI: 459 $[MH]^+$

Elemental analysis:	Found	C 50.3	H 5.3	N 10.1
$C_{23}H_{24}N_4O_3ClF$ 1.4H_2O 1.8HCl	Requires	C 50.2	H 5.2	N 10.2%

Example 67

Diethyl azodicarboxylate (368µl, 2.34mmol) was added dropwise to a mixture of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (284mg, 0.78mmol), (prepared

as described for the starting material in Example 48), triphenylphosphine (613mg, 2.34mmol) and 4-(2-hydroxyethyl)-3-morpholinone (170mg, 1.17mmol), (EP580402A2), in methylene chloride (10ml) under nitrogen. The mixture was stirred for 2.5 hours at ambient temperature, the insolubles were removed by filtration. The filtrate was purified by pouring it directly on to a column of silica and eluting with methylene chloride/ethyl acetate/methanol (60/35/5). The purified product was triturated with ether and collected by filtration. The solid was dissolved in methylene chloride containing a few drops of methanol and 3.8M ethereal hydrogen chloride (0.5ml) was added. The volatiles were removed by evaporation and the residue was triturated with ether, collected by filtration and dried under vacuum to give of **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(3-oxomorpholino)ethoxy)quinazoline** hydrochloride (108mg, 26%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.56(t, 2H); 3.8-3.9(m, 4H); 4.06(s, 3H); 4.06(s, 2H); 4.4(t, 2H); 7.35(s, 1H); 7.5-7.6(m, 2H); 7.8(d, 1H); 8.13(s, 1H); 8.87(s, 1H)

MS - ESI: 491 $[MH]^+$

Elemental analysis:	Found	C 47.1	H 4.1	N 10.5
$C_{21}H_{20}N_4O_4BrF\ 0.3H_2O\ 0.95HCl$	Requires	C 47.5	H 4.1	N 10.5%

Example 68

Diethyl azodicarboxylate (283µl, 1.8mmol) was added dropwise to a mixture of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (218mg, 0.6mmol), (prepared as described for the starting material in Example 48), triphenylphosphine (95µl, 0.84mmol) and 1-(2-hydroxyethyl)-2-pyrrolidinone (95µl, 0.84 mmol) in methylene chloride (8ml) under nitrogen. The mixture was stirred for 4 hours at ambient temperature, and then purified by pouring it directly on to a column of silica and eluting with methylene chloride/acetonitrile/methanol (60/32.5/7.5). The purified product was triturated with ether and collected by filtration. The solid was dissolved in methylene chloride/methanol (1/1) and 2M ethereal hydrogen chloride (1ml) was added. The volatiles were removed by evaporation, the residue was triturated with ether, collected by filtration and dried under vacuum to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(2-oxopyrrolidin-1-yl)ethoxy)quinazoline** hydrochloride (182mg, 60%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.9-2.0(m, 2H); 2.24(t, 2H); 3.53(t, 2H); 3.7(t, 2H); 4.01(s, 3H); 4.34(t, 2H); 7.36(s, 1H); 7.5-7.6(m, 2H); 7.75(d, 1H); 8.16(s, 1H); 8.87(s, 1H)

MS - ESI: 477 $[MH]^+$

Elemental analysis:	Found	C 50.2	H 4.3	N 10.9
$C_{21}H_{20}N_4O_3BrF$ 0.8HCl	Requires	C 50.0	H 4.2	N 11.1%

Example 69

Diethyl azodicarboxylate (236µl, 1.5mmol) was added dropwise to a mixture of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (182mg, 0.5mmol), (prepared as described for the starting material in Example 48), triphenylphosphine (393mg, 1.5mmol) and 2-(2-methoxyethoxy)ethanol (84µl, 0.7mmol) in methylene chloride (7ml) under nitrogen. The mixture was stirred for 4 hours at ambient temperature, the reaction mixture was purified by pouring it directly on to a column of silica and eluting with ethyl acetate/petroleum ether (9/1 followed by 10/0). The purified product was triturated with ether and collected by filtration. The solid was dissolved in methylene chloride/methanol and 2M ethereal hydrogen chloride (1ml) was added. The mixture was concentrated by evaporation and the precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(2-methoxyethoxy)ethoxy)quinazoline** hydrochloride (84mg, 34%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.26(s, 3H); 3.47(m, 2H); 3.64(m, 2H); 3.85(m, 2H); 4.02(s, 3H); 4.35(m, 2H); 7.35(s, 1H); 7.5-7.7(m, 2H); 7.82(d, 1H); 8.12(s, 1H); 8.87(s, 1H)

MS - ESI: 468 $[MH]^+$

Elemental analysis:	Found	C 47.5	H 4.4	N 8.7
$C_{20}H_{21}N_3O_4BrF$ 0.65H_2O 0.65HCl	Requires	C 47.9	H 4.6	N 8.3%

Example 70

Diethyl azodicarboxylate (567µl, 3.6mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (383mg, 1.2mmol), (prepared as described for the starting material in Example 2), 4-(3-hydroxypropyl)-3,5-dioxomorpholine

(291mg, 1.68mmol) and triphenylphosphine (944mg, 3.6mmol) in methylene chloride (10ml) under nitrogen. The mixture was stirred at ambient temperature for 6 hours and the insolubles were removed by filtration. The filtrate was purified by pouring it directly on to a column of silica and eluting with methylene chloride/acetonitrile/methanol (60/34/6 followed by 60/24/16 and 60/16/24). The semi-purified product was repurified by column chromatography eluting with methylene chloride/acetonitrile/methanol (5/4/1). The purified product was dissolved in methylene chloride/methanol, 2M ethereal hydrogen chloride (1ml) was added and the volatiles were removed by evaporation. The residue was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-7-(3-(3,5-dioxomorpholino)propoxy)-6-methoxyquinazoline** hydrochloride (56mg, 10%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.0-2.1(m, 2H); 3.35(t, 2H); 3.98(s, 2H); 4.01(s, 3H); 4.24(t, 2H); 7.33(s, 1H); 7.45(d, 1H); 7.62(t, 1H); 7.68(d, 1H); 8.13(s, 1H); 8.87(s, 1H)

MS - ESI: 475 $[MH]^+$

Elemental analysis:	Found	C 48.9	H 4.4
$C_{22}H_{20}N_4O_5ClF$ 1.4H_2O 1HCl	Requires	C 49.2	H 4.5

The starting material was prepared as follows:

A solution of diglycolic anhydride (2.32g, 20mmol) in 3-amino-1-propanol (6ml) was refluxed at 180°C for 3 hours. The volatiles were removed by evaporation and the residue was purified by column chromatography on silica eluting with methylene chloride/methanol (8/2) to give 4-(3-hydroxypropyl)-3,5-dioxomorpholine (3.46g, 99%).

^{1}H NMR Spectrum: ($CDCl_3$) 2.75-2.8(m, 2H); 3.1(br s, 1H); 3.45-3.5(m, 2H); 3.75(t, 2H); 4.04(s, 2H)

MS - (EI): 174 $[MH]^+$

Example 71

Diethyl azodicarboxylate (472µl, 3mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (319.5mg, 1mmol), (prepared as described for the starting material in Example 2), triphenylphosphine (786mg, 3mmol) and 4-(2-hydroxyethyl)-3,5-dioxomorpholine (223mg, 1.4mmol) in methylene chloride (10ml)

under nitrogen. The mixture was stirred at ambient temperature for 4.5 hours and the insolubles were removed by filtration. The solvent was removed from the filtrate by evaporation and the residue was purified by column chromatography eluting with methylene chloride/acetonitrile/methanol (85/12.5/2.5). The purified product was dissolved in methylene chloride, 2M ethereal hydrogen chloride (1ml) was added and the mixture was diluted with ether. The resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-7-(2-(3,5-dioxomorpholino)ethoxy)-6-methoxyquinazoline** hydrochloride (97mg, 20%).

^{1}H NMR ($DMSOd_6$; CF_3COOD) 4.0(s, 3H); 4.19(d, 2H); 4.39(t, 2H); 4.45(s, 4H); 7.35(s, 1H); 7.45(d, 1H); 7.67(t, 1H); 7.69(d, 1H); 8.12(s, 1H); 8.87(s, 1H)

MS - ESI: 461 $[MH]^+$

Elemental analysis:	Found	C 49.9	H 3.9	N 11.1
$C_{21}H_{18}N_4O_5ClF\ 0.5H_2O\ 0.9HCl$	Requires	C 50.2	H 4.0	N 11.1%

The starting material was prepared as follows:

Ethanolamine (2.44g, 40mmol) was added dropwise to a solution of diglycolic anhydride (2.32g, 20mmol) in pyridine (10ml). The mixture was stirred for 5 minutes at ambient temperature and then heated at reflux for 2 hours. The volatiles were removed by evaporation and the residue was heated at 180°C for 2 hours. The reaction mixture was allowed to cool and was purified by column chromatography eluting with methylene chloride/methanol (9/1) to give 4-(2-hydroxyethyl)-3,5-dioxomorpholine (400mg, 12.5%)

^{1}H NMR Spectrum: ($CDCl_3$) 1.6(br s, 1H); 3.8(t, 2H); 4.05(t, 2H); 4.4(s, 4H)

MS - EI: 160 $[MH]^+$

Example 72

Diethyl azodicarboxylate (378µl, 2.4mmol) was added dropwise to a mixture of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (292mg, 0.8mmol), (prepared as described for the starting material in Example 48), triphenylphosphine (629mg, 2.4mmol) and 2-(2-morpholinoethoxy)ethanol (196mg, 1.12mmol), (prepared as described for the starting material in Example 53), in methylene chloride (10ml) under nitrogen. The mixture was stirred for 3.5 hours at ambient temperature and the mixture was purified by pouring it

directly on to a column of silica and eluting with methylene chloride/acetonitrile/methanol (6/3/1). The purified product was dissolved in methylene chloride/methanol and the insolubles were removed by filtration. 2M Ethereal hydrogen chloride (1ml) was added to the filtrate and the volatiles were removed by evaporation. The residue was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(2-morpholinoethoxy)ethoxy)quinazoline** hydrochloride (232mg, 49%).

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD) 3.1-3.2(m, 2H); 3.35-3.4(br s, 2H); 3.45(d, 2H); 3.75(t, 2H); 3.9-4.0(m,6H); 4.02(s, 3H); 4.4(br s, 2H); 7.45(s, 1H); 7.5-7.6(m, 2H); 7.8(m, 1H); 8.22(s, 1H); 8.87(s, 1H)

MS - ESI: 523 $[MH]^+$

Elemental analysis:	Found	C 46.3	H 4.9	N 9.2
$C_{23}H_{26}N_4O_4BrF$ 0.5H_2O 1.8HCl	Requires	C 46.3	H 4.9	N 9.4%

Example 73

Diethyl azodicarboxylate (220µl, 1.4mmol) was added dropwise to a mixture of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (170mg, 0.46mmol), (prepared as described for the starting material in Example48), triphenylphosphine (367mg, 1.4mmol) and 3-(1,1-dioxothiomorpholino)-1-propanol (135mg, 0.7mmol) in methylene chloride (4ml) under nitrogen. The mixture was stirred for 1 hour at ambient temperature and further triphenylphosphine (61mg, 0.23mmol), 3-(1,1-dioxothiomorpholino)-1-propanol (30mg, 0.23mmol) and diethyl azodicarboxylate (37µl, 0.23mmol) were added. The mixture was stirred for 1 hour at ambient temperature and the mixture was purified by pouring it on to a column of silica and eluting with methylene chloride/methanol (95/5). The purified product was dissolved in methylene chloride/methanol, 2.2M ethereal hydrogen chloride (1ml) was added and the volatiles were removed by evaporation. The residue was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-bromo-2-fluoroanilino)-7-(3-(1,1-dioxothiomorpholino)propoxy)-6-methoxyquinazoline** hydrochloride (138mg, 47%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.3-2.4(m, 2H); 3.5(t, 2H); 3.7-3.8(br s, 4H); 3.85(br s, 4H); 4.03(s, 3H); 4.35(t, 2H); 7.4(s, 1H); 7.5-7.6(m, 2H); 7.8(d, 1H); 8.21(s, 1H); 8.88(s, 1H)

MS - ESI: 539 $[MH]^+$

Elemental analysis:	Found	C 42.1	H 4.6	N 8.6
$C_{22}H_{24}N_4O_4BrFS$ 1.1H_2O 1.85HCl	Requires	C 42.2	H 4.5	N 8.9%

The starting material was prepared as follows:

A mixture of 3-amino-1-propanol (650µl, 8.4mmol) and vinyl sulphone (1g, 8.4mmol) was heated at 110°C for 45 minutes. The mixture was allowed to cool and was purified by column chromatography eluting with methylene chloride/methanol (95/5) to give 3-(1,1-dioxothiomorpholino)-1-propanol (800mg, 90%).

^{1}H NMR Spectrum: ($CDCl_3$) 1.7-1.8(m, 2H); 2.73(t, 2H); 3.06(br s, 8H); 3.25(s, 1H); 3.78(t, 2H)

MS - ESI: 194 $[MH]^+$

Example 74

A solution of 2-methoxyethylsulphonyl chloride (42mg, 0.26mmol), (J. Amer. Chem. Soc. 1992, 114, 1743-1749), in acetonitrile (1ml) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylaminoethoxy)quinazoline (94mg, 0.25mmol), (prepared as described for the starting material in Example 60), and triethylamine (80µl, 0.5mmol) in acetonitrile (15ml). The mixture was stirred for 10 minutes at ambient temperature, the volatiles were removed by evaporation and the residue was partitioned between methylene chloride and water. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (97/3). The purified product was dissolved in methylene chloride (5ml) and 2.2M ethereal hydrogen chloride (2ml) was added and the volatiles were removed by evaporation. The residue was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-7-(2-([N-methyl-N-(2-methoxyethylsulphonyl)]amino)ethoxy)-6-methoxyquinazoline** hydrochloride (86mg, 64%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.96(s, 3H); 3.28(s, 3H); 3.47(t, 2H); 3.6-3.7(m, 4H); 4.02(s, 3H); 4.37(t, 2H); 7.37(s, 1H); 7.46(d, 1H); 7.64(t, 1H); 7.7(d, 1H); 8.15(s, 1H); 8.88(s, 1H)

MS - ESI 499 $[MH]^+$

Elemental analysis:	Found	C 47.2	H 4.9	N 10.2
$C_{21}H_{24}N_4O_5ClFS$ 1HCl	Requires	C 47.1	H 4.7	N 10.5%

Example 75

Using a method analogous to that in Example 74, a mixture of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylaminoethoxy)quinazoline (102mg, 0.27mmol), (prepared as described for the starting material in Example 60), and triethylamine (0.1ml, 0.72mmol) in acetonitrile (17ml) was treated with 3-morpholinopropylsulphonyl chloride (75mg, 0.28mmol), (WO 930181), to give, after purification and hydrochloride salt formation, **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-([N-methyl-N-(3-morpholinopropylsulphonyl)]amino)ethoxy)quinazoline** hydrochloride (96mg, 54%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.1-2.2(m, 2H); 3.0(s, 3H); 3.05-3.15(m, 2H); 3.2-3.3(m, 2H); 3.3-3.4(m, 2H); 3.45(d, 2H); 3.65-3.8(m, 4H); 3.95(d, 2H); 4.03(s, 3H); 4.39(t, 2H); 7.42(s, 1H); 7.45(d, 1H); 7.65(t, 1H); 7.7(d, 1H); 8.2(s, 1H); 8.9(s, 1H)

MS - ESI: 568 $[MH]^+$

Example 76

Diethyl azodicarboxylate (0.18ml, 1.14mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (111mg, 0.35mmol), (prepared as described for the starting material in Example 2), triphenylphosphine (312mg, 1.19mmol) and (S)-1-(3-hydroxypropyl)-2-(N,N-dimethylcarbamoyl)pyrrolidine (84mg, 0.42mmol) in methylene chloride (10ml) cooled at 0°C under nitrogen. The mixture was stirred for 15 minutes at 0°C, the mixture was allowed to warm to ambient temperature and was then stirred for 22 hours. Further (S)-1-(3-hydroxypropyl)-2-(N,N-dimethylcarbamoyl)pyrrolidine (10mg, 0.05mmol), triphenylphosphine (35mg, 0.13mmol) and diethyl azodicarboxylate (20µl, 0.13mmol) were added and the mixture was stirred for a further 2 hours. The mixture was partitioned between water and methylene chloride and the aqueous phase was adjusted to pH2

with 2M hydrochloric acid. The aqueous layer was separated, adjusted to pH9 with sodium hydrogen carbonate and was extracted with methylene chloride. The combined organic extracts were washed with water and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/methanol (85/15 followed by 75/25 and 60/40). The purified product was dissolved in methylene chloride (5ml) and methanol (1ml), 3.9M ethereal hydrogen chloride (0.5ml) was added and the mixture was diluted with ether. The resulting precipitate was collected by filtration, washed with ether, and dried under vacuum to give **(S)-4-(4-chloro-2-fluoroanilino)-7-(3-(2-(N,N-dimethylcarbamoyl)pyrrolidin-1-yl)propoxy)-6-methoxyquinazoline** hydrochloride (86mg, 32%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.8-1.95(m, 2H); 2.1-2.3(m, 4H); 2.92(s, 3H); 3.0(s, 3H); 3.2-3.45(m, 3H); 3.75-3.85(m, 1H); 4.0(s, 3H); 4.32(t, 2H); 4.75(t, 1H); 7.4(s, 1H); 7.45(d, 1H); 7.65(t, 1H); 7.7(d, 1H); 8.25(s, 1H); 8.9(s, 1H)

MS - ESI: 502 $[MH]^+$

Elemental analysis:	Found	C 50.2	H 5.5	N 11.6
$C_{25}H_{29}N_5O_3ClF$ $1H_2O$ 2HCl	Requires	C 50.6	H 5.6	N 11.8%

The starting material was prepared as follows:

A mixture of (S)-2-(N,N-dimethylcarbamoyl)pyrrolidine (426mg, 3mmol), (Chem. pharm. Bull. 1973, 21, 2112-2116), 3-bromo-1-propanol (0.41ml, 4.5mmol) and potassium carbonate (829mg, 6mmol) in acetonitrile (6ml) was heated at reflux for 8 hours. The mixture was allowed to cool and was partitioned between methylene chloride and water. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography, eluting with methylene chloride/methanol (a gradient from 90/10 to 60/40) to give (S)-1-(3-hydroxypropyl)-2-(N,N-dimethylcarbamoyl)pyrrolidine (290mg, 48%).

^{1}H NMR Spectrum: ($CDCl_3$; CD_3COOD) 1.8-2.1(m, 4H); 2.2-2.3(m, 1H); 2.6-2.7(m,1H); 3.0(s, 3H); 3.10(s, 3H); 3.4-3.6(m, 3H); 3.75-3.85(m, 3H); 5.05(m, 1H)

MS - ESI: 223 $[MNa]^+$

Example 77

The following illustrate representative pharmaceutical dosage forms containing the compound of formula I, or a pharmaceutically acceptable salt thereof (hereafter compound X), for therapeutic or prophylactic use in humans:

(a)	Tablet I		mg/tablet
	Compound X		100
	Lactose Ph.Eur		182.75
	Croscarmellose sodium		12.0
	Maize starch paste (5% w/v paste)		2.25
	Magnesium stearate		3.0
(b)	Tablet II		mg/tablet
	Compound X		50
	Lactose Ph.Eur		223.75
	Croscarmellose sodium		6.0
	Maize starch		15.0
	Polyvinylpyrrolidone (5% w/v paste)		2.25
	Magnesium stearate		3.0
(c)	Tablet III		mg/tablet
	Compound X		1.0
	Lactose Ph.Eur		93.25
	Croscarmellose sodium		4.0
	Maize starch paste (5% w/v paste)		0.75
	Magnesium stearate		1.0
(d)	Capsule		mg/capsule
	Compound X		10
	Lactose Ph.Eur		488.5
	Magnesium stearate		1.5

(e) Injection I (50 mg/ml)

Compound X	5.0% w/v
1N Sodium hydroxide solution	15.0% v/v
0.1N Hydrochloric acid (to adjust pH to 7.6)	
Polyethylene glycol 400	4.5% w/v
Water for injection to 100%	

(f) Injection II (10 mg/ml)

Compound X	1.0% w/v
Sodium phosphate BP	3.6% w/v
0.1N Sodium hydroxide solution	15.0% v/v
Water for injection to 100%	

(g) Injection III (1mg/ml,buffered to pH6)

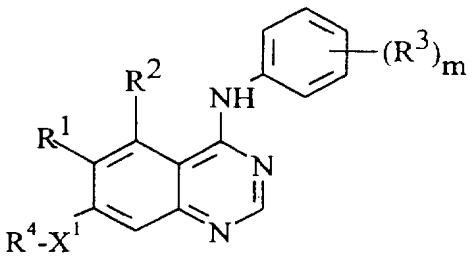
Compound X	0.1% w/v
Sodium phosphate BP	2.26% w/v
Citric acid	0.38% w/v
Polyethylene glycol 400	3.5% w/v
Water for injection to 100%	

Note

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate.

CLAIMS

1. A quinazoline derivative of the formula I:



(I)

[wherein:

m is an integer from 1 to 2;

R^1 represents hydrogen, hydroxy, halogeno, nitro, trifluoromethyl, cyano, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkylthio, or $-NR^5R^6$ (wherein R^5 and R^6, which may be the same or different, each represents hydrogen or C_{1-3}alkyl);

R^2 represents hydrogen, hydroxy, halogeno, methoxy, amino or nitro;

R^3 represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkanoyloxy, trifluoromethyl, cyano, amino or nitro;

X^1 represents -O-, $-CH_2-$, -S-, -SO-, $-SO_2-$, $-NR^7CO-$, $-CONR^8-$, $-SO_2NR^9-$, $-NR^{10}SO_2-$ or $-NR^{11}-$ (wherein R^7, R^8, R^9, R^{10} and R^{11} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

R^4 is selected from one of the following thirteen groups:

1) C_{1-5}alkylR^{12} (wherein R^{12} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to C_{1-5}alkyl through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl) or C_{1-5}alkylR^{13} (wherein R^{13} is a group selected from pyrrolidin-1-yl, imidazolidin-1-yl and thiomorpholino, which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-}

$_4$alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

2) C_{2-5}alkenylR^{14} (wherein R^{14} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

3) C_{2-5}alkynylR^{15} (wherein R^{15} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

4) C_{1-5}alkylX^2C_{1-5}alkylX^3R^{16} (wherein X^2 and X^3 which may be the same or different are each -O-, -S-, -SO-, -SO_2-, -NR^{17}CO-, -$CONR^{18}$-, -SO_2NR^{19}-, -$NR^{20}SO_2$- or -NR^{21}- (wherein R^{17}, R^{18}, R^{19}, R^{20} and R^{21} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be -CH_2- when R^4 is C_{1-5}alkylX^2C_{1-5}alkylX^3R^{16};

5) C_{1-5}alkylX^4COR^{22} (wherein X^4 represents -O- or -NR^{23}- (wherein R^{23} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{22} represents -$NR^{24}R^{25}$ or -OR^{26} (wherein R^{24}, R^{25} and R^{26} which may be the same or different each represents hydrogen, C_{1-4}alkyl or C_{1-3}alkoxyC_{2-3}alkyl));

6) C_{1-5}alkylX^5R^{27} (wherein X^5 represents -O-, -S-, -SO-, -SO_2-, -OCO-, -NR^{28}CO-, -$CONR^{29}$-, -SO_2NR^{30}-, -$NR^{31}SO_2$- or -NR^{32}- (wherein R^{28}, R^{29}, R^{30}, R^{31} and R^{32} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) or X^5 is carbonyl, and R^{27} represents cyclopentyl, cyclohexyl or a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which cyclopentyl, cyclohexyl or heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl or R^{27} is C_{1-3}alkyl with the proviso that when R^{27} is C_{1-3}alkyl, X^5 is -S-, -SO-, -SO_2-, -SO_2NR^{30}- or -$NR^{31}SO_2$- and X^1 is not -CH_2-);

7) C_{1-3}alkoxyC_{2-4}alkyl provided that X^1 is -S-, -SO- or -SO_2-;

8) C_{1-3}alkoxyC_{2-4}alkyl or C_{1-4}alkyl provided that X^1 is -O-;

9) C_{1-5}alkylX^6C_{1-5}alkylR^{33} (wherein X^6 represents -O-, -S-, -SO-, -SO_2-, -NR^{34}CO-, -$CONR^{35}$-, -SO_2NR^{36}-, -$NR^{37}SO_2$- or -NR^{38}- (wherein R^{34}, R^{35}, R^{36}, R^{37} and R^{38} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{33} represents cyclopentyl, cyclohexyl or a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which cyclopentyl, cyclohexyl or heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

10) R^{39} (wherein R^{39} is a group selected from pyrrolidin-3-yl, piperidin-3-yl and piperidin-4-yl which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

11) C_{1-5}alkylR^{40} (wherein R^{40} is piperazin-1-yl which bears at least one substituent selected from C_{1-4}alkanoyl, C_{1-4}alkoxycarbonyl, C_{1-4}hydroxyalkyl and -$CONR^{41}R^{42}$ (wherein R^{41} and R^{42} each independently represents hydrogen or C_{1-4}alkyl);

12) C_{1-5}alkylR^{43} (wherein R^{43} is morpholino which may bear one or two substituents selected from oxo, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl) with the proviso that when R^4 is C_{1-5}alkylR^{43}, X^1 is -S-, -SO-, -SO_2-, -SO_2NR^9- or -$NR^{10}SO_2$-; and

13) C_{1-5}alkylR^{44} (wherein R^{44} is morpholino which bears at least one and optionally two substituents selected from oxo, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

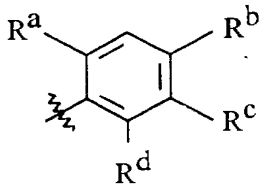
with the further proviso that when R^4 is selected from group 8) R^1 and/or R^2 is/are nitro or at least one R^3 is C_{1-3}alkanoyloxy;]

and salts thereof.

2. A quinazoline derivative as claimed in claim 1 wherein R^1 represents hydrogen, hydroxy, cyano, nitro, trifluoromethyl, methyl, ethyl, methoxy or ethoxy.

3. A quinazoline derivative as claimed in claim 1 or claim 2 wherein R^2 is hydrogen.

4. A quinazoline derivative as claimed in any one of the preceding claims wherein the phenyl group bearing $(R^3)_m$ is of the formula II:



(II)

wherein:

R^a represents hydrogen, methyl, fluoro or chloro;

R^b represents hydrogen, methyl, methoxy, bromo, fluoro or chloro;

R^c represents hydrogen or hydroxy;

R^d represents hydrogen, fluoro or chloro.

5. A quinazoline derivative as claimed in any one of the preceding claims wherein X^1 represents -O-, -S-, $-NR^7CO-$, $-NR^{10}SO_2-$ or $-NR^{11}-$ (wherein R^7, R^{10} and R^{11} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

6. A quinazoline derivative as claimed in any one of the preceding claims wherein R^4 is selected from one of the following eleven groups:

1) C_{1-4}alkylR^{12} (wherein R^{12} is a group selected from 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, pyrrolidin-2-yl, pyrrolidin-3-yl, piperidin-2-yl, piperidin-3-yl, piperidin-4-yl, morpholin-2-yl, morpholin-3-yl and piperazin-2-yl which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-

$_3$alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or C_{2-4}alkylR^{45} (wherein R^{45} is a group selected from imidazolidin-1-yl, pyrrolidin-1-yl and thiomorpholino which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

2) 1-R^{46}prop-1-en-3-yl, 1-R^{46}but-2-en-4-yl, 1-R^{46}but-1-en-3-yl, 1-R^{46}pent-2-en-4-yl or 2-R^{46}pent-3-en-5-yl (wherein R^{46} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to the alkenyl group through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or 1-R^{47}but-2-en-4-yl, 1-R^{47}pent-2-en-4-yl or 2-R^{47}pent-3-en-5-yl (wherein R^{47} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to the alkenyl group through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

3) 1-R^{48}prop-1-yn-3-yl, 1-R^{48}but-2-yn-4-yl, 1-R^{48}but-1-yn-3-yl, 1-R^{48}pent-2-yn-4-yl or 2-R^{48}pent-3-yn-5-yl (wherein R^{48} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to the alkynyl group through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or 1-R^{49}but-2-yn-4-yl, 1-R^{49}pent-2-yn-4-yl or 2-R^{49}pent-3-yn-5-yl (wherein R^{49} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to the alkynyl group through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-}

$_3$hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

4) C_{2-3}alkylX^2C_{1-3}alkylX^3R^{16} (wherein X^2 and X^3 are as defined in claim 1 and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be $-CH_2-$ when R^4 is C_{2-3}alkylX^2C_{1-3}alkylX^3R^{16};

5) C_{2-3}alkylX^4COR^{22} (wherein X^4 is as defined in claim 1 and R^{22} represents $-NR^{24}R^{25}$ or $-OR^{26}$ (wherein R^{24}, R^{25} and R^{26} which may be the same or different each represents hydrogen, C_{1-4}alkyl or C_{1-2}alkoxyethyl));

6) C_{2-3}alkylX^5R^{27} (wherein X^5 is as defined in claim 1 and R^{27} represents a group selected from cyclopentyl, cyclohexyl, pyrrolidinyl and piperidinyl which group is linked to X^5 through a carbon atom and which group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, C_{1-2}alkoxy, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl or R^{27} is C_{1-3}alkyl with the proviso that when R^{27} is C_{1-3}alkyl, X^5 is -S-, -SO-, $-SO_2-$, $-SO_2NR^{30}-$ or $-NR^{31}SO_2-$ and X1 is not $-CH_2-$);

7) C_{1-2}alkoxyC_{2-3}alkyl provided that X^1 is -S-, -SO- or $-SO_2-$;

8) C_{2-3}alkylX^6C_{2-3}alkylR^{33} (wherein X^6 is as defined in claim 1 and R^{33} represents a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl, and C_{1-3}alkoxycarbonyl);

9) C_{2-3}alkylR^{40} (wherein R^{40} is piperazin-1-yl which bears at least one substituent selected from acetyl, C_{1-2}alkoxycarbonyl, C_{1-2}hydroxyalkyl and $CONR^{41}R^{42}$ (wherein R^{41} and R^{42} each independently represents hydrogen or C_{1-2}alkyl);

10) C_{2-3}alkylR^{43} (wherein R^{43} is morpholino which may bear one or two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl) with the proviso that when R^4 is C_{2-3}alkylR^{43}, X^1 is -S-, -SO-, $-SO_2-$, $-SO_2NR^9-$ or $-NR^{10}SO_2-$; and

11) C_{2-3}alkylR^{44} (wherein R^{44} is morpholino which bears at least one and optionally two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl).

7. A quinazoline derivative as claimed in claim 6 wherein R^4 is selected from one of the following nine groups:

1) C_{1-3}alkylR^{12} (wherein R^{12} is a group selected from 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, pyrrolidin-2-yl, pyrrolidin-3-yl, piperidin-2-yl, piperidin-3-yl, piperidin-4-yl, morpholin-2-yl, morpholin-3-yl and piperazin-2-yl which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, C_{1-2}alkoxy, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl) or C_{2-3}alkylR^{45} (wherein R^{45} is a group selected from imidazolidin-1-yl, pyrrolidin-1-yl and thiomorpholino which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, C_{1-2}alkoxy, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl);

2) 1-R^{50}but-2-en-4-yl (wherein R^{50} is a group selected from imidazolidin-1-yl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, piperidin-4-yl, pyrrolidin-1-yl, pyrrolidin-3-yl, piperazin-1-yl, morpholino, thiomorpholino and piperidino which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, C_{1-2}alkoxy, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl);

3) 1-R^{51}but-2-yn-4-yl (wherein R^{51} is a group selected from imidazolidin-1-yl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, piperidin-4-yl, pyrrolidin-1-yl, pyrrolidin-3-yl, piperazin-1-yl, morpholino, thiomorpholino and piperidino which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, C_{1-2}alkoxy, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl);

4) C_{2-3}alkylX^2C_{1-3}alkylX^3R^{16} (wherein X^2 and X^3 are as defined in claim 1 and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be $-CH_2-$ when R^4 is C_{2-3}alkylX^2C_{1-3}alkylX^3R^{16};

5) C_{1-2}alkoxyC_{2-3}alkyl provided that X^1 is -S-, -SO- or -SO_2-;

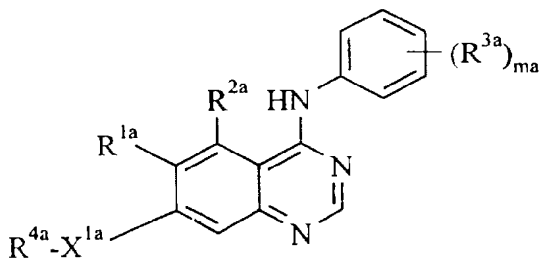
6) 2-(3,3-dimethylureido)ethyl, 3-(3,3-dimethylureido)propyl, 2-(3-methylureido)ethyl, 3-(3-methylureido)propyl, 2-ureidoethyl, 3-ureidopropyl, 2-(N,N-dimethylcarbamoyloxy)ethyl, 3-(N,N-dimethylcarbamoyloxy)propyl, 2-(N-methylcarbamoyloxy)ethyl, 3-(N-methylcarbamoyloxy)propyl, 2-(carbamoyloxy)ethyl, 3-(carbamoyloxy)propyl, 2-(1,3,3-trimethylureido)ethyl, 3-(1,3,3-trimethylureido)propyl, 2-(isopropoxycarbonylamino)ethyl, 3-(isopropoxycarbonylamino)propyl, 2-(isobutoxycarbonylamino)ethyl, 3-(isobutoxycarbonylamino)propyl, 2-(t-butoxycarbonylamino)ethyl or 3-(t-butoxycarbonylamino)propyl;

7) C_{2-3}alkylX^5R^{27} (wherein R^{27} is C_{1-2}alkyl and X^5 is -S-, -SO-, -SO_2-, -SO_2NR^{30}- or -$NR^{31}SO_2$- and with the proviso that X^1 is not -CH_2-);

8) C_{2-3}alkylX^6C_{2-3}alkylR^{33} (wherein X^6 is as defined in claim 1 and R^{33} represents a group selected from morpholino, 2-oxopyrrolidin-1-yl, pyrrolidin-1-yl, piperidino, piperazin-1-yl and 4-methylpiperazin-1-yl); and

9) C_{2-3}alkylR^{43} (wherein R^{43} is morpholino which may bear one or two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl) with the proviso that when R^4 is C_{2-3}alkylR^{43}, X^1 is -S-, -SO-, -SO_2-, -SO_2NR^9- or -$NR^{10}SO_2$-.

8. A compound as claimed in claim 1 of the formula Ia:



(Ia)

[wherein:

R^{1a} is hydrogen or methoxy;

R^{2a} is hydrogen;

the phenyl group bearing $(R^{3a})_{ma}$ is the 4-chloro-2-fluorophenyl group or the 4-bromo-2-fluorophenyl group;

X^{1a} is -O-, -S-, $-NR^{5a}CO-$ or $-NR^{6a}SO_2-$ (wherein R^{5a} and R^{6a} each independently represents hydrogen or C_{1-2}alkyl);

R^{4a} is selected from one of the following eleven groups:

1) C_{1-4}alkylR^{7a} (wherein R^{7a} is a group selected from 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, pyrrolidin-2-yl, pyrrolidin-3-yl, piperidin-2-yl, piperidin-3-yl, piperidin-4-yl, morpholin-2-yl, morpholin-3-yl and piperazin-2-yl which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or C_{2-4}alkylR^{8a} (wherein R^{8a} is a group selected from imidazolidin-1-yl, pyrrolidin-1-yl and thiomorpholino which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

2) 1-R^{9a}prop-1-en-3-yl, 1-R^{9a}but-2-en-4-yl, 1-R^{9a}but-1-en-3-yl, 1-R^{9a}pent-2-en-4-yl or 2-R^{9a}pent-3-en-5-yl (wherein R^{9a} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to the alkenyl group through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or 1-R^{10a}but-2-en-4-yl, 1-R^{10a}pent-2-en-4-yl or 2-R^{10a}pent-3-en-5-yl (wherein R^{10a} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to the alkenyl group through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

3) 1-R^{11a}prop-1-yn-3-yl, 1-R^{11a}but-2-yn-4-yl, 1-R^{11a}but-1-yn-3-yl, 1-R^{11a}pent-2-yn-4-yl or 2-R^{11a}pent-3-yn-5-yl (wherein R^{11a} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to the alkynyl group through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or 1-R^{12a}but-2-yn-4-yl, 1-R^{12a}pent-2-yn-4-yl or 2-R^{12a}pent-3-yn-5-yl (wherein R^{12a} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to the alkynyl group through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

4) C_{2-3}alkyl$X^{2a}C_{1-3}$alkyl$X^{3a}R^{13a}$ (wherein X^{2a} and X^{3a} which may be the same or different each represents -O-, -S-, -SO-, -SO_2-, -NR^{14a}CO-, or -NR^{15a}- (wherein R^{14a} and R^{15a} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl) and R^{13a} represents hydrogen or C_{1-3}alkyl);

5) C_{2-3}alkyl$X^{4a}COR^{16a}$ (wherein X^{4a} represents -O- or -NR^{17a}- (wherein R^{17a} represents hydrogen, C_{1-3}alkyl or C_{1-2}alkoxyethyl) and R^{16a} represents -$NR^{18a}R^{19a}$ or -OR^{20a} (wherein R^{18a}, R^{19a} and R^{20a} which may be the same or different each represents hydrogen, C_{1-4}alkyl or C_{1-2}alkoxyethyl));

6) C_{2-3}alkyl$X^{5a}R^{21a}$ (wherein X^{5a} represents carbonyl, -O-, -S-, -SO-, -SO_2-, -NR^{22a}CO-, -$NR^{23a}SO_2$- or -NR^{24a}- (wherein R^{22a}, R^{23a} and R^{24a} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl) and R^{21a} represents a group selected from cyclopentyl, cyclohexyl, pyrrolidinyl and piperidinyl which group is linked to X^{5a} through a carbon atom and which group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, C_{1-2}alkoxy, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl or R^{21a} is C_{1-3}alkyl with the proviso that when R^{21a} is C_{1-3}alkyl, X^{5a} is -S-, -SO-, -SO_2- or -$NR^{23a}SO_2$-);

7) C_{1-2}alkoxyC_{2-3}alkyl provided that X^{1a} is -S-;

8) C_{2-3}alkyl$X^{6a}$$C_{2-3}$alkyl$R^{25a}$ (wherein X^{6a} represents -O-, -S-, -SO-, -SO_2-, -NR^{26a}CO-, -$NR^{27a}SO_2$- or -NR^{28a}- (wherein R^{26a}, R^{27a} and R^{28a} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl) and R^{25a} represents a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl, and C_{1-3}alkoxycarbonyl);

9) C_{2-3}alkylR^{29a} (wherein R^{29a} is piperazin-1-yl which bears at least one substituent selected from acetyl, C_{1-2}alkoxycarbonyl, C_{1-2}hydroxyalkyl and $CONR^{30a}R^{31a}$ (wherein R^{30a} and R^{31a} each independently represents hydrogen or C_{1-2}alkyl);

10) C_{2-3}alkylR^{32a} (wherein R^{32a} is morpholino which may bear one or two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl) with the proviso that when R^{4a} is C_{2-3}alkylR^{32a}, X^{1a} is -S- or -$NR^{6a}SO_2$- (wherein R^{6a} is as defined herein); and

11) C_{2-3}alkylR^{33a} (wherein R^{33a} is morpholino which bears at least one and optionally two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl);]

and salts thereof.

9. A quinazoline derivative as claimed in claim 1 selected from:-

4-(4-chloro-2-fluoroanilino)-7-(1,3-dioxolan-2-ylmethoxy)-6-methoxyquinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-morpholinobut-2-yn-1-yloxy)quinazoline,

(E)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-morpholinobut-2-en-1-yloxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-7-(3-(2,6-dimethylmorpholino)propoxy)-6-methoxyquinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-([N-methyl-N-methylsulphonyl]amino)propoxy)quinazoline,

7-(2-[N-tert-butoxycarbonylamino]ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-([N-methyl-N-methylsulphonyl]amino)propoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-oxoimidazolidin-1-yl)ethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(3-oxomorpholino)ethoxy)quinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(3-oxomorpholino)ethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-thiomorpholinoethoxy)quinazoline,
(S)-4-(4-bromo-2-fluoroanilino)-7-(3-(2-carbamoylpyrrolidin-1-yl)propoxy)-6-methoxyquinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(2-oxopyrrolidin-1-yl)propoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-oxopyrrolidin-1-yl)ethoxy)quinazoline,
(S)-7-(3-(2-carbamoylpyrrolidin-1-yl)propoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-morpholinoethoxy)ethoxy)quinazoline and
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-(2-oxopyrrolidin-1-yl)propoxy)quinazoline
and salts thereof.

10. A quinazoline derivative as claimed in claim 1 selected from:-
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-2-(2-methoxyethoxy)ethoxyquinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-yl)methoxyquinazoline,
4-(4-bromo-2-fluoroanilino)-7-(3-(1,1-dioxothiomorpholino)propoxy)-6-methoxyquinazoline,
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(2-methoxyethoxy)ethoxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-pyrrolidin-1-ylethoxy)ethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-[4-methylpiperazin-1-yl]ethoxy)ethoxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropylthio)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-([N-methyl-N-methoxyacetyl]amino)ethoxy)quinazoline and
4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(2-oxopyrrolidin-1-yl)ethoxy)quinazoline
and salts thereof.

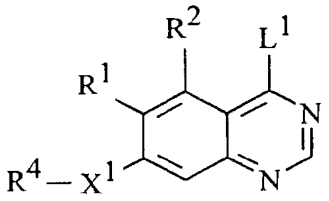
11. A quinazoline derivative as claimed in claim 1 selected from:-
(E)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-(pyrrolidin-1-yl)but-2-en-1-yloxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(methylsulphonyl)propoxy)quinazoline,
(S)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-yl)methoxyquinazoline and
(R)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-yl)methoxyquinazoline
and salts thereof.

12. A quinazoline derivative as claimed in claim 1 selected from:-
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(methylsulphonyl)propoxy)quinazoline
and salts thereof.

13. A quinazoline derivative as claimed in any one of the preceding claims in the form of a pharmaceutically acceptable salt.

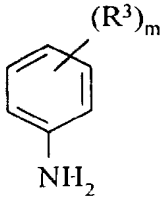
14. A process for the preparation of a quinazoline derivative of formula I or salt thereof (as defined in claim 1) which comprises:-

(a) the reaction of a compound of the formula III:



(III)

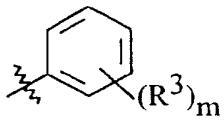
(wherein R^1, R^2, X^1 and R^4 are as defined in claim 1 and L^1 is a displaceable moiety), with a compound of the formula IV:



(IV)

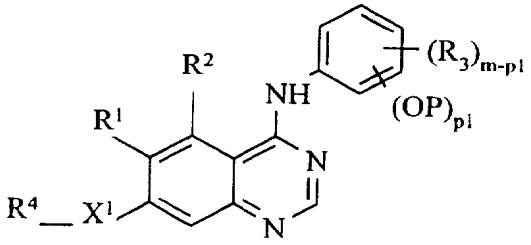
(wherein R^3 and m are as defined in claim 1) whereby to obtain compounds of the formula I and salts thereof;

(b) for the preparation of compounds of formula I and salts thereof in which the group of formula IIa:



(IIa)

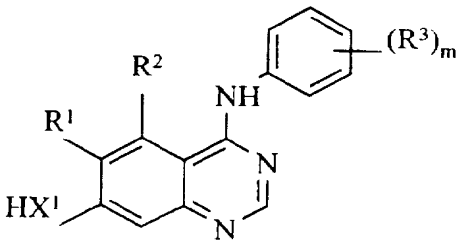
(wherein R^3 and m are as defined in claim 1) represents a phenyl group carrying one or more hydroxy groups, the deprotection of a compound of formula V:



(V)

(wherein X^1, m, R^1, R^2, R^3 and R^4 are as defined in claim 1, P represents a phenolic hydroxy protecting group and p1 is an integer from 1 to 5 equal to the number of protected hydroxy groups and such that m-p1 is equal to the number of R^3 substituents which are not protected hydroxy);

(c) for the preparation of those compounds of formula I and salts thereof wherein the substituent X^1 is -O-, -S-, $-NR^{11}$-, $-SO_2$-, $-CONR^8$- or $-SO_2NR^9$-, the reaction of a compound of the formula VI:



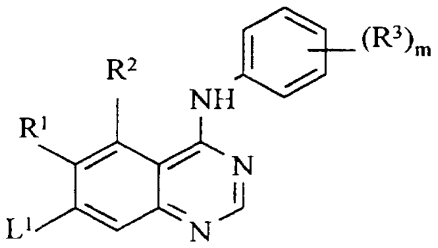
(VI)

(wherein m, X^1, R^1, R^2 and R^3 are as defined in claim 1) with a compound of formula VII:

$$R^4\text{-}L^1 \qquad \text{(VII)}$$

(wherein R^4 is as defined in claim 1 and L^1 is as defined herein);

(d) the reaction of a compound of the formula VIII:



(VIII)

with a compound of the formula IX:

$$R^4\text{-}X^1\text{-}H \qquad (IX)$$

(wherein R^1, R^2, R^3, R^4, m and X^1 are as defined in claim 1 and L^1 is as defined herein);

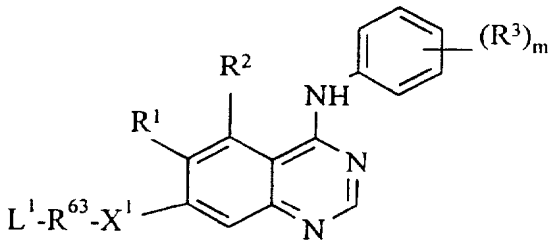
(e) for the preparation of compounds of formula I and salts thereof wherein R^4 is $C_{1-5}alkylR^{53}$, [wherein R^{53} is selected from one of the following three groups:

1) X^7R^{27} (wherein X^7 represents -O-, -S-, $-SO_2-$, $-NR^{54}CO-$, $-NR^{55}SO_2-$ or $-NR^{56}-$ (wherein R^{54}, R^{55} and R^{56} each independently represents hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$) and R^{27} is as defined in claim 1);

2) $X^8C_{1-5}alkylX^3R^{16}$ (wherein X^8 represents -O-, -S-, $-SO_2-$, $-NR^{57}CO-$, $-NR^{58}SO_2-$ or $-NR^{59}-$ (wherein R^{57}, R^{58} and R^{59} each independently represents hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$) and X^3 and R^{16} are as defined in claim 1); and

3) $X^9C_{1-5}alkylR^{33}$ (wherein X^9 represents -O-, -S-, $-SO_2-$, $-NR^{60}CO-$, $-NR^{61}SO_2-$ or $-NR^{62}-$ (wherein R^{60}, R^{61} and R^{62} each independently represents hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$) and R^{33} is as defined in claim 1);],

the reaction of a compound of the formula X:



(X)

(wherein X^1, R^1, R^2, R^3 and m are as defined in claim 1, L^1 is as defined herein and R^{63} is C_{1-5}alkyl) with a compound of the formula XI:

$$R^{53}\text{-}H \qquad \text{(XI)}$$

(wherein R^{53} is as defined herein) to give a compound of the formula I;
the preparation of compounds of the formula I wherein R^4 is C_{2-5}alkylR^{45}. (wherein R^{45} is a group selected from imidazolidin-1-yl, pyrrolidin-1-yl and thiomorpholino. which group may bear one or two substituents selected from oxo, hydroxy, halogeno. C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl), the reaction of a compound of formula X (wherein R^{63} is C_{2-5}alkyl) with a compound of the formula XIa:

$$R^{45}\text{-}H \qquad \text{(XIa)}$$

(wherein R^{45} is as defined herein) to give a compound of the formula I;

(f) for the preparation of those compounds of the formula I and salts thereof wherein the substituent R^1 is represented by $-NR^5R^6$, where one or both of R^5 and R^6 are C_{1-3}alkyl, the reaction of compounds of formula I wherein the substituent R^1 is an amino group with an alkylating agent;

(g) for the preparation of compounds of formula I and salts thereof wherein one or more of the substituents R^1, R^2 or R^3 is an amino group, the reduction of a

corresponding compound of formula I wherein the substituent(s) at the corresponding position(s) of the quinazoline and/or aniline ring is/are a nitro group(s);
and when a pharmaceutically acceptable salt of a quinazoline derivative of formula I is required, reaction of the compound obtained with an acid or base whereby to obtain the desired pharmaceutically acceptable salt.

15. A pharmaceutical composition which comprises as active ingredient a compound of formula I as defined in claim 1 or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable excipient or carrier.

16. A method for producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal in need of such treatment which comprises administering to said animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof as defined in claim 1.

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/GB 97/02588

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D239/94 C07D405/12 C07D403/12 C07D401/12 A61K31/505

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 566 226 A (ZENECA) 20 October 1993 cited in the application see claims	1-3,8, 13-16
P,X	WO 97 22596 A (ZENECA) 26 June 1997 see page 40 - page 155	1-3,8, 13-16
P,A	WO 97 32856 A (ZENECA) 12 September 1997 see claims	1-3,8, 13-16

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
12 January 1998	20/01/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Francois, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte· onal Application No
PCT/GB 97/02588

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		CA 2086968 A	21-07-93
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ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
19/043,275	**02/05/2025 07:37:02 PM Z ET**	**50535-710.304**

Title of Invention

METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	5486	FILED BY	Jennifer Vail
PATENT CENTER #	69068752	AUTHORIZED BY	Victoria Cheng
CUSTOMER #	21971	FILING DATE	-
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Alexis HOWERTON

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
DA / 232415	**E202525J37508955**	**Jennifer Vail**

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
1832	FILING AN INFORMATION DISCLOSURE STATEMENT THAT CAUSES THE CUMULATIVE NUMBER OF APPLICANT-PROVIDED ITEMS OF INFORMATION TO EXCEED 50 BUT NOT EXCEED 100	200.00	1	200.00
			TOTAL AMOUNT:	**$200.00**

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New Applications Under 35 U.S.C. 111

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(71) Applicant: **NEUROCRINE BIOSCIENCES, INC.** [US/US]; 12780 El Camino Real, San Diego, California 92130-1102 (US).

(72) Inventor: **GRIGORIADIS, Dimitri, E.**; 10626 Ginger-wood Cove, San Diego, California 92130 (US).

(74) Agents: **ROSOK, Mae, Joanne** et al.; Seed Intellectual Property Law Group PLLC, Suite 5400, 701 Fifth Avenue, Seattle, Washington 98104-7064 (US).

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(54) **Title**: CRF1 RECEPTOR ANTAGONISTS FOR THE TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA

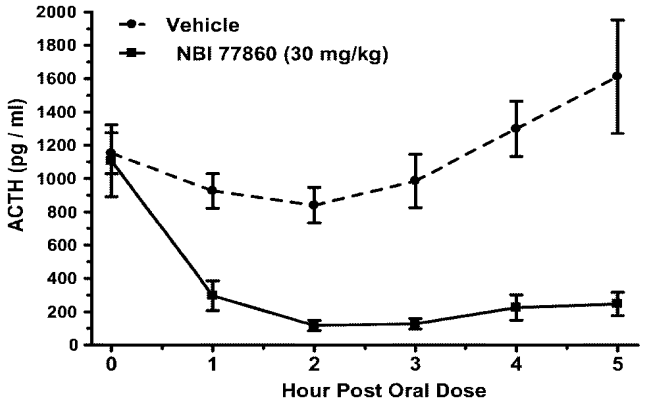

FIG. 2

(57) **Abstract**: CRF_1 receptor antagonists have the potential to directly inhibit ACTH release in patients with CAH and thereby allow normalization of androgen production while using lower, more physiologic doses of hydrocortisone, and thus reducing treatment-associated side effects.

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TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA

BACKGROUND

Technical Field

Compositions and methods for treating congenital adrenal hyperplasia are described herein.

Description of the Related Art

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive genetic disorders that result in little or no cortisol biosynthesis. The most frequent form of the disease is 21-hydroxylase deficiency caused by mutations in the *CYP21A2* gene located on chromosome 6p21, which accounts for approximately 95% of CAH cases (*see, e.g.,* Speiser et al., *Int. J. Pediatr. Endocrinol.* 2010:494173 (2010) for a review). These mutations can range from complete loss of enzyme activity required for synthesis of cortisol in the adrenal cortex to a spectrum of partial loss, which results in disease severity that is a direct consequence of a specific mutation. This continuum of 21-hydroxylase deficiency has been broadly classified into salt-wasting and simple-virilizing forms, grouped as classical CAH, and the milder form known as non-classic CAH (NCCAH) or "late-onset" CAH, which is usually diagnosed in late childhood or early adulthood. Non-classic CAH patients are either homozygous or compound heterozygotes, often with a classical CAH allele. These patients have sufficient enzyme activity (> 20-50% of normal) such that they do not have salt-wasting or cortisol deficiency and have normal genitalia at birth, and many remain asymptomatic throughout life (Trapp et al., *Steroids* 77(4):342-46 (2012)). In the less frequent form of the disease, which accounts for 5% of cases, mutation of the 11β-hydroxylase gene *CYP11B1* results in CAH (11β-OH CAH).

Both genetic mutations result in congenital adrenal hyperplasia, cortisol deficiency and excessive adrenocorticotropic hormone (ACTH) production with overproduction of androgens. These patients require lifelong management with

glucocorticoids and the attendant problems associated with such treatment. Accordingly, a significant need exists for treatment regimens to improve the health, well-being, quality of life, and to manage related disorders in patient with CAH.

BRIEF SUMMARY

Corticotropin-releasing factor (CRF) activates the CRF_1 receptor, a class B G protein-coupled receptor (GPCR). CRF_1 antagonists have the potential to directly inhibit ACTH release in patients with CAH, thereby allowing normalization of androgen production while using lower, more physiologic doses of hydrocortisone, and reducing treatment-associated side effects.

In one embodiment, a method is provided for treating CAH by administering to a subject in need thereof an effective amount of a CRF_1 antagonist, including (but not limited to) bedtime administration.

In a more specific embodiment, the CRF_1 antagonist has a dissociation half-life ($t_{1/2}$) in excess of 30 minutes, and in another embodiment in excess of 40 minutes, and in another embodiment in excess of 50 minutes.

Embodiment 1. A method for treating Congenital Adrenal Hyperplasia (CAH) by administering to a subject in need thereof a CRF_1 receptor antagonist having a dissociation half-life in excess of 30 minutes.

Embodiment 2. The method of Embodiment 1 wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 40 minutes.

Embodiment 3. The method of Embodiment 1 wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 50 minutes.

Embodiment 4. The method of any one of Embodiments 1-3 wherein the CRF_1 receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

Embodiment 5. The method of any one of Embodiments 1-3 wherein the CRF_1 receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543,

antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

Embodiment 6. The method of any one of Embodiments 1-5 wherein the CRF_1 receptor antagonist is administered at bedtime.

Embodiment 7. The method of any one of Embodiments 1-6 wherein the CRF_1 receptor antagonist is administered at or before the expected circadian release of ACTH.

Embodiment 8. The method of Embodiment 7 wherein the CRF_1 receptor antagonist is administered 3-4 hours before the expected circadian release of ACTH.

Embodiment 9. A method for reducing 17-OHP and ACTH levels in a subject who has Congenital Adrenal Hyperplasia (CAH), said method comprising administering to the subject a CRF_1 receptor antagonist at bedtime.

Embodiment 10. The method of Embodiment 9, wherein the CRF_1 receptor antagonist is administered at or before the expected circadian release of ACTH.

Embodiment 11. The method of Embodiment 9 or Embodiment 10, wherein the CRF_1 receptor antagonist is administered 3-4 hours before the expected circadian release of ACTH.

Embodiment 12. The method of any one of Embodiments 9-11, wherein the CRF_1 receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

Embodiment 13. The method of any one of Embodiments 9-11, wherein the CRF_1 receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

Embodiment 14. A CRF_1 receptor antagonist for use in treating Congenital Adrenal Hyperplasia (CAH), wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 30 minutes.

Embodiment 15. The CRF_1 receptor antagonist of 14, wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 40 minutes.

Embodiment 16. The CRF_1 receptor antagonist of Embodiment 14, wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 50 minutes.

Embodiment 17. The CRF_1 receptor antagonist of any one of Embodiments 14-16, wherein the CRF_1 receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

Embodiment 18. The CRF_1 receptor antagonist of any one of Embodiments 14-16, wherein the CRF_1 receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

Embodiment 19. The CRF_1 receptor antagonist of any one of Embodiments 14-18, wherein the CRF_1 receptor antagonist is suitable for administration at bedtime.

Embodiment 20. The CRF_1 receptor antagonist of any one of Embodiments 14-19, wherein the CRF_1 receptor antagonist is suitable for administration at or before the expected circadian release of ACTH.

Embodiment 21. The CRF_1 receptor antagonist of any one of Embodiments 14-20, wherein the CRF_1 receptor antagonist is suitable for administration 3-4 hours before the expected circadian release of ACTH.

In other embodiments, the methods and uses described above and herein further comprise reducing the amount of a glucocorticoid or mineralocorticoid by at least 10%, 15%, 20%, 30%, 40%, 50%, 60% from the recommended daily dose of GC (such as hydrocortisone (HC), prednisone, prednisolone, dexamethasone, or fludrocortisone) administered to a full grown subject (*e.g.,* a human subject) who has CAH. In other embodiments, the methods and uses described above and herein further comprise reducing the amount of a glucocorticoid or mineralocorticoid by at least 10%, 15%, 20%, 30%, 40%, 50%, 60% from the recommended daily dose of GC (e.g.,

hydrocortisone) or mineralocorticoid (e.g., fludrocortisone) administered to a growing subject (*e.g.,* a human subject) who has CAH.

These and other embodiments will be apparent upon reference to the following detailed description. To this end, various references are set forth herein that describe in more detail certain background information, procedures, compounds and compositions, and are each hereby incorporated by reference in their entirety.

Terms not specifically defined herein should be given the meanings that would be given to them by one of skill in the art in light of the disclosure and the context. As used in the specification, however, unless specified to the contrary, the terms have the meaning indicated. Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment. Thus, the appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

Also, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a non-human animal" may refer to one or more non-human animals, or a plurality of such animals, and reference to "a cell" or "the cell" includes reference to one or more cells and equivalents thereof (*e.g.,* plurality of cells) known to those skilled in the art, and so forth. When steps of a method are described or claimed, and the steps are described as occurring in a particular order, the description of a first step occurring (or being performed) "prior to" (*i.e.,* before) a second step has the same meaning if rewritten to state that the second step occurs (or is performed) "subsequent" to the first step. The term "about" when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range may vary between 1% and 15% of the stated number or numerical range. It should also be noted that the term "or" is generally

employed in its sense including "and/or" unless the content clearly dictates otherwise. The term, "at least one," for example, when referring to at least one compound or to at least one composition, has the same meaning and understanding as the term, "one or more."

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic of steroid synthetic pathways in the adrenal gland. The most common form of CAH is caused by 21-hydroxylase (also called 21-α hydroxylase) deficiency resulting in a decrease of cortisol and increase in androgens such as testosterone and estrogen. A more rare type of classical CAH is 11β-hydroxylase deficiency.

Figure 2 presents a graph illustrating the effect of Compound I (NBI-77860) on ACTH concentration in adrenalectomized rats. Rats received 30 mg/kg of Compound I (NBI-77860) orally. Data are presented as mean plasma concentration of ACTH (±SEM).

Figures 3A-C present graphs illustrating the effect of CRF_1 receptor antagonists, differentiated by their dissociation half-life, on ACTH concentration in adrenalectomized rats.

Figure 4 presents the study design schematic for the clinical trial described in Example 6 that was designed to assess the safety, tolerability and plasma exposure of NBI-77860, as well as the effect of this compound on endogenous levels of HPA (hypothalamic-pituitary-adrenal) axis hormones.

Figure 5 presents the data for mean 17-OHP (upper panel) and ACTH levels (lower panel) throughout the 24-hour postdose period for the clinical trial described in Example 6.

Figure 6 presents the response of a specific individual subject for 17-OHP (upper panel) and ACTH levels (lower panel) following administration of 300 mg and 600 mg NBI-77860 and placebo over time.

DETAILED DESCRIPTION

As described herein, CRF_1 receptor antagonists have been found to directly inhibit ACTH release in patients with CAH and thereby allow normalization of androgen production. Administration of a CRF_1 receptor antagonist permits use of lower, more physiologic doses of hydrocortisone in subjects with CAH and thus reduces treatment-associated side effects.

Newborn screening for CAH is performed by immunoassay to measure 17-OHP levels in heel-stick capillary blood specimens obtained within the first 72 hours of life. The blood sample is analyzed for 17-OHP by commercially available dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA; PerkinElmer, Waltham Massachusetts) (White et al., J. Pediatr. 163:10-12 (2013)).

Second-tier screening tests utilizing biochemical and molecular genetic testing methods, performed between 8 and 14 days of life, are employed by nine states in the United States and strongly recommended by an additional 5 states. The biochemical method includes immunoassay with organic solvent extraction or liquid chromatography followed by tandem mass spectrometry to measure steroid ratios of 17-OHP, androstenedione, and 21-deoxycortisol to cortisol (*see, e.g.,* Speiser et al., *Int. J. Pediatr. Endocrinol.* 2010:494173, 2010). The genetic screen looks for *CYP21A2* mutations that are associated with CAH. While not widely employed in the U.S., the addition of a second screening could potentially improve the sensitivity of the overall screening process, where sensitivity of the first screen alone is approximately 72%.

In absence of results from the newborn screening, female infants with classical CAH are typically identified due to the presence of ambiguous genitalia. Males have normal genitalia at birth and therefore are not diagnosed unless newborn screening is conducted or other medical complications come to attention. Infants who are not initially diagnosed with CAH and suffer from the salt-wasting form of the disease are later diagnosed in the setting of poor weight gain, vomiting, hyperkalemia and hyponatremia within the first few weeks of life.

Treatment of CAH is based on normalization of hormone and steroid levels using a variety of medications from diagnosis in infancy through adulthood.

Glucocorticoids are the current standard treatment in CAH and are used both to correct the endogenous cortisol deficiency and for reducing the elevated ACTH levels from the pituitary, which drives increased androgen production. Unlike the treatment of Addison's disease (adrenal insufficiency), in which cortisol replacement is sufficient, the treatment of CAH must also reduce ACTH production, to control the subsequent androgen excess as well. Thus, the goals of glucocorticoid treatment include cortisol replacement and suppression of ACTH to prevent virilization and menstrual disturbances in women and to inhibit testicular adrenal rest tumors in men. Mineralocorticoid replacement is needed to achieve normal plasma renin activity for maintenance of regular blood pressure, electrolyte balance, and volume status in those patients with the salt-wasting form of CAH.

The regimen of glucocorticoid treatment must support normal physiology and also ensure that sufficient cortisol is available during events that may elicit a strong stress response (*e.g.,* intercurrent illness, exercise, hypotension). Careful monitoring is also necessary to avoid the development of iatrogenic Cushing's syndrome due to glucocorticoid overtreatment in an effort to adequately suppress androgen production, or Addisonian syndrome due to under-treatment. Overtreatment with mineralocorticoids may cause hypertension while under-treatment may lead to low blood pressure, salt loss, fatigue and increased requirements for glucocorticoids. Typical laboratory tests for monitoring treatment efficacy include measurement of plasma concentrations of 17-OHP, androstenedione, testosterone, renin activity, and electrolytes.

Adult patients with CAH have an increased prevalence of risk factors for cardiovascular disease including obesity, hypertension, and insulin resistance (*see, e.g.,* Kim et al., *Semin. Reprod. Med.* 27(4):316-21 (2009)). A study of a large cohort of pediatric and adult CAH patients (n=244) demonstrated that patients are prescribed a variety of glucocorticoid treatment regimens yet frequently suffer from poor hormonal control and the aforementioned adverse outcomes (*see, e.g.,* Finkielstain et al., *J. Clin. Endocrinol Metab.* 97(12):4429-38 (2012)).

Treatment of CAH includes efforts to normalize the cortisol deficiency with glucocorticoids (usually hydrocortisone in children but often more potent agents with narrow therapeutic indices, such as dexamethasone, in adults) and, if necessary for salt-wasting, mineralocorticoids (usually fludrocortisone). The glucocorticoid doses required to achieve sufficient suppression of excess androgens, however, are usually well above the normal physiologic dose used for cortisol replacement alone as in patients with Addison's disease. This increased exposure to glucocorticoids can lead to iatrogenic Cushing's syndrome, increased cardiovascular risk factors, glucose intolerance, and decreased bone mineral density in CAH patients (*see, e.g.,* Elnecave et al., *J. Pediatr. Endocrinol. Metab.* 21:1155-62 (2008); King et al., *J. Clin. Endocrinol. Metab.* 91(3):8656-59 (2006); Migeon et al., *Endocrinol. Metab. Clin. North Am.* 30:193-206 (2001)).

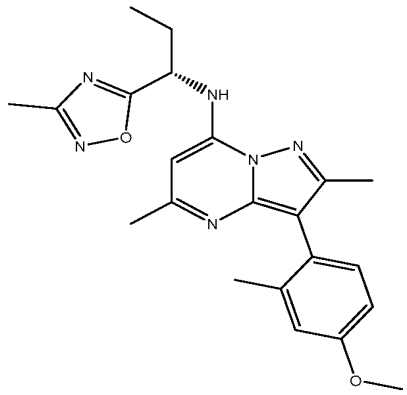
Corticotropin-releasing factor (CRF) was isolated from ovine hypothalami and identified as a 41-amino acid peptide. CRF has been found to produce profound alterations in endocrine, nervous, and immune system function. CRF is believed to be the major physiological regulator of the basal and stress-induced release of adrenocorticotropic hormone ("ACTH"), ß-endorphin, and other pro-opiomelanocortin ("POMC")-derived peptides from the anterior pituitary (*see, e.g.,* Vale et al., *Science 213*:1394-1397, 1981). Secretion of CRF causes release of ACTH from corticotrophs in the anterior pituitary via binding to the CRF_1 receptor, a member of the class B family of G-protein coupled receptors.

Due to the physiological significance of CRF, the development of biologically-active small molecules having significant CRF_1 receptor binding activity and which are capable of antagonizing the CRF_1 receptor remains a desirable goal and has been the subject of ongoing research and development for the treatment of anxiety, depression, irritable bowel syndrome, post-traumatic stress disorder, and substance abuse.

The pituitary hormone ACTH, under the control of hypothalamic corticotropin-releasing factor (CRF), stimulates uptake of cholesterol and drives the synthesis of pregnenolone initiating steroidogenesis in the adrenal gland (*see* Figure 1).

The adrenal cortex is comprised of three zones, which produce distinct classes of hormones many of which are driven by ACTH mobilizing cholesterol through this pathway. Deficiencies in these enzymes as a result of mutation or deletion cause the substrate concentrations to increase. In the most common form of CAH resulting from mutations or deletions in the 21-hydroxylase gene (CYP21A2), potent androgens are produced by the adrenal because of the accumulation of the steroid precursors, progesterone and 17-hydroxyprogesterone (17-OHP). Plasma levels of 17-OHP can reach 10-1000 times the normal concentration in these cases. These increases result in the overproduction of androgens, specifically androstenedione, testosterone, and dihydroxytestosterone causing virilization in females. In addition, 21-hydroxylase deficiency in CAH causes insufficient biosynthesis of glucocorticoids and mineralocorticoids, specifically cortisol and aldosterone. Cortisol is a critical negative feedback regulator of hypothalamic CRF secretion and pituitary ACTH release. The lack of glucocorticoid synthesis and release eliminates the restraint on the hypothalamus and pituitary, which causes ACTH levels to increase. The excessive ACTH stimulation causes hypertrophy of the zona fasciculata and zona reticularis resulting in adrenal hyperplasia.

In an embodiment, a CRF_1 receptor antagonist useful for the treatment of CAH is NBI-77860, 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine (also referred to as "Compound I" herein), and which has the following structure.



Compound I

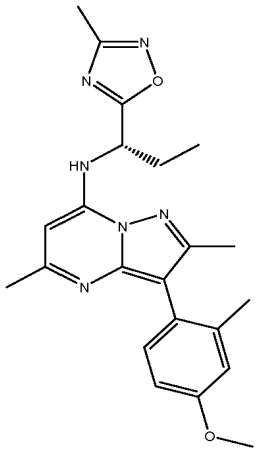
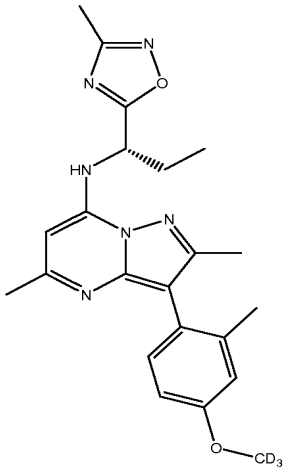
Compound I is a potent CRF_1 antagonist possessing a binding pKi = 8.2 (*see, e.g.,* Tellew et al., *Bioorg. Med. Chem. Lett.* 20:7259, 2010 and Int'l. Patent Appl. Publ. No. WO 2006/044958, both of which references are incorporated by reference herein in their entirety). As described herein, Compound I has potent ACTH lowering effects as shown in adrenalectomized rats.

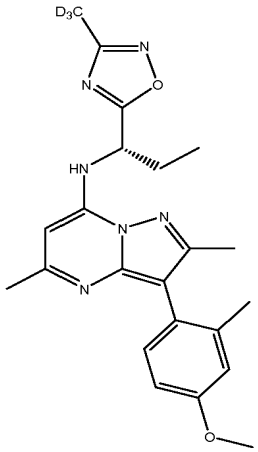
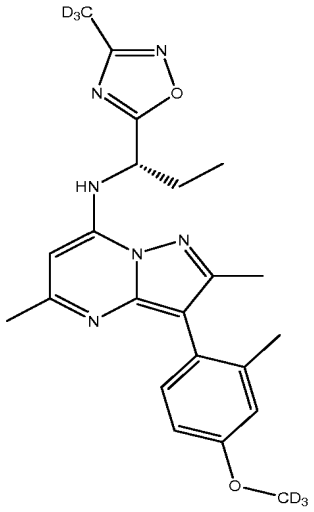
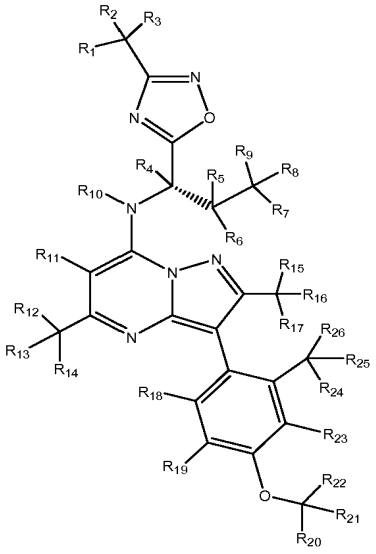
In another embodiment, the CRF_1 receptor antagonist useful for the treatment of CAH is a small molecule antagonist as described in U.S. 6,586,456, US 6,806,282, US 6,531,475, US 6,664,261, US 6,610,678, WO 98/08846, WO 98/11075, WO 99/10350, WO 2000/059888, WO 2006/044821, WO 2006/102194, WO 2006/107784, WO 2006/116412, WO 2006/126718, WO 2007/069565, WO 2007/069671, WO 2008/036541, WO 2008/036579, WO 2008/051533, WO 2008/082003, WO 2008/083070, WO 2008/136377, WO 2009/008552, WO 2009/144632, WO 2010/014280, WO 2010/014687, WO 2010/015628, WO 2010/015655, WO 2010/062718, WO 2010/096426, WO 2011/043387, WO 2011/092293, WO 2011/095450, WO 2011/092290, and WO 2011/043381.

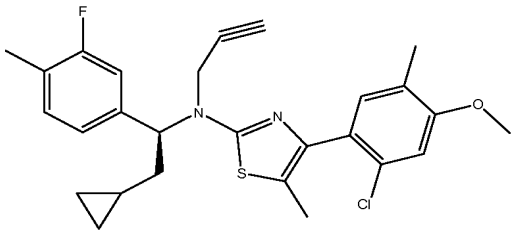
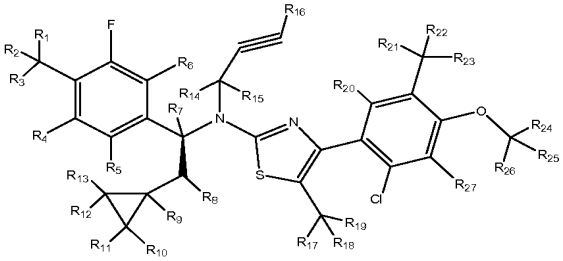
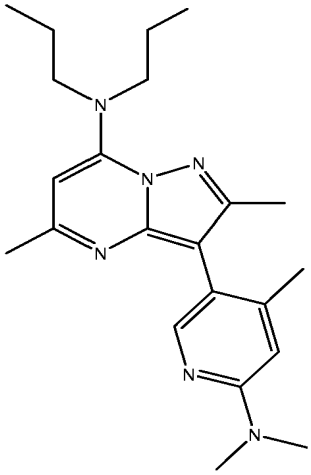
In still another embodiment, the CRF_1 receptor antagonist is NBI-30775, CP-316,311, pexacerfont, emicerfont, SSR-125543 [4-(2-chloro-4-methoxy-5-methylphenyl)-*N*-[(1*S*)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-*N*-(2-propyn-1-yl)-2-thiazolamine], SSR-126374, ONO-2333, NBI-34041, JNJ-19567470, GSK586529, PF-00572778, CP-376395, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), and DMP904.

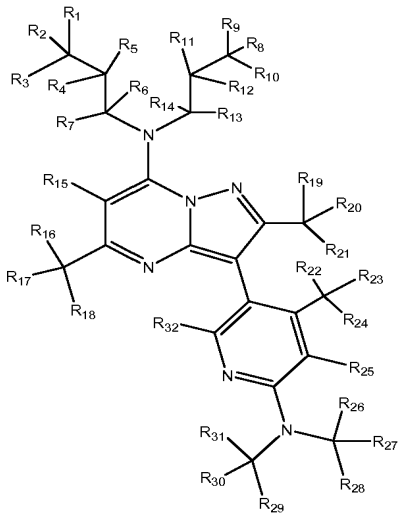
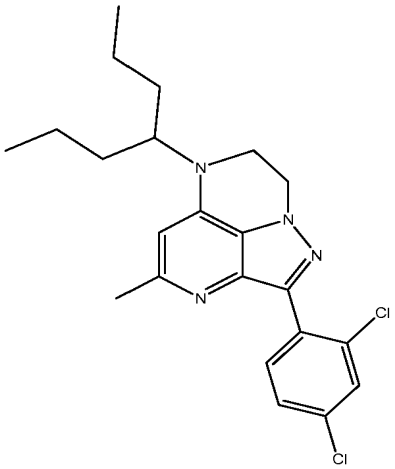
In yet another embodiment, the CRF_1 receptor antagonist has a dissociation half-life ($t_{1/2}$) in excess of 30 minutes, and in another embodiment in excess of 40 minutes, and in another embodiment in excess of 50 minutes. The dissociation half-life of a particular CRF_1 receptor antagonist is determined by the technique disclosed in Example 3. Representative CRF_1 receptor antagonists of these embodiments include Compound I, NBI-30775, NBI-34041, SSR-125543A, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), and DMP904.

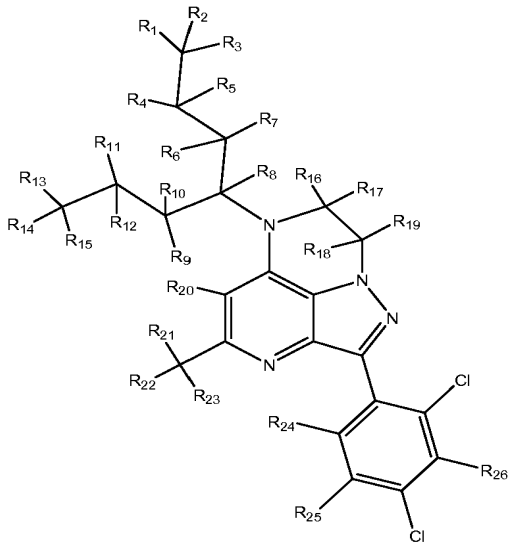
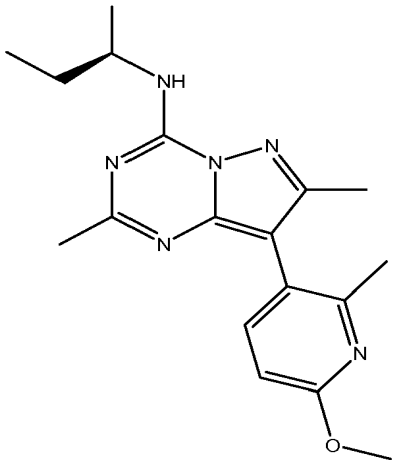
With respect to the compounds described herein, it should also be understood that when a particular position is designated as hydrogen, that such hydrogen (H) may be replaced with deuterium (D). Incorporation of deuterium in place of hydrogen is known to produce significant effects on the physiological and pharmacological activities of the substituted compound. To this end, it should be understood that deuterium replacement of hydrogen means that the abundance of deuterium at that position is substantially greater than the natural abundance of deuterium. Accordingly, in one embodiment representative compounds include the following.

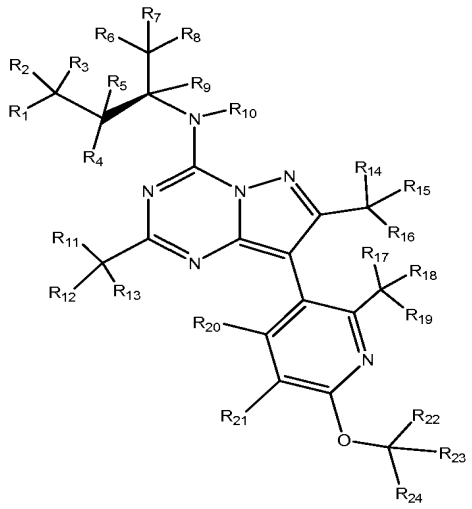
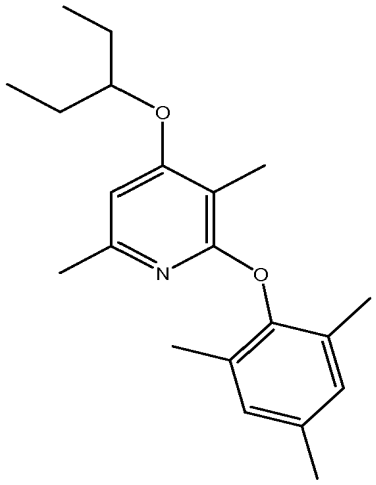
Cpd.	Structure	Chemical Name
NBI-77860		2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine
Deuterated NBI-77860		2,5-dimethyl-3-[2-methyl-4-(d_3-methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine

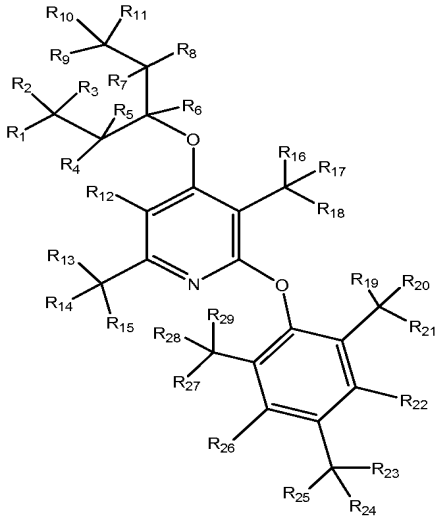
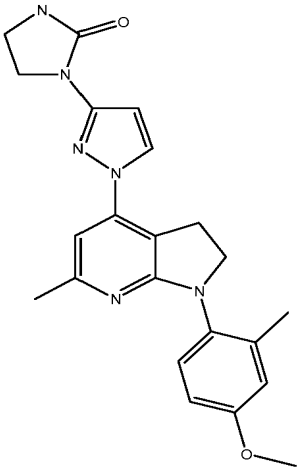
Cpd.	Structure	Chemical Name
Deuterated NBI-77860		2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-d_3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a] pyrimidin-7-amine
Deuterated NBI-77860		2,5-dimethyl-3-[2-methyl-4-(d_3-methyloxy)phenyl]-N-[(1S)-1-(3-d_3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine
Deuterated NBI-77860		

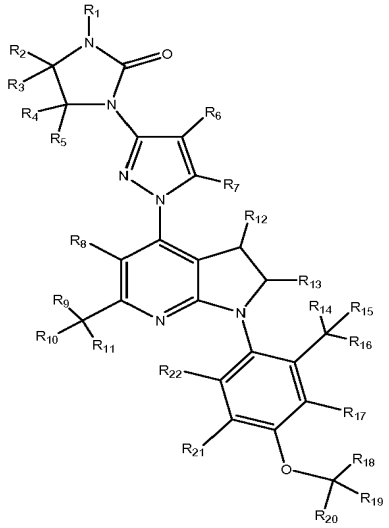
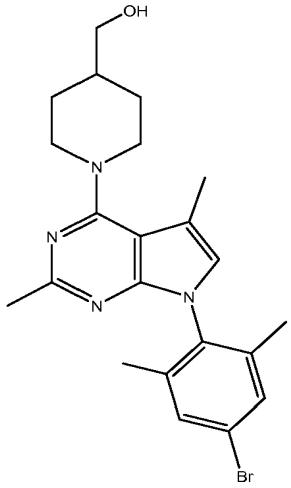
Cpd.	Structure	Chemical Name
	R_1-R_{26} independently H or D	
SSR-125543		4-(2-chloro-4-methoxy-5-methylphenyl)-*N*-[(1*S*)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-*N*-(2-propyn-1-yl)-2-thiazolamine
Deuterated SSR-125543	 R_1-R_{27} independently H or D	
NBI-30775		5-[7-(dipropylamino)-2,5-dimethylpyrazolo[1,5-a]pyrimidin-3-yl]-N,N,4-trimethylpyridin-2-amine

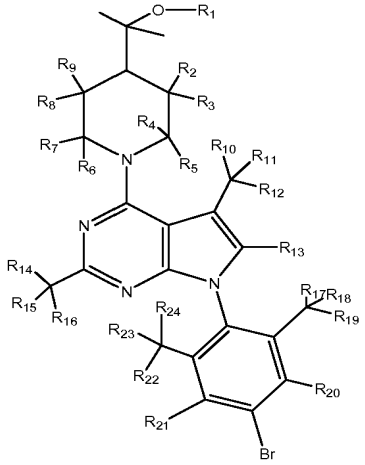
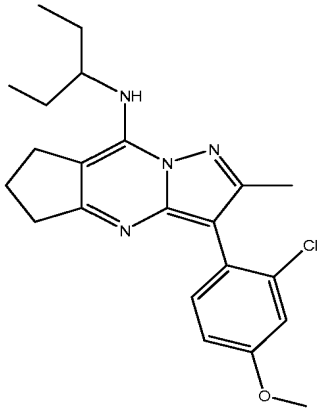
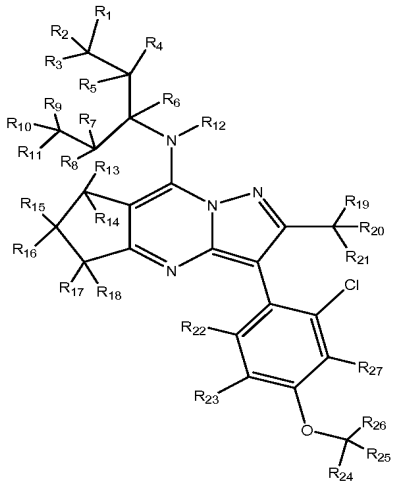
Cpd.	Structure	Chemical Name
Deuterated NBI-30775	 R_1-R_{32} independently H or D	
NBI-34041		3-(2,4-dichlorophenyl)-9-(heptan-4-yl)-6-methyl- 1,2,5,9-tetraazatricyclo[6.3.1.$0^{4,12}$]dodeca- 2,4(12),5,7-tetraene

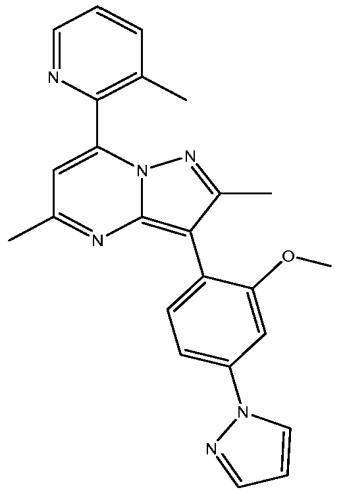
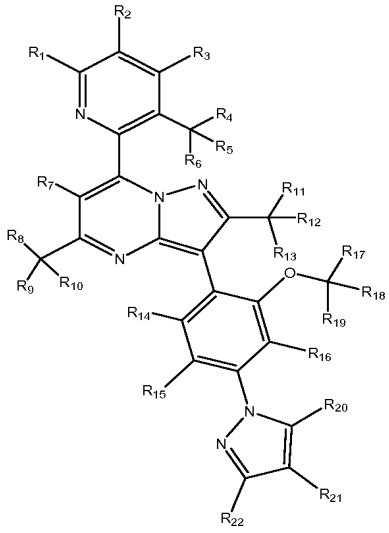
Cpd.	Structure	Chemical Name
Deuterated NBI-34041	 R_1-R_{26} independently H or D	
Pexacerfont		N-[(2R)-butan-2-yl]-8-(6-methoxy-2-methylpyridin-3-yl)-2,7-dimethylpyrazolo[1,5-a][1,3,5]triazin-4-amine

Cpd.	Structure	Chemical Name
Deuterated Pexacerfont	 R_1-R_{24} independently H or D	
CP-316,311		3,6-dimethyl-4-(pentan-3-yloxy)-2-(2,4,6-trimethylphenoxy)pyridine

Cpd.	Structure	Chemical Name
Deuterated CP-316,311	 R_1-R_{29} independently H or D	
GSK876008		

Cpd.	Structure	Chemical Name
Deuterated GSK876008	 R_1-R_{22} independently H or D	
CRA5626/R3 17573		

Cpd.	Structure	Chemical Name
Deuterated CRA5626/R3 17573	 R_1-R_{24} independently H or D	
ONO2333Ms		10-(2-chloro-4-methoxyphenyl)-11-methyl-N-(pentan-3-yl)-1,8,12-triazatricyclo[7.3.0.0^{3,7}] dodeca- 2,7,9,11-tetraen-2-amine
Deuterated ONO2333	 R_1-R_{27} independently H or D	

Cpd.	Structure	Chemical Name
NBI76169		
Deuterated NBI76169	 R_1-R_{22} independently H or D	

In another embodiment, any of the aforementioned compounds may incorporate stable or radioactive isotopes. Accordingly, also contemplated is use of isotopically-labeled compounds identical to those described herein, wherein one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into these compounds include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine and chlorine, such as, but not limited to

deuterium as discussed above (2H), as well as 3H, 13C, 14C, 15N, 18O, 17O, 31P, 32P, 35S, 18F, and 36Cl, respectively. Certain isotopically-labeled compounds, for example those into which radioactive isotopes such as 3H and 14C are incorporated, are also useful in drug or substrate tissue distribution assays. Tritiated hydrogen (3H) and carbon-14 (14C) isotopes are particularly preferred for their ease of preparation and detectability. Substitution with heavier isotopes such as deuterium (2H) can provide certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dose requirements and, therefore, may be preferred in some circumstances. Isotopically-labeled compounds can generally be prepared by performing procedures routinely practiced in the art.

Effect on Plasma ACTH in Adrenalectomized Rats

Adrenalectomy eliminates circulating corticosterone (the primary glucocorticoid) in rats and removes the negative feedback control of the HPA axis at both the hypothalamic and pituitary (corticotroph cells) levels and thus chronically elevates plasma ACTH (*see, e.g.,* Mims et al., *J. Natl. Med. Assoc.* 69:145-47 (1977)). Intravenous injection of peptide CRF_1 receptor antagonists have been demonstrated to reduce the high plasma ACTH levels in adrenalectomized (ADX) rats (*see, e.g.,* Rivier et al., *J. Med. Chem.* 12:42:3175-82 (1999)). These findings were recapitulated with the small molecule NBI-77860 (Compound I). In adrenalectomized rats, Compound I has potent capability for lowering ACTH. The maximum reduction in ACTH correlated with peak plasma concentrations of NBI-77860; however, the duration of the ACTH-lowering effect exceeded drug plasma exposure. In adrenalectomized rats, a predictable relationship therefore exists between integrated plasma exposure of NBI-77860 and *in vivo* efficacy following oral administration.

The effectiveness of a compound as a CRF receptor antagonist may be determined by various assay methods. CRF antagonists described herein may be capable of inhibiting the specific binding of CRF to its receptor and consequently antagonizing activities associated with CRF. A compound may be assessed for activity as a CRF antagonist by one or more generally accepted assays including the assay

described in the Examples. CRF antagonists useful for the methods described herein include compounds that demonstrate affinity for CRF receptor.

Without wishing to be bound by theory, in the treatment of CAH, CRF receptor antagonists would potentially block the release of ACTH from pituitary corticotrophs, thereby decreasing the production of androgens, and allow a more refined treatment paradigm for replacement of cortisol. Animal and human studies have shown the pharmacologic effect of Compound I (NBI-77860) on ACTH release. Standard biomarker assessments used by endocrinologists when monitoring treatment efficacy may be used for monitoring the effects of this CRF_1 receptor antagonist. Plasma levels of 17-OHP, androstenedione, testosterone, cortisol and ACTH, as well as urinary metabolites of these steroids, are easily measured in both children and adults giving rapid and meaningful data regarding treatment impact.

Pharmaceutical Compositions and Methods of Treatment

The present disclosure further provides for pharmaceutical compositions comprising any one of the CRF antagonist compounds described herein and a pharmaceutically acceptable excipient for use in the methods for treating CAH. A pharmaceutically acceptable excipient is a physiologically and pharmaceutically suitable non-toxic and inactive material or ingredient that does not interfere with the activity of the active ingredient; an excipient also may be called a carrier. The CRF antagonist compounds may be formulated in a pharmaceutical composition for use in treatment or preventive (or prophylactic) treatment (*e.g.,* reducing exacerbation of CAH disease, or occurrence or recurrence of one or more symptoms of the disease). The methods and excipients described herein are exemplary and are in no way limiting. Pharmaceutically acceptable excipients are well known in the pharmaceutical art and described, for example, in Rowe *et al.*, Handbook of Pharmaceutical Excipients: A Comprehensive Guide to Uses, Properties, and Safety, 5th Ed., 2006, and in *Remington: The Science and Practice of Pharmacy* (Gennaro, 21st Ed. Mack Pub. Co., Easton, PA (2005)). Examples of pharmaceutically acceptable excipients include sterile saline and phosphate buffered saline at physiological pH. Preservatives, stabilizers, dyes, buffers,

and the like may be provided in the pharmaceutical composition. In addition, antioxidants and suspending agents may also be used.

Therapeutic and/or prophylactic benefit includes, for example, an improved clinical outcome, both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow or retard (lessen) an undesired physiological change or disorder, or to prevent or slow or retard (lessen) the expansion or severity of such disorder. As discussed herein, beneficial or desired clinical results from treating a subject include, but are not limited to, abatement, lessening, or alleviation of symptoms that result from or are associated the disease, condition, or disorder to be treated; decreased occurrence of symptoms; improved quality of life; longer disease-free status (*i.e.,* decreasing the likelihood or the propensity that a subject will present symptoms on the basis of which a diagnosis of a disease is made); diminishment of extent of disease; stabilized (*i.e.,* not worsening) state of disease; delay or slowing of disease progression; amelioration or palliation of the disease state; and remission (whether partial or total), whether detectable or undetectable; and/or overall survival. "Treatment" can also mean prolonging survival when compared to expected survival if a subject were not receiving treatment. Subjects in need of treatment include those who already have the condition or disorder as well as subjects prone to have or at risk of developing the disease, condition, or disorder, and those in which the disease, condition, or disorder is to be prevented (*i.e.,* decreasing the likelihood of occurrence of the disease, disorder, or condition). A subject may be a human or non-human mammal (e.g., rat, mouse, dog, cat, livestock, zoo animal).

Optimal doses may generally be determined using experimental models and/or clinical trials. The optimal dose may depend upon the body mass, weight, or blood volume of the subject. In general, the amount of a compound described herein, that is present in a dose ranges from about 0.1 mg to about 30 mg per kg weight of the subject. In certain embodiments, a single dose is about 50-1000 mg. The use of the minimum dose that is sufficient to provide effective therapy is usually preferred. Subjects may generally be monitored for therapeutic effectiveness by clinical evaluation and using assays suitable for the condition being treated or

prevented, which assays will be familiar to those having ordinary skill in the art and are described herein. The level of a compound that is administered to a subject may be monitored by determining the level of the compound in a biological fluid, for example, in the blood, blood fraction (*e.g.,* serum), and/or in the urine, and/or other biological sample from the subject. Any method practiced in the art to detect the compound may be used to measure the level of compound during the course of a therapeutic regimen.

The dose of a composition comprising at least one of the compounds described herein for treating CAH or a related disease or disorder may depend upon the subject's condition, that is, stage of the disease, severity of symptoms caused by the disease, general health status, as well as age, gender, and weight, and other factors apparent to a person skilled in the medical art. Similarly, the dose of the compound may be determined according to parameters understood by a person skilled in the medical art.

The pharmaceutical compositions described herein that comprise at least one of the CRF_1 antagonist compounds described herein may be administered to a subject in need by any one of several routes that effectively deliver an effective amount of the compound. Such administrative routes include, for example, oral, parenteral, enteral, rectal, intranasal, buccal, sublingual, intramuscular, and transdermal. Compositions administered by these routes of administration and others are described in greater detail herein.

Administration of the compounds or compositions disclosed includes nighttime administration or administration prior to sleep (*i.e.,* bedtime administration). As used herein, bedtime administration refers to dosing intended to deliver clinically relevant concentrations of the CRF_1 antagonist at or before (such as 2-5 hours before) the expected circadian release of ACTH. Since this ACTH release is typically at 1-2 A.M., and since most orally administered drugs have a Tmax of several hours, dosing at 10 P.M., for example, which is 3-4 hours in advance of the expected circadian release of ACTH is desirable. This same pre-pulse bedtime dosing may be adapted for shift workers (*e.g.,* those who work at night and sleep during the day), in which case

administration will not necessarily occur at nighttime. Administration is therefore dependent upon the expected circadian release of ACTH, and can vary depending upon the individual's (*i.e.,* subject, patient) particular work and sleep patterns. In certain embodiments, a CRF_1 receptor antagonist described herein (for example, NBI-77860 or any one of NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904) is administered between about 2-5 hours prior to (*i.e.,* before, in advance of) the expected circadian release of ACTH. In other embodiments, the CRF_1 receptor antagonist is administered to the subject between about 2-4 hours or 3-5 hours prior to the expected circadian release of ACTH. In a more specific embodiment, CRF_1 receptor antagonist is administered to the subject between about 3-4 hours prior to the expected circadian release of ACTH.

Also provided herein is a method for reducing 17-OHP and/or ACTH levels in a subject (*i.e.,* patient, individual) who has CAH by administering a CRF_1 receptor antagonist. In certain embodiments, the antagonist is administered at or before the expected circadian release of ACTH. In other certain embodiments, the CRF_1 receptor antagonist as administered between about 2-4 hours or between about 3-5 hours prior to the expected circadian release of ACTH. In a more specific embodiment, CRF_1 receptor antagonist is administered to the subject between about 3-4 hours prior to the expected circadian release of ACTH.

The methods described herein that comprise administering a CRF_1 receptor antagonist to a female subject in need thereof in the manner described herein that causes a decrease in level of ACTH and 17-OHP in the subject may result in decreased release of androgens such as testosterone and androstenedione. The dose of glucocorticoids may concomitantly be decreased by a clinically significant amount, which in turn results in decreased side effects.

The amount of glucocorticoids (GC) and mineralocorticoids (MC) for maintenance therapy in patients who have CAH and who are young and growing and for maintenance therapy in patients who are full grown patients is known to a person skilled in the art. For example, guidelines are described in Speiser et al. (*J. Clin.*

Endocrinol. Metab. 95:4133-60 (2010), incorporated by reference in its entirety) and shown in the Tables 1 and 2 therein. Particularly, for young, growing patients who have CAH, persons skilled in the art appreciate that too high dosing with GCs can impede statural growth in the patient. Accordingly, the methods described herein for treating a patient with a CRF_1 receptor antagonist may comprise reducing the dose of a GC in a clinically significant manner.

In certain embodiments, methods for treating CAH by administering a CRF_1 receptor antagonist may further comprise administering a GC at a dose lower than the currently recommended dose of a GC for treating a subject who has CAH. When the subject is a fully grown patient, the dose of a GC, such as the dose of hydrocortisone (HC), prednisone, prednisolone, dexamethasone, or fludrocortisone recommended for maintenance therapy in a fully grown subject may be decreased by about 10%, 15%, 20%, 30%, 40%, 50%, 60% or more from the recommended doses of 15-25 mg/day HC; 5-7.5 mg/day prednisone, 4-6 mg/day prednisolone; 0.25-0.5 mg/day dexamethasone, or 0.05-0.2 mg/day of fludrocortisone. In a subject who is a growing patient with CAH, the recommended dose of GCs, such as HC may be decreased from the total recommended dose of 10-15 mg/m^2 per day and/or the total dose of fludrocortisone of 0.05-0.2 mg/day may each be decreased by about 10%, 15%, 20%, 30%, 40%, 50%, 60% or more in a subject who receives a CRF_1 receptor antagonist as described herein. In an embodiment, the methods described herein comprising administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of ACTH levels relative to placebo. In a specific embodiment, administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of ACTH levels relative to placebo, wherein that reduction is at least 25%. In another embodiment, administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of ACTH levels relative to placebo, wherein that reduction is at least 50%. See Speiser et al., *supra*, for guidelines regarding administering glucocorticoids and mineralocorticoids.

In another embodiment, the methods described herein comprising administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of 17-OHP levels relative to placebo. In another specific embodiment, administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of 17-OHP levels relative to placebo wherein that reduction is at least 25%. In still another embodiment, administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of 17-OHP levels relative to placebo wherein that reduction is at least 50%.

In an embodiment, the methods described herein comprising administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of both ACTH and 17-OHP levels relative to placebo. In a certain embodiment, administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of both ACTH and 17-OHP levels relative to placebo wherein the reduction is at least 25%. In another specific embodiment, administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of both ACTH and 17-OHP levels relative to placebo wherein the reduction is at least 50%.

The pharmaceutical compositions may be in the form of a solution. Alternatively, they may be in the form of a solid, such as powder, tablets, or the like. A composition comprising any one of the compounds described herein may be formulated for sustained or slow release. Such compositions may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain the compound dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Excipients for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. The amount of active compound contained within a sustained release formulation depends

upon the site of implantation, the rate and expected duration of release, and the nature of the condition to be treated or prevented.

For oral formulations, at least one of the compounds described herein can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders; with disintegrators; with lubricants; and if desired, with diluents, buffering agents, moistening agents, preservatives, coloring agents, and flavoring agents. The compounds may be formulated with a buffering agent to provide for protection of the compound from low pH of the gastric environment and/or an enteric coating. A compound included in the compositions may be formulated for oral delivery with a flavoring agent, *e.g.*, in a liquid, solid or semi-solid formulation and/or with an enteric coating. Oral formulations may be provided as gelatin capsules, which may contain the active compound along with powdered carriers, such as lactose, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similar carriers and diluents may be used to make compressed tablets.

EXAMPLES

EXAMPLE 1

CRF RECEPTOR BINDING ACTIVITY

CRF antagonists as used in the methods described herein may be evaluated for binding activity to the CRF receptor by a standard radioligand binding assay as generally described by Grigoriadis et al. (*see, e.g., Mol. Pharmacol* vol 50, pp 679-686, 1996) and Hoare et al. (*see, e.g., Mol. Pharmacol* 63: 751-765, 2003.) By utilizing radiolabeled CRF ligands, the assay may be used to evaluate the binding activity of the compounds described herein with any CRF receptor subtype.

Briefly, the binding assay involves the displacement of a radiolabeled CRF ligand from the CRF receptor. More specifically, the binding assay is performed in 96-well assay plates using 1-10 μg cell membranes from cells stably transfected with

human CRF receptors. Each well receives about 0.05 mL assay buffer (*e.g.*, Dulbecco's phosphate buffered saline, 10 mM magnesium chloride, 2 mM EGTA) containing compound of interest or a reference ligand (for example, sauvagine, urocortin I, or CRF), 0.05 mL of [^{125}I] tyrosine - sauvagine (final concentration ~150 pM or approximately the K_D as determined by Scatchard analysis) and 0.1 mL of a cell membrane suspension containing the CRF receptor. The mixture is incubated for 2 hours at 22°C followed by separation of the bound and free radioligand by rapid filtration over glass fiber filters. Following three washes, the filters are dried and radioactivity (Auger electrons from ^{125}I) is counted using a scintillation counter. All radioligand binding data may be analyzed using the non-linear least-squares curve-fitting programs Prism (GraphPad Software Inc) or XL*fit* (ID Business Solutions Ltd).

EXAMPLE 2

CRF_1 Receptor Agonist Activity

As reported in Fleck *et al.* (*J. Pharmacology and Experimental Therapeutics*, 341(2):518-531, 2012) (hereinafter "Fleck *et al.*" and incorporated by reference in it's entirely) the activity of previously identified CRF_1 receptor antagonists are presented. Such activity is reported as the kinetically derived affinity (K_i) calculated from the association (k_1) and dissociation (k_{-1}) rate constants by the following equation:

$$K_i = k_{-1}/k_1$$

Also as reported in Fleck *et al.*, the kinetic K_i of the CRF_1 receptor antagonists listed in Table 1 below have been reported:

Table 1

Representative CRF_1 Receptor Antagonists

Ligand	**k_1 ($10^6 M^{-1}min^{-1}$)**	**Kinetic K_i (nM)**
NBI-27914	9.4 ± 3	25

Ligand	k_1 ($10^6 M^{-1}min^{-1}$)	Kinetic K_i (nM)
CP-316,311	13 ± 2	12
NBI-46200	6.2 ± 2	22
DMP696	7.7 ± 2	9.5
pexacerfont	2.6 ± 0.1	19
NBI-35965	20 ± 2	2.3
ONO-2333Ms	4.4 ± 2.2	15
antalarmin	3.4 ± 0.6	3.9
NBI-34041	8.3 ± 2.0	1.7
DMP904	18 ± 1	0.38
NBI-30775	14 ± 2.0	0.36
SSR125543A	33 ± 5	0.049

By this same technique, the kinetic Ki of Compound I (NBI-77860) was found to be as follows:

Table 1 (cont.)

Ligand	k_1 ($10^6 M^{-1}min^{-1}$)	Kinetic K_i (nM)
NBI-77860	0.24 ± 0.05	48 ± 9

EXAMPLE 3

DISSOCIATION HALF-LIFE ($T_{1/2}$) OF CRF_1 RECEPTOR ANTAGONISTS

The dissociation half-life ($t_{1/2}$) of a CRF_1 receptor antagonist as used in the methods described herein is evaluated by the technique described in Fleck *et al.* As described therein, the dissociation rate constant for labeled and unlabeled ligands is

denoted as k_{-1}, while the half-life of drug dissociation from the receptor ($t_{1/2}$), which is equal to the median residence time, is calculated from the dissociation rate constant (k_{-1}) by the following equation:

$$t_{1/2} = 0.693/k_{-1}$$

As reported in Fleck *et al.*, the dissociation half-life ($t_{1/2}$) of the CRF_1 receptor antagonists listed in Table 2 below have been reported.

Table 2

Dissociation Half-Life of Representative Compounds

Ligand	**k_{-1} (min^{-1})**	**Dissociation $t_{1/2}$ (min)**
NBI 27914	0.27 ± 0.07	2.6
CP-316,311	0.17 ± 0.04	4.1
NBI-46200	0.13 ± 0.002	5.3
DMP696	0.095 ± 0.02	7.3
pexacerfont	0.049 ± 0.001	14
NBI-35965	0.048 ± 0.005	16
ONO-2333Ms	0.063 ± 0.029	17
antalarmin	0.013 ± 0.002	53
NBI-34041	0.013 ± 0.002	53
DMP904	0.0072 ± 0.002	96
NBI-30775	0.0054 ± 0.0006	130
SSR125543A	0.0016 ± 0.0003	430

By this same technique, the dissociation half-life of Compound I (NBI-77860) was found to be as follows.

Table 2 (cont.)

Ligand	k_{-1}, min^{-1}	Dissociation $t_{1/2}$, min
NBI-77860	0.012 ± 0.0003	58

Accordingly, CRF_1 receptor antagonists that have a dissociation half-life ($t_{1/2}$) in excess of 30 minutes include (but are not limited to) antalarim, NBI-34041, DMP904, NBI-30775, SSR125543A, and NBI-77860 (Compound I). These same compounds are also representative of CRF_1 receptor antagonists having a dissociation half-life ($t_{1/2}$) in excess of 40 minutes, and a dissociation half-life ($t_{1/2}$) in excess of 50 minutes.

EXAMPLE 4

LOWERING OF ACTH IN ADRENALECTOMIZED RATS

Compound I (NBI-77860) (*see, e.g.,* Tellew et al., *Bioorg. Med. Chem. Lett.* 2010, 20:7259; WO2006044958) is a potent CRF_1 antagonist possessing a binding pKi of 8.2, a kinetic Ki of 49 nM (Table 1 above), and a Dissociation $t_{1/2}$ of 58 minutes (Table 2 above).

Intravenous injection of peptide CRF_1 receptor antagonists have been demonstrated to reduce the high plasma ACTH levels in adrenalectomized (ADX) rats (*see, e.g.,* Rivier et al., *J. Med. Chem.* 12:42:3175-82 (1999)). These findings were recapitulated with the small molecule NBI-77860. When administered orally to ADX rats (n=6/group), 30 mg/kg single doses of NBI-77860 significantly lowered the plasma ACTH levels for up to 5 hours (*see* Figure 2). The duration of efficacy correlated with peak plasma concentrations, with a time course exceeding drug plasma exposure. In adrenalectomized rats, a predictable relationship exists between integrated plasma exposure of NBI-77860 and *in vivo* efficacy following oral administration.

Fleck *et al.* also report the effects of CRF_1 receptor antagonists differentiated by their dissociation half-life on plasma ACTH levels in adrenalectomized rats; namely, NBI 30775, NBI 34041 and NBI 35965. At the highest

dose (10 mg/kg), all three ligands reduced ACTH acutely (1 hr post-injection, Figure 3A-C). After a longer duration, a clear difference emerged between NBI 35965 and the other two ligands. The ACTH level returned to the vehicle level by 2 hr for NBI 35965 (Fig. 3C), whereas the response was sustained for 4-6 hr for NBI 30775 and NBI 34041 (Fig. 3A and 3B).

EXAMPLE 5

PHARMACODYNAMIC EFFECTS OF COMPOUND I IN HUMANS

In this study, pharmacodynamic effects of NBI-77860 (Compound I) on human subjects were evaluated by observing the effect of single oral doses on HPA axis response produced by metyrapone (0.04 g/kg) with respect to placebo and to a single dose of alprazolam (0.75 mg). Metyrapone blocks cortisol synthesis in the adrenal, thereby mimicking the cortisol deficiency of CAH, and is associated with an acute reflex rise ACTH levels.

The primary analysis was performed by determining the ACTH values between 30 min and 4 h post dose (AUC (30 min to 4 h)). In this analysis, when compared to placebo, the following observations were made. A significant reduction of ACTH AUC (30 min to 4 h) was observed with the alprazolam and the NBI-77860, 400 mg treatments. A non-significant reduction in ACTH AUC (30 min to 4 h) was seen with the NBI-77860, 50 mg treatment. An increase in AUC (30 min to 4 h) was seen with the NBI-77860, 10 mg treatment.

In the analysis performed on the ACTH values between 2 h to 4 h post-dose (AUC (2 h to 4 h)) (the period when exposure reached the C_{max} value), there was a significant reduction of ACTH levels in the NBI-77860, 400 mg and 50 mg treatment periods compared with the placebo treatment period; however, the reduction was not seen with the NBI-77860 at the 10 mg dose.

EXAMPLE 6

CLINICAL STUDY

In this study, NBI-77860 (Compound I) was evaluated in the clinical study entitled "A Phase 1, Single-Blind, Placebo-Controlled, Fixed-Sequence, Single-Dose Study to Evaluate the Safety and Tolerability of NBI-77860 in Adult Females with Congenital Adrenal Hyperplasia" (IND 117,388). The study was a single-blind, placebo-controlled, single center, fixed-sequence, single-dose clinical trial in adult female classical CAH patients. It was designed to assess the safety, tolerability, and plasma exposure of NBI-77860, as well as the effect of this compound on endogenous levels of HPA axis hormones.

A total of 8 female subjects, ages 19 to 58, with a medical diagnosis of classical 21-hydroxylase deficiency CAH were administered single bedtime doses (hs) of NBI-77860 300 mg, 600 mg, and placebo during three separate treatment periods (see study design schematic in Figure 4). The subjects' usual morning dose of concurrent steroidal treatment was delayed until after the 16-hour postdose blood samples were collected (*i.e.*, until approximately 1400h).

The PD endpoints for this study included the HPA axis biomarkers of interest in this patient population; namely, 17-hydroxyprogesterone (17-OHP; as the primary PD endpoint), adrenocorticotropic hormone (ACTH), androstenedione, testosterone, and serum cortisol levels. The initial analysis of PD variables was an examination of the grouped subject data for each biomarker, expressed as a mean percent change from predose levels for the two active dosing conditions relative to the placebo condition. The data for mean 17-OHP and ACTH levels throughout the 24-hour postdose period are provided in Figure 5.

Consistent and clinically meaningful reductions from predose levels of both 17-OHP and ACTH were observed throughout the postdose period following administration of NBI-77860 relative to placebo in these CAH patients. In addition to the group mean data, individual responses were evaluated and treatment "responders" were conservatively defined as those subjects with at least a 50% decrease in 17-OHP and ACTH under active NBI-77860 relative to placebo during the peak morning period.

This responder analysis yielded a sizeable responder rate of 50% in the study (none of these subjects were responders during the initial placebo treatment period). Furthermore, the 300 mg dose yielded nearly identical effects on 17-OHP and ACTH as the 600 mg dose. An example of an individual subject response for these biomarkers is provided in Figure 6.

The various embodiments described above can be combined to provide further embodiments. All U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, including U.S. Provisional Application Serial Nos. 61,929,941, filed January 21, 2014; 61,981,033, filed April 17, 2014; and 62/069,155, filed October 27, 2014, are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

I claim the following:

1. A method for treating Congenital Adrenal Hyperplasia (CAH) by administering to a subject in need thereof a CRF_1 receptor antagonist having a dissociation half-life in excess of 30 minutes.

2. The method of claim 1 wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 40 minutes.

3. The method of claim 1 wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 50 minutes.

4. The method of claim 1 wherein the CRF_1 receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

5. The method of claim 1 wherein the CRF_1 receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

6. The method of any one of claims 1-5 wherein the CRF_1 receptor antagonist is administered at bedtime.

7. The method of any one of claims 1-5 wherein the CRF_1 receptor antagonist is administered at or before the expected circadian release of ACTH.

8. The method of claim 7 wherein the CRF_1 receptor antagonist is administered 3-4 hours before the expected circadian release of ACTH.

9. A method for reducing 17-OHP and ACTH levels in a subject who has Congenital Adrenal Hyperplasia (CAH), said method comprising administering to the subject a CRF_1 receptor antagonist at bedtime.

10. The method of claim 9, wherein the CRF_1 receptor antagonist is administered at or before the expected circadian release of ACTH.

11. The method of claim 9, wherein the CRF_1 receptor antagonist is administered 3-4 hours before the expected circadian release of ACTH.

12. The method of any one of claims 9-11, wherein the CRF_1 receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

13. The method of any one of claims 9-11, wherein the CRF_1 receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

14. A CRF_1 receptor antagonist for use in treating Congenital Adrenal Hyperplasia (CAH), wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 30 minutes.

15. The CRF_1 receptor antagonist of claim 14, wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 40 minutes.

16. The CRF_1 receptor antagonist of claim 14, wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 50 minutes.

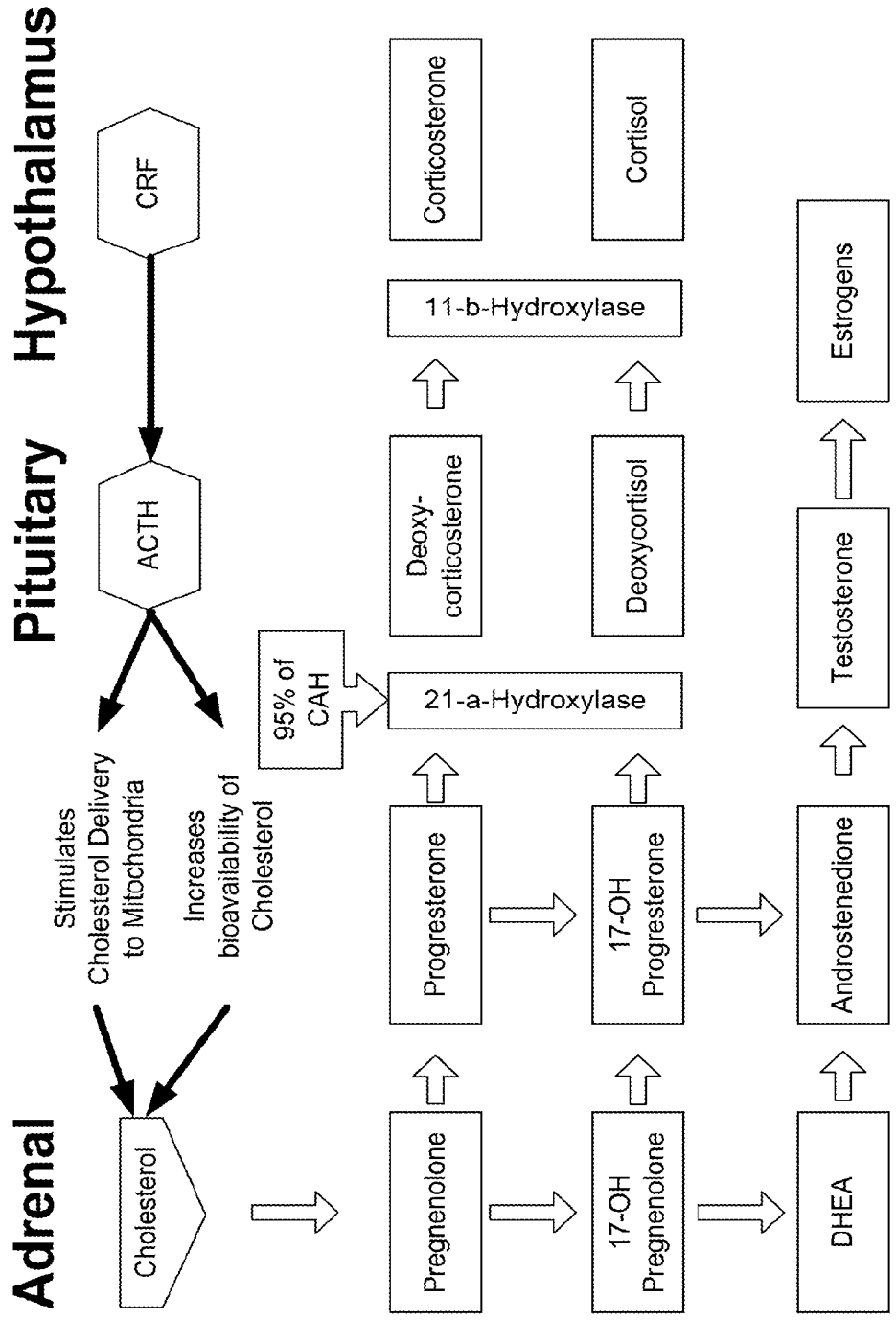
17. The CRF_1 receptor antagonist of claim 14, wherein the CRF_1 receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

18. The CRF_1 receptor antagonist of claim 14, wherein the CRF_1 receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

19. The CRF_1 receptor antagonist of any one of claims 14-18, wherein the CRF_1 receptor antagonist is suitable for administration at bedtime.

20. The CRF_1 receptor antagonist of any one of claims 14-18, wherein the CRF_1 receptor antagonist is suitable for administration at or before the expected circadian release of ACTH.

21. The CRF_1 receptor antagonist of any one of claims 14-18, wherein the CRF_1 receptor antagonist is suitable for administration 3-4 hours before the expected circadian release of ACTH.


Adrenal
Pituitary
Hypothalamus
CRF
ACTH
Stimulates Cholesterol Delivery to Mitochondria
Increases bioavailability of Cholesterol
95% of CAH
Cholesterol
Pregnenolone
Progresterone
17-OH Pregnenolone
17-OH Progresterone
DHEA
Androstenedione
21-a-Hydroxylase
11-b-Hydroxylase
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Deoxycortisol
Corticosterone
Cortisol
Testosterone
Estrogens

FIG. 1

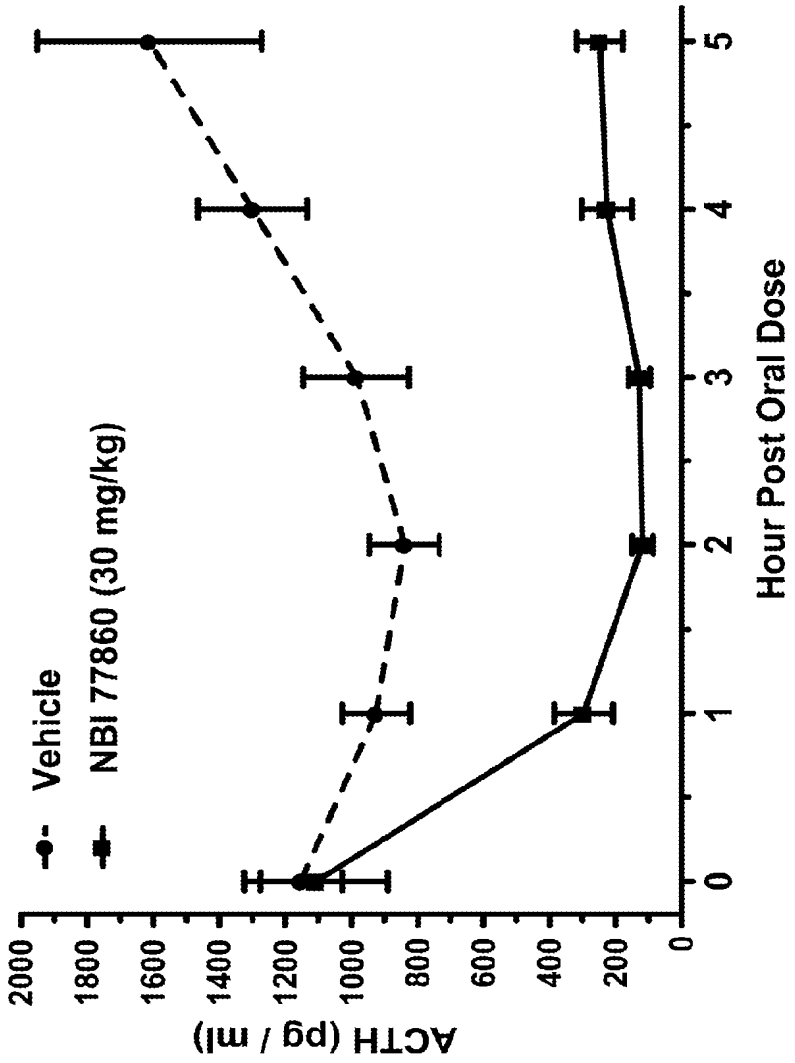

ACTH (pg / ml)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
1
2
3
4
5
Hour Post Oral Dose
Vehicle
NBI 77860 (30 mg/kg)

FIG. 2

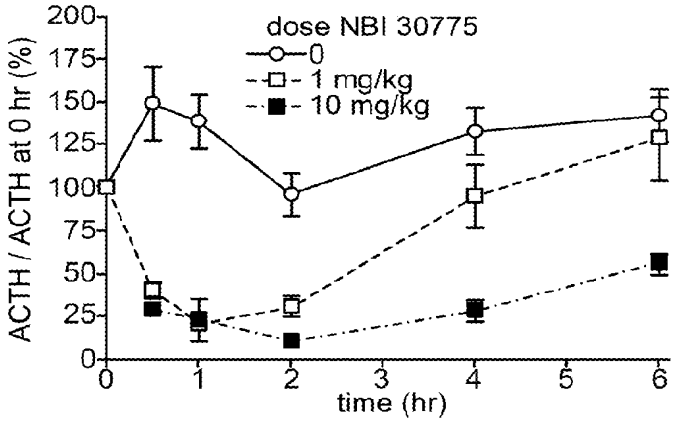


FIG. 3A

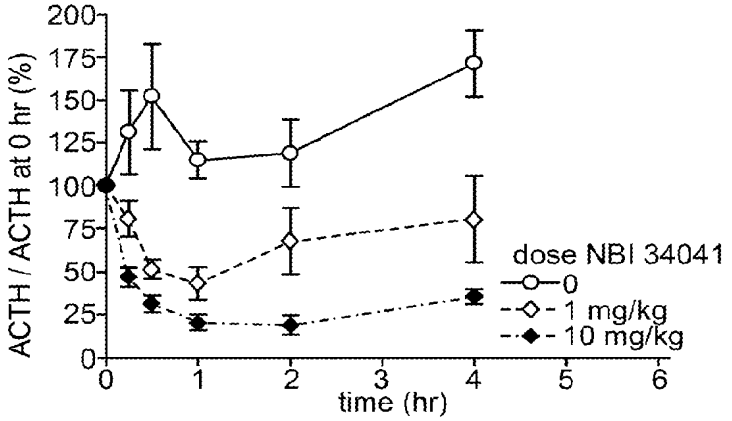


FIG. 3B

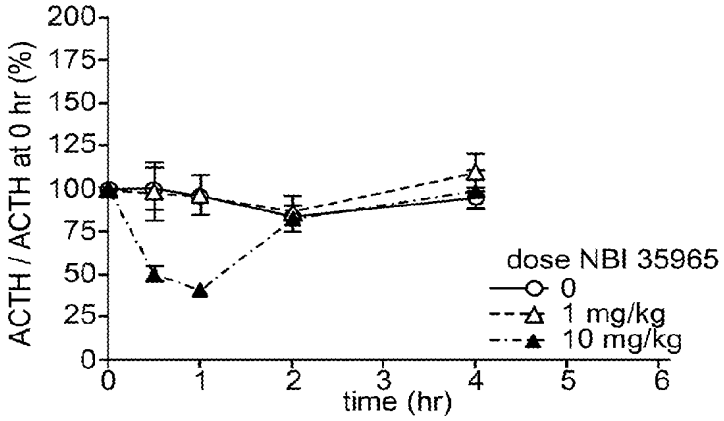


FIG. 3C

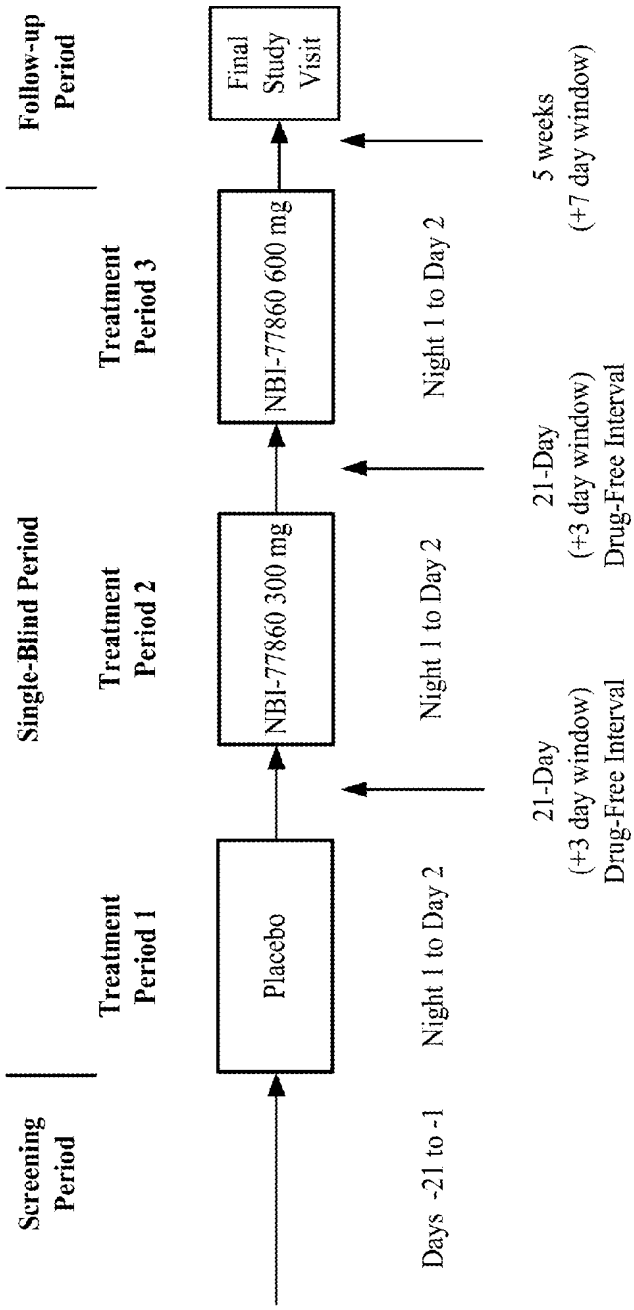
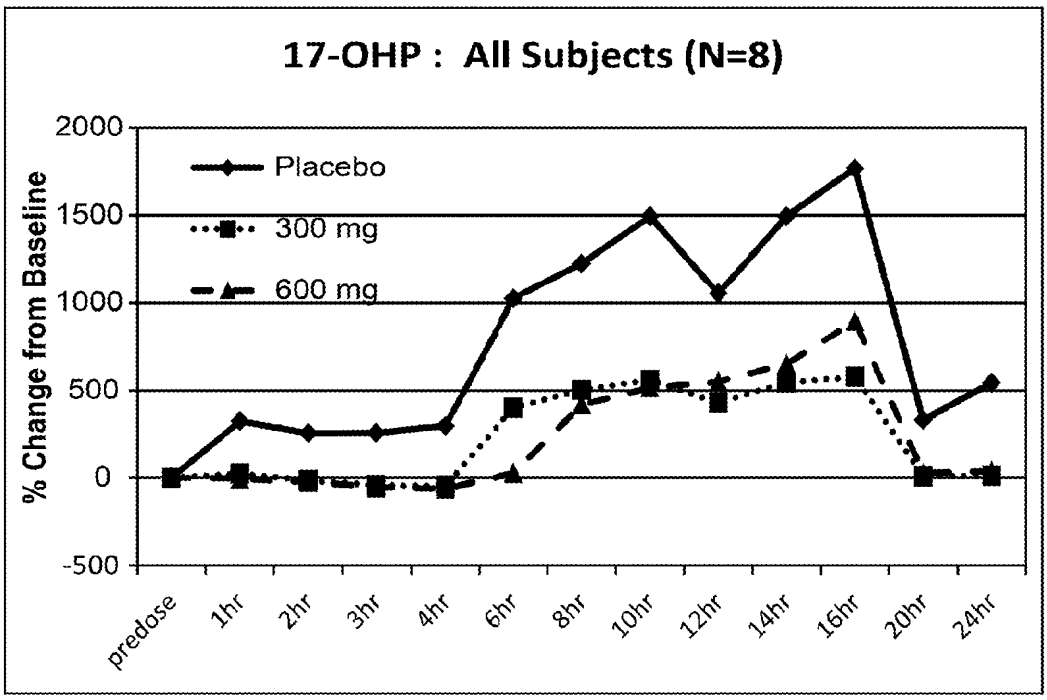

Screening Period
Single-Blind Period
Follow-up Period
Treatment Period 1
Treatment Period 2
Treatment Period 3
Placebo
NBI-77860 300 mg
NBI-77860 600 mg
Final Study Visit
Days -21 to -1
Night 1 to Day 2
Night 1 to Day 2
Night 1 to Day 2
21-Day (+3 day window) Drug-Free Interval
21-Day (+3 day window) Drug-Free Interval
5 weeks (+7 day window)

FIG. 4


17-OHP : All Subjects (N=8)
% Change from Baseline
2000
1500
1000
500
0
-500
Placebo
300 mg
600 mg
predose
1hr
2hr
3hr
4hr
6hr
8hr
10hr
12hr
14hr
16hr
20hr
24hr

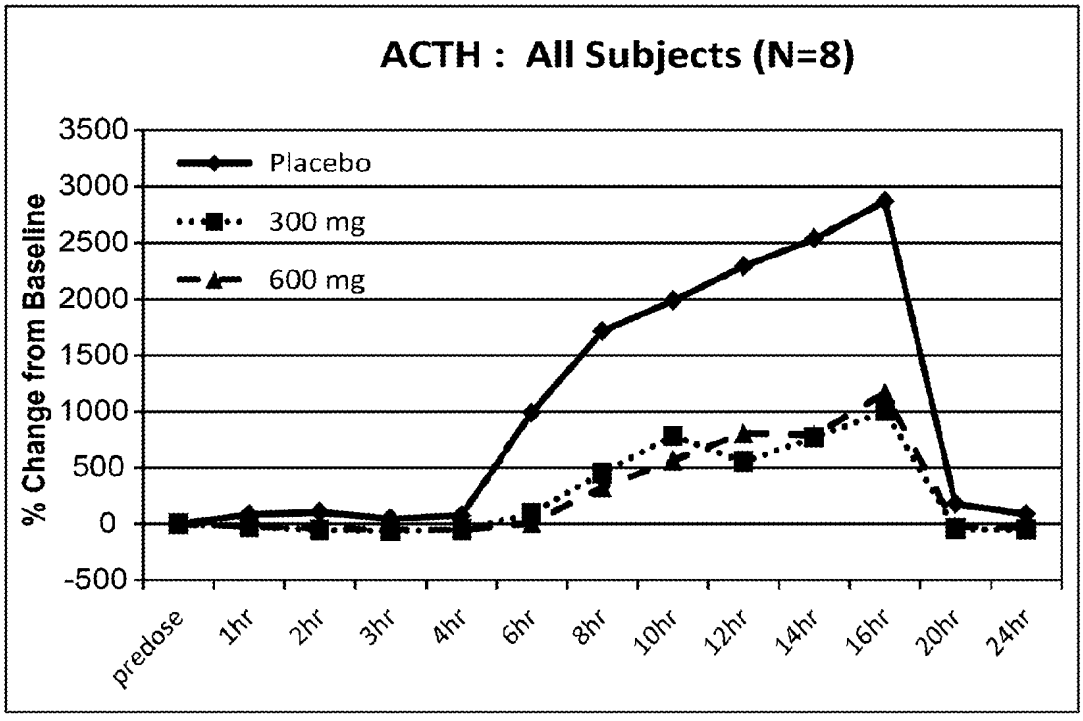

ACTH : All Subjects (N=8)
% Change from Baseline
3500
3000
2500
2000
1500
1000
500
0
-500
Placebo
300 mg
600 mg
predose
1hr
2hr
3hr
4hr
6hr
8hr
10hr
12hr
14hr
16hr
20hr
24hr

FIG. 5

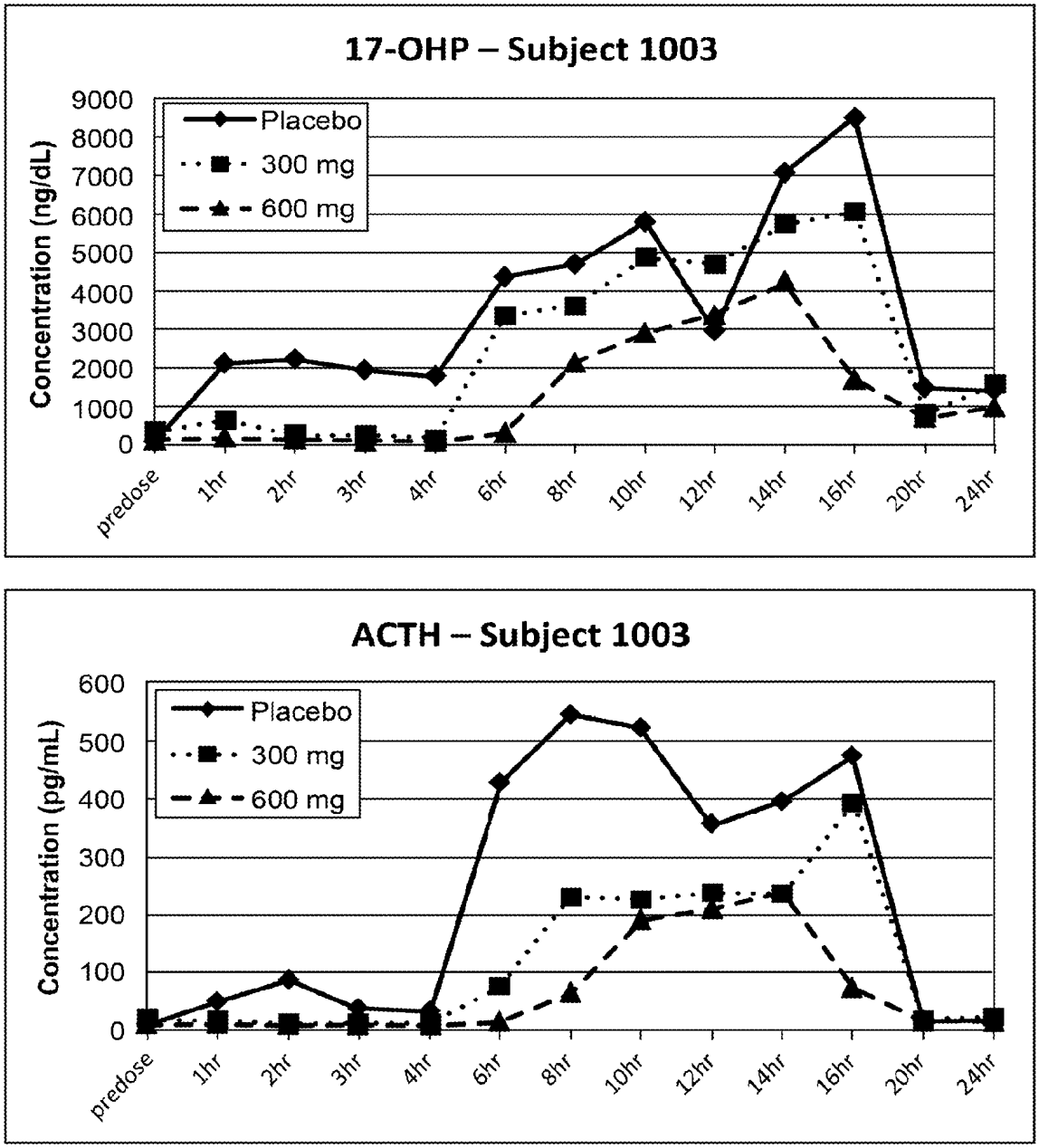

17-OHP – Subject 1003
Concentration (ng/dL)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Placebo
300 mg
600 mg
predose
1hr
2hr
3hr
4hr
6hr
8hr
10hr
12hr
14hr
16hr
20hr
24hr
ACTH – Subject 1003
Concentration (pg/mL)
600
500
400
300
200
100
0
Placebo
300 mg
600 mg
predose
1hr
2hr
3hr
4hr
6hr
8hr
10hr
12hr
14hr
16hr
20hr
24hr

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/012315

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/427 A61K31/4985 A61K31/519
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 8 030 304 B2 (CHEN ZHAOGEN [US] ET AL) 4 October 2011 (2011-10-04) cited in the application	1-3, 14-16
Y	column 2, lines 13-46 column 2, line 56 -----	1-21
X	ZOUMAKIS E ET AL: "Corticotropin-releasing hormone receptor antagonists", EUROPEAN JOURNAL OF ENDOCRINOLOGY, vol. 155, no. Suppl. 1, November 2006 (2006-11), pages S85-S91, XP002738177, ISSN: 0804-4643	1-3,5, 14-16,18
Y	page S86, left-hand column, paragraph 3; table 1 ----- -/--	1-21

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 April 2015	21/04/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Engl, Brigitte

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KAREN J LOECHNER ET AL: "Alternative Strategies for the Treatment of Classical Congenital Adrenal Hyperplasia: Pitfalls and Promises", INTERNATIONAL JOURNAL OF PEDIATRIC ENDOCRINOLOGY, BIOMED CENTRAL LTD, LONDON, UK, vol. 2010, no. 1, 8 June 2010 (2010-06-08) , page 670960, XP021109266, ISSN: 1687-9856, DOI: 10.1186/1687-9856-2010-670960 page 2, left-hand column, paragraph 1 -----	1-21
Y	NEWFIELD ET AL: "ACTH receptor blockade: A novel approach to treat congenital adrenal hyperplasia, or Cushing's disease", MEDICAL HYPOTHESES, EDEN PRESS, PENRITH, US, vol. 74, no. 4, 1 April 2010 (2010-04-01), pages 705-706, XP026975604, ISSN: 0306-9877 [retrieved on 2010-03-18] the whole document -----	1-21
Y	JOHN E TELLEW ET AL: "Discovery of NBI-77860/GSK561679, a potent corticotropin-releasing factor (CRF) receptor antagonist with improved pharmacokinetic properties", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, AMSTERDAM, NL, vol. 20, no. 24, 19 October 2010 (2010-10-19), pages 7259-7264, XP028129093, ISSN: 0960-894X, DOI: 10.1016/J.BMCL.2010.10.095 [retrieved on 2010-10-24] cited in the application page 7264, left-hand column, lines 17-21 -----	1-21
Y	CHEN C ET AL: "NBI 30775 (R121919), an orally active antagonist of the corticotropin-releasing factor (CRF) type-1 receptor for the treatment of anxiety and depression", DRUG DEVELOPMENT RESEARCH 200508 US, vol. 65, no. 4, August 2005 (2005-08), pages 216-226, XP002738174, ISSN: 0272-4391 page 219, left-hand column, line 28 - page 220, left-hand column, line 4 ----- -/--	1-21

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WEBSTER E L ET AL: "In vivo and in vitro characterization of Antalarmin, a nonpeptide corticotropin releasing hormone (CRH) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation", ENDOCRINOLOGY, THE ENDOCRINE SOCIETY, US, vol. 137, no. 12, 1 January 1996 (1996-01-01), pages 5747-5750, XP000651739, ISSN: 0013-7227, DOI: 10.1210/EN.137.12.5747 the whole document -----	1-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/012315

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AT 481405 T	15-10-2010
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		WO 2008036579 A1	27-03-2008

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C07C 13/547, A61K 31/165, 31/22, 31/19

A1

(11) International Publication Number: **WO 00/40529**

(43) International Publication Date: 13 July 2000 (13.07.00)

(21) International Application Number: PCT/GB99/04436

(22) International Filing Date: 24 December 1999 (24.12.99)

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(71) Applicant *(for all designated States except US)*: ANGIOGENE PHARMACEUTICALS LTD. [GB/GB]; Aston Rowant, Watlington, Oxfordshire OX9 5SX (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: DAVIS, Peter, David [GB/GB]; Aston Rowant, Watlington, Oxfordshire OX9 5SX (GB). ARNOULD, Jean-Claude [FR/FR]; Z.I. la Pompelle, BP 1050, F-51689 Reims Cedex 2 (FR). BOYLE, Francis, Thomas [GB/GB]; Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).

(74) Agent: BILL, Kevin; Global Intellectual Property, Patents, Astrazeneca PLC, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).

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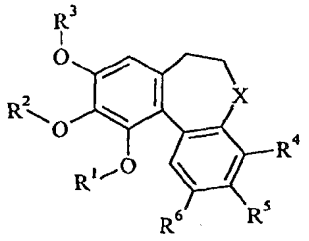

(I)

(57) Abstract

The invention relates to the use of compounds of formula (I): wherein X is –C(O)–, –C(S)–, –C=NOH, or –CH(R^7)– wherein R^7 is hydrogen, hydroxy, C_{1-7}alkoxy, $-OR^8$ or $-NR^8R^9$ (wherein R^8 is a group $-Y^1R^{10}$ (wherein Y^1 is a direct bond, –C(O)–, –C(S)–, –S–, –C(O)O, $-C(O)NR^{11}$–, $-SO_2$– or $-SO_2NR^{12}$– (wherein R^{11} and R^{12}, which may be the same or different, each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{10} is as defined herein, and R^9 includes hydrogen; R^1, R^2 and R^3 are as defined herein and are preferably methyl; R^4, R^5 and R^6 are as defined herein with the proviso that R^5 is not hydroxy, alkoxy, substituted alkoxy, $-OPO_3H_2$, –O–C_{1-7}alkanoyl or benzyloxy; and salts thereof in the manufacture of a medicament for use in the production of a vascular damaging effect in warm-blooded animals such as humans. The present invention further relates to compounds of the formula (I), pharmaceutical compositions containing them, processes for their preparation and to a method of treatment using the compounds to produce a vascular damaging effect in a warm-blooded animal such as a human. The compounds of formula (I) and the pharmaceutically acceptable salts thereof may be useful in the treatment of a number of disease states including cancer and rheumatoid arthritis.

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COLCHINOL DERIVATIVES AS VASCULAR DAMAGING AGENTS

The present invention relates to vascular damaging agents, in particular to the use of compounds of the invention in the manufacture of medicaments for use in the production of antiangiogenic effects in warm-blooded animals such as humans, to processes for the preparation of such compounds, to pharmaceutical compositions containing such compounds as active ingredient, to methods for the treatment of disease states associated with angiogenesis and to the use of such compounds as medicaments.

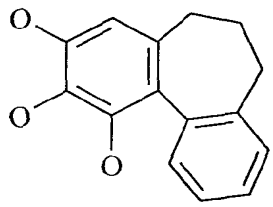
Normal angiogenesis plays an important role in a variety of processes including embryonic development, wound healing and several components of female reproductive function. Undesirable or pathological angiogenesis has been associated with disease states including diabetic retinopathy, psoriasis, cancer, rheumatoid arthritis, atheroma, Kaposi's sarcoma and haemangioma (Fan et al, 1995, Trends Pharmacol. Sci. 16: 57-66; Folkman, 1995, Nature Medicine 1: 27-31). Formation of new vasculature by angiogenesis is a key pathological feature of several diseases (J. Folkman, New England Journal of Medicine 333, 1757-1763 (1995)). For example, for a solid tumour to grow it must develop its own blood supply upon which it depends critically for the provision of oxygen and nutrients; if this blood supply is mechanically shut off the tumour undergoes necrotic death. Neovascularisation is also a clinical feature of skin lesions in psoriasis, of the invasive pannus in the joints of rheumatoid arthritis patients and of atherosclerotic plaques. Retinal neovascularisation is pathological in macular degeneration and in diabetic retinopathy.

Reversal of neovascularisation by damaging the newly-formed vascular endothelium is expected to have a beneficial therapeutic effect. The present invention is based on the discovery of tricyclic compounds that surprisingly specifically damage newly formed vasculature without affecting the normal, established vascular endothelium of the host species, a property of value in the treatment of disease states associated with angiogenesis such as cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation, endometriosis, dysfunctional uterine bleeding and ocular diseases with retinal vessel proliferation.

Compounds of the present invention are colchinol derivatives. Colchinol derivatives for example *N*-acetyl-colchinol are known. Anti-tumour effects have been noted on animal

models (see for example - Jnl. Natl. Cancer Inst. 1952, 13, 379-392). However, the effect studied was that of gross damage (haemorrhage, softening and necrosis) and there is no suggestion of treatment of inappropriate angiogenesis by destruction of neovasculature.

A search of Chemical Abstracts (post 1955) based on the substructure:



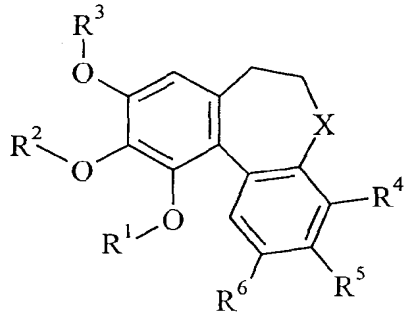
revealed a number of colchinol related structures. To the extent that any of these compounds have been studied for anti-cancer activity it is because tubulin-binding agents like colchinol might be expected to be anti-mitotic and therefore to have a direct effect on tumour cells. Some compounds which bind tubulin have been shown to have anti-vascular effects when given at their maximum tolerated dose (MTD) (S.A. Hill et al. Eur. J Cancer, 29A, 1320-1324 (1993)) but other tubulin-binding agents have no vascular-damaging activity even when administered at the MTD, for example docetaxel (Lancet, 1994, 344, 1267-1271). Based on this information and in the course of the work on the present invention, the issue of the relevance of tubulin-binding properties to possible effectiveness as anti-vascular agent was studied but no predictability was found. No correlation between the potency of tubulin interaction and effectiveness as an anti-vascular agent is apparent. Certain compounds structurally related to those of the present invention but not of the present invention, have been found to have a therapeutic window (ratio of MTD to minimum effective dose (MED)) too small for potential clinical effectiveness.

The presence of tubulin-binding properties is then not predictive for antivascular activity. Compounds which have strong tubulin-binding activity give rise to antimitotic effects *in vivo*. The effects of this are most noticeable on proliferating tissue and give rise to undesirable effects, for example on the proliferative tissue of the gut and bone marrow. Compounds which have vascular damaging activity but weak tubulin-binding activity would therefore be useful in the treatment of diseases involving angiogenesis.

It is believed, though this is not limiting on the invention, that the use of compounds of the invention damages newly-formed vasculature, for example the vasculature of tumours,

thus effectively reversing the process of angiogenesis as compared to known anti-angiogenic agents which tend to be less effective once the vasculature has formed.

According to one aspect of the present invention there is provided the use of a compound of the formula I:



(I)

wherein

X is

-C(O)-, -C(S)-, -C=NOH, or -CH(R^7)- wherein R^7 is hydrogen, hydroxy, C_{1-7}alkoxy, -OR^8 or -NR^8R^9 (wherein R^8 is a group -Y^1R^{10} (wherein Y^1 is a direct bond, -C(O)-, -C(S)-, -S-, -C(O)O-, -C(O)NR^{11}-, -SO_2- or -SO_2NR^{12}- (wherein R^{11} and R^{12}, which may be the same or different, each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{10} is selected from one of the following nine groups:

1) hydrogen, C_{1-7}alkyl, C_{3-7}cycloalkyl, C_{1-4}alkylY^8C_{1-4}alkyl wherein Y^8 is as defined hereinafter, or phenyl,

(which alkyl, cycloalkyl, alkylY^8alkyl or phenyl group may bear one or more substituents selected from:

halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, carboxy, carbamoyl, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, phenyl, nitro, sulphate, phosphate,

Z^1 (wherein Z^1 represents a 5-6 membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}aminoalkyl, C_{1-7}alkanoyl, cyanoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and Z^2 (wherein Z^2 is a 5-6-membered saturated

heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}aminoalkyl, C_{1-7}alkanoyl, cyanoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl)),

C_{1-4}alkylZ^1 (wherein Z^1 is as defined hereinbefore), and

a group $-Y^2R^{13}$ (wherein Y^2 is $-NR^{14}C(O)-$ or $-O-C(O)-$ (wherein R^{14} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{13} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{15} wherein R^{15} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{16}R^{17}$ and $-NR^{18}COR^{19}$ (wherein R^{16}, R^{17}, R^{18} and R^{19}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)));

2) R^{15} wherein R^{15} is as defined hereinbefore;

3) C_{2-7}alkenylR^{15} (wherein R^{15} is as defined hereinbefore);

4) C_{3-7}alkynylR^{15} (wherein R^{15} is as defined hereinbefore));

5) Z^1 (wherein Z^1 is as defined hereinbefore);

6) C_{1-7}alkylZ^1 (wherein Z^1 is as defined hereinbefore);

7) C_{1-7}alkylY^8Z^1 (wherein Z^1 is as defined hereinbefore and Y^8 is $-C(O)-$, $-NR^{59}C(O)-$, $-NR^{59}C(O)C_{1-4}$alkyl-, $-C(O)NR^{60}-$ or $-C(O)NR^{60}C_{1-4}$alkyl-, (wherein R^{59} and R^{60}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl, C_{1-3}hydroxyalkyl or C_{1-3}alkoxyC_{2-3}alkyl));

8) $(C_{1-7}alkyl)_cY^9Z^3$ (wherein c is 0 or 1, Z^3 is an amino acid group and Y^9 is a direct bond, $-C(O)-$ or $-NR^{61}-$ (wherein R^{61} is hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)); and

9) C_{1-7}alkylR^{15} (wherein R^{15} is as defined hereinbefore);

and R^9 is hydrogen, C_{1-7}alkyl or C_{3-7}cycloalkyl, which alkyl or cycloalkyl group may bear one or more substituents selected from C_{1-4}alkoxy and phenyl);

R^1, R^2 and R^3 are each independently

hydrogen, PO_3H_2, sulphate, C_{3-7}cycloalkyl, C_{2-7}alkenyl, C_{2-7}alkynyl, C_{1-7}alkanoyl, a group $R^{20}C_{1-7}$alkyl (wherein R^{20} is phenyl which may bear one or more substituents selected from C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}aminoalkyl and C_{1-4}hydroxyalkoxy), C_{1-7}alkyl or C_{1-7}alkylsulphonyl

(which alkyl or alkylsulphonyl group may bear one or more substituents selected from: halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-Y^2R^{21}$ (wherein Y^2 is $-NR^{22}C(O)-$ or $-O-C(O)-$ (wherein R^{22} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{21} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{23} wherein R^{23} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{24}R^{25}$ and $-NR^{26}COR^{27}$ (wherein R^{24}, R^{25}, R^{26} and R^{27}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)));

with the proviso that at least two of R^1, R^2 and R^3 are C_{1-7}alkyl;

R^4, R^5 and R^6 are each independently selected from:

hydrogen, $-OPO_3H_2$, phosphonate, cyano, halogeno, nitro, amino, carboxy, carbamoyl, hydroxy, C_{1-7}alkoxy, C_{1-7}alkanoyl, C_{1-7}thioalkoxy, C_{1-7}alkyl,

(which alkyl group may bear one or more substituents selected from: halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, sulphate, phosphate and a group $-Y^3R^{28}$ (wherein Y^3 is $-NR^{29}C(O)-$ or $-O-C(O)-$ (wherein R^{29} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{28} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}

$_4$hydroxyalkoxy, carboxy, cyano, -CONR^{31}R^{32} and -NR^{31}COR^{32} (wherein R^{31}, R^{32}, R^{33} and R^{34}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))), and

a group -Y^{4}R^{35}

(wherein Y^4 is -C(O)-, -OC(O)-, -O-, -SO-, -SO$_2$-, -OSO$_2$-, -NR^{36}-, -C_{1-4}alkylNR^{36}-, -C_{1-4}alkylC(O)-, -NR^{37}C(O)-, -OC(O)O-, -C(O)NR^{38}- or -NR^{39}C(O)O- (wherein R^{36}, R^{37}, R^{38} and R^{39}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and

R^{35} is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate, hydroxy, amino, C_{1-7}alkyl, C_{1-7}alkoxy, C_{1-7}alkanoyl, C_{1-7}alkylamino, di(C_{1-7}alkyl)amino, aminoC_{1-7}alkylamino, C_{1-7}alkylaminoC_{1-7}alkylamino, C_{1-7}alkanoylaminoC_{1-7}alkyl, di(C_{1-7}alkyl)aminoC_{1-7}alkylamino, C_{1-7}alkylphosphate, C_{1-7}alkylphosphonate, C_{1-7}alkylcarbamoylC_{1-7}alkyl,

(which alkyl, alkoxy, alkanoyl, alkylamino, dialkylamino, aminoalkylamino, alkylaminoalkylamino, alkanoylaminoalkyl, dialkylaminoalkylamino, alkylphosphate, alkylphosphonate or alkylcarbamoylalkyl, may bear one or more substituents selected from:

halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y^{5}R^{40} (wherein Y^5 is -NR^{41}C(O)-, -C(O)NR^{42}-, -C(O)-O- or -O-C(O)- (wherein R^{41} and R^{42} which may be the same or different each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{40} is C_{1-7}alkyl, C_{3-7}cycloalkyl, carboxyC_{1-7}alkyl or a group R^{43} wherein R^{43} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, -CONR^{44}R^{45} and -NR^{46}COR^{47} (wherein R^{44}, R^{45}, R^{46} and R^{47}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))),

R^{48} (wherein R^{48} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from

hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, di(C_{1-4}alkyl)aminoC_{1-4}alkyl, di(C_{1-4}hydroxyalkyl)aminoC_{1-4}alkyl, di(C_{1-4}aminoalkyl)aminoC_{1-4}alkyl, C_{1-4}hydroxyalkoxy, carboxy, C_{1-4}carboxyalkyl, phenyl, cyano, -$CONR^{49}R^{50}$, -$NR^{51}COR^{52}$ (wherein R^{49}, R^{50}, R^{51} and R^{52}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and C_{1-4}alkylR^{53} (wherein R^{53} is as defined hereinafter),

C_{1-7}alkylR^{48} (wherein R^{48} is as defined hereinbefore),

R^{53} (wherein R^{53} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}carboxyalkyl, C_{1-4}aminoalkyl, di(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{54} (wherein R^{54} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl)), or

$(CH_2)_aY^6(CH_2)_bR^{53}$ (wherein R^{53} is as defined hereinbefore, a is 0, or an integer 1-4, b is 0 or an integer 1-4 and Y^6 represents a direct bond, -O-, -C(O)-, -NR^{55}-, -NR^{56}C(O)- or -C(O)NR^{57}- (wherein R^{55}, R^{56}, and R^{57}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl), and wherein one or more of the $(CH_2)_a$ or $(CH_2)_b$ groups may bear one or more substituents selected from hydroxy, amino and halogeno));

with the proviso that R^5 is not hydroxy, alkoxy, substituted alkoxy (wherein R^5 is Y^4R^{35} and Y^4 is -O- and R^{35} is C_{1-7}alkyl bearing one or more substituents selected from the list given hereinbefore), -OPO_3H_2, -O-C_{1-7}alkanoyl or benzyloxy;

and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof in the manufacture of a medicament for use in the production of a vascular damaging effect in warm-blooded animals such as humans.

According to a further aspect of the present invention there is provided the use of a compound of the formula I as defined hereinbefore and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof in the manufacture of a medicament for use in the production of a vascular damaging effect at less than the maximum tolerated dose in warm-blooded animals such as humans.

Conveniently X is -C(O)-, -C(S)- or -CH(R^7)- wherein R^7 is hydrogen, hydroxy, -OR^8 or -NR^8R^9 (wherein R^8 is a group -Y^1R^{10} (wherein Y^1 is a direct bond, -C(O)-, -C(S)-, -S-, -C(O)O-, -C(O)NR^{11}-, -SO_2- or -SO_2NR^{12}- (wherein R^{11} and R^{12}, which may be the same or different, each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{10} is selected from one of the following seven groups:

1) hydrogen, C_{1-7}alkyl, C_{3-7}cycloalkyl, C_{1-4}alkylY^8C_{1-4}alkyl wherein Y^8 is as defined hereinafter, or phenyl,

(which alkyl, cycloalkyl, alkylY^8alkyl or phenyl group may bear one or more substituents selected from:

halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, carboxy, carbamoyl, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, phenyl, nitro, sulphate, phosphate,

Z^1 (wherein Z^1 represents a 5-6 membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}aminoalkyl, C_{1-7}alkanoyl, cyanoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and Z^2 (wherein Z^2 is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}aminoalkyl, C_{1-7}alkanoyl, cyanoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl)),

C_{1-4}alkylZ^1 (wherein Z^1 is as defined hereinbefore), and

a group -Y^2R^{13} (wherein Y^2 is -$NR^{14}C(O)$- or -O-C(O)- (wherein R^{14} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{13} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{15} wherein R^{15} is a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, -$CONR^{16}R^{17}$ and -$NR^{18}COR^{19}$ (wherein R^{16}, R^{17}, R^{18} and R^{19}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)));

2) R^{15} wherein R^{15} is as defined hereinbefore;

3) Z^1 (wherein Z^1 is as defined hereinbefore);

4) C_{1-7}alkylZ^1 (wherein Z^1 is as defined hereinbefore);

5) C_{1-7}alkylY^8Z^1 (wherein Z^1 is as defined hereinbefore and Y^8 is -C(O)-, -$NR^{59}C(O)$-, -$NR^{59}C(O)C_{1-4}$alkyl-, -C(O)NR^{60}- or -C(O)$NR^{60}C_{1-4}$alkyl-, (wherein R^{59} and R^{60}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl, C_{1-3}hydroxyalkyl or C_{1-3}alkoxyC_{2-3}alkyl));

6) $(C_{1-7}alkyl)_cY^9Z^3$ (wherein c is 0 or 1, Z^3 is an amino acid group and Y^9 is a direct bond, -C(O)- or -NR^{61}- (wherein R^{61} is hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)); and

7) C_{1-7}alkylR^{15} (wherein R^{15} is as defined hereinbefore));

and R^9 is hydrogen, C_{1-7}alkyl or C_{3-7}cycloalkyl, which alkyl or cycloalkyl group may bear one or more substituents selected from C_{1-4}alkoxy and phenyl).

Advantageously X is -CH(R^7)- wherein R^7 is -OR^8 or -NR^8R^9 (wherein R^8 is a group -Y^1R^{10} (wherein Y^1 is -C(O)-, -C(O)O- or -C(O)NR^{11}- (wherein R^{11} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{10} is as defined hereinbefore) and R^9 is as defined hereinbefore).

Preferably X is -CH(R^7)- wherein R^7 is -OR^8 or -NR^8R^9 (wherein R^8 is a group -Y^1R^{10} (wherein Y^1 is -C(O)- or -C(O)O- and R^{10} is as defined hereinbefore) and R^9 is as defined hereinbefore).

In one embodiment of the present invention preferably X is -C(O)-, -CH_2-, -CH(OH)- or -CH($NHC(O)CH_3$)-.

In one embodiment of the present invention more preferably X is -CH($NHC(O)CH_3$)-.

Conveniently R^1, R^2 and R^3 are each independently

hydrogen, PO_3H_2, sulphate, C_{1-7}alkyl, C_{3-7}cycloalkyl, C_{2-7}alkenyl, C_{2-7}alkynyl, C_{1-7}alkanoyl, C_{1-7}alkylsulphonyl or a group $R^{20}C_{1-7}$alkyl (wherein R^{20} is phenyl which may bear one or more substituents selected from C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}aminoalkyl and C_{1-4}hydroxyalkoxy); with the proviso that at least two of R^1, R^2 and R^3 are C_{1-7}alkyl.

Preferably R^1, R^2 and R^3 are each independently C_{1-4}alkyl.

More preferably R^1, R^2 and R^3 are each methyl.

Conveniently R^4 is

hydrogen, cyano, halogeno, nitro, amino, hydroxy, C_{1-7}alkoxy, C_{1-7}thioalkoxy, C_{1-7}alkanoyl or C_{1-7}alkyl,

(which alkyl group may bear one or more substituents selected from halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-Y^3R^{28}$ (wherein Y^3 is $-NR^{29}C(O)-$ or $-O-C(O)-$ (wherein R^{29} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{28} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{31}R^{32}$ and $-NR^{31}COR^{32}$ (wherein R^{31}, R^{32}, R^{33} and R^{34}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))).

Preferably R^4 is hydrogen, hydroxy, halogeno, cyano, amino or C_{1-7}alkanoyl.

More preferably R^4 is hydrogen.

Conveniently R^5 and R^6 are each independently selected from:

hydrogen, $-OPO_3H_2$, phosphonate, cyano, halogeno, nitro, amino, carboxy, carbamoyl, hydroxy, C_{1-7}alkoxy, C_{1-7}alkanoyl, C_{1-7}thioalkoxy, C_{1-7}alkyl,

(which alkyl group may bear one or more substituents selected from: halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, sulphate, phosphate and a group $-Y^3R^{28}$ (wherein Y^3 is $-NR^{29}C(O)-$ or $-O-C(O)-$ (wherein R^{29} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{28} is C_{1-7}alkyl, C_{3-}

$_7$cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{31}R^{32}$ and $-NR^{31}COR^{32}$ (wherein R^{31}, R^{32}, R^{33} and R^{34}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))), and

a group $-Y^4R^{35}$

(wherein Y^4 is -C(O)-, -OC(O)-, -O-, -SO-, $-SO_2-$, $-OSO_2-$, $-NR^{36}-$, $-C_{1-4}$alkyl$NR^{36}-$, $-C_{1-4}$alkylC(O)-, $-NR^{37}C(O)-$, -OC(O)O-, $-C(O)NR^{38}-$ or $-NR^{39}C(O)O-$ (wherein R^{36}, R^{37}, R^{38} and R^{39}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and

R^{35} is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate, hydroxy, amino, C_{1-7}alkyl, C_{1-7}alkoxy, C_{1-7}alkanoyl, C_{1-7}alkylamino, di(C_{1-7}alkyl)amino, aminoC_{1-7}alkylamino, C_{1-7}alkylaminoC_{1-7}alkylamino, C_{1-7}alkanoylaminoC_{1-7}alkyl, di(C_{1-7}alkyl)aminoC_{1-7}alkylamino, C_{1-7}alkylphosphate, C_{1-7}alkylphosphonate, C_{1-7}alkylcarbamoylC_{1-7}alkyl,

(which alkyl, alkoxy, alkanoyl, alkylamino, dialkylamino, aminoalkylamino, alkylaminoalkylamino, alkanoylaminoalkyl, dialkylaminoalkylamino, alkylphosphate, alkylphosphonate or alkylcarbamoylalkyl, may bear one or more substituents selected from:

halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-Y^5R^{40}$ (wherein Y^5 is $-NR^{41}C(O)-$, $-C(O)NR^{42}-$, -C(O)-O- or -O-C(O)- (wherein R^{41} and R^{42} which may be the same or different each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{40} is C_{1-7}alkyl, C_{3-7}cycloalkyl, carboxyC_{1-7}alkyl or a group R^{43} wherein R^{43} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-}

$_4$alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, -$CONR^{44}R^{45}$ and -$NR^{46}COR^{47}$ (wherein R^{44}, R^{45}, R^{46} and R^{47}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))),

R^{48} (wherein R^{48} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from

hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, di(C_{1-4}alkyl)aminoC_{1-4}alkyl, di(C_{1-4}hydroxyalkyl)aminoC_{1-4}alkyl, di(C_{1-4}aminoalkyl)aminoC_{1-4}alkyl, C_{1-4}hydroxyalkoxy, carboxy, C_{1-4}carboxyalkyl, phenyl, cyano, -$CONR^{49}R^{50}$, -$NR^{51}COR^{52}$ (wherein R^{49}, R^{50}, R^{51} and R^{52}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and C_{1-4}alkylR^{53} (wherein R^{53} is as defined hereinafter),

C_{1-7}alkylR^{48} (wherein R^{48} is as defined hereinbefore),

R^{53} (wherein R^{53} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}carboxyalkyl, C_{1-4}aminoalkyl, di(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{54} (wherein R^{54} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl)), or

$(CH_2)_aY^6(CH_2)_bR^{53}$ (wherein R^{53} is as defined hereinbefore, a is 0, or an integer 1-4, b is 0 or an integer 1-4 and Y^6 represents a direct bond, -O-, -C(O)-, -NR^{55}-, -NR^{56}C(O)- or -C(O)NR^{57}- (wherein R^{55}, R^{56}, and R^{57}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl), and wherein one or more of the $(CH_2)_a$ or $(CH_2)_b$ groups may bear one or more substituents selected from hydroxy, amino and halogeno));

with the proviso that R^5 is not hydroxy, alkoxy, substituted alkoxy (wherein R^5 is Y^4R^{35} and Y^4 is -O- and R^{35} is C_{1-7}alkyl bearing one or more substituents selected from the list given hereinbefore), $-OPO_3H_2$, -O-C_{1-7}alkanoyl or benzyloxy.

In another embodiment of the present invention conveniently R^5 and R^6 are each independently selected from:

hydrogen, $-OPO_3H_2$, cyano, halogeno, nitro, amino, carboxy, hydroxy, C_{1-7}alkoxy, C_{1-7}alkanoyl, C_{1-7}thioalkoxy, C_{1-7}alkyl,

(which alkyl group may bear one or more substituents selected from:

halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, sulphate, phosphate and a group $-Y^3R^{28}$ (wherein Y^3 is $-NR^{29}C(O)-$ or -O-C(O)- (wherein R^{29} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{28} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{31}R^{32}$ and $-NR^{31}COR^{32}$ (wherein R^{31}, R^{32}, R^{33} and R^{34}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))), and

a group $-Y^4R^{35}$

(wherein Y^4 is -C(O)-, -OC(O)-, -O-, -SO-, $-SO_2-$, $-OSO_2-$, $-NR^{36}-$, $-NR^{37}C(O)-$, -OC(O)O-, $-C(O)NR^{38}-$ or $-NR^{39}C(O)O-$ (wherein R^{36}, R^{37}, R^{38} and R^{39}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{35} is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate, C_{1-7}alkyl, C_{1-7}alkoxy, C_{1-7}alkanoyl, aminoC_{1-7}alkylamino, C_{1-7}alkylaminoC_{1-7}alkylamino, di(C_{1-7}alkyl)aminoC_{1-7}alkylamino, C_{1-7}alkylphosphate

(which alkyl, alkoxy, alkanoyl, aminoalkylamino, alkylaminoalkylamino, dialkylaminoalkylamino, or alkylphosphate may bear one or more substituents selected from:

halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy,

phenyl, nitro, sulphate, phosphate and a group $-Y^5R^{40}$ (wherein Y^5 is $-NR^{41}C(O)-$, $-C(O)NR^{42}-$, $-C(O)-O-$ or $-O-C(O)-$ (wherein R^{41} and R^{42} which may be the same or different each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{40} is C_{1-7}alkyl, C_{3-7}cycloalkyl, carboxyC_{1-7}alkyl or a group R^{43} wherein R^{43} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{44}R^{45}$ and $-NR^{46}COR^{47}$ (wherein R^{44}, R^{45}, R^{46} and R^{47}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))),

R^{48} (wherein R^{48} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, phenyl, cyano, $-CONR^{49}R^{50}$ and $-NR^{51}COR^{52}$ (wherein R^{49}, R^{50}, R^{51} and R^{52}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)), or

R^{53} (wherein R^{53} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{54} (wherein R^{54} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl)));

with the proviso that R^5 is not hydroxy, alkoxy, substituted alkoxy, $-OPO_3H_2$, $-O-C_{1-7}$alkanoyl or benzyloxy.

Preferably R^6 is hydrogen, halogeno, amino, carboxy, hydroxy, C_{1-7}alkoxy or a group Y^4R^{35}

(wherein Y^4 is -C(O)-, -O- or -OSO_2- and R^{35} is C_{1-7}alkyl, C_{1-7}alkoxy (which alkyl or alkoxy may bear one or more substituents selected from halogeno), R^{48} (wherein R^{48} is a benzyl group) or R^{53} (wherein R^{53} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms selected independently from O, S and N)).

Particularly R^6 is hydrogen, $C(O)OCH_3$ or methoxy, especially $C(O)OCH_3$ or methoxy. More preferably R^6 is hydrogen.

Preferably R^5 is hydrogen, halogeno, amino, carboxy, carbamoyl, C_{1-7}alkanoyl, C_{1-7}thioalkoxy, or

a group -Y^4R^{35}

(wherein Y^4 is -C(O)-, -OC(O)-, -O-, -SO-, -OSO_2-, -NR^{36}-, -$NR^{37}C(O)$- or -$C(O)NR^{38}$- (wherein R^{36}, R^{37} and R^{38}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and

R^{35} is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, C_{1-7}alkyl, C_{1-7}alkoxy, C_{1-7}alkanoyl, C_{1-7}alkanoylaminoC_{1-7}alkyl,

(which alkyl, alkoxy, alkanoyl, alkanoylaminoalkyl may bear one or more substituents selected from:

halogeno, amino, hydroxy, carboxy, and a group -Y^5R^{40} (wherein Y^5 is -C(O)-O- or -O-C(O)- and R^{40} is C_{1-7}alkyl or a group R^{43} wherein R^{43} is a benzyl group),

R^{48} (wherein R^{48} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from

hydroxy, fluoro, amino, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, di(C_{1-4}alkyl)aminoC_{1-4}alkyl, di(C_{1-4}hydroxyalkyl)aminoC_{1-4}alkyl, di(C_{1-4}aminoalkyl)aminoC_{1-4}alkyl, C_{1-4}hydroxyalkoxy, carboxy, C_{1-4}carboxyalkyl, cyano, -$CONR^{49}R^{50}$, -$NR^{51}COR^{52}$ (wherein R^{49}, R^{50}, R^{51} and R^{52}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and C_{1-4}alkylR^{53} (wherein R^{53} is as defined hereinafter),

C_{1-7}alkylR^{48} (wherein R^{48} is as defined hereinbefore),

R^{53} (wherein R^{53} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, fluoro, chloro, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}carboxyalkyl, C_{1-4}aminoalkyl, di(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{54} (wherein R^{54} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl)), or

$(CH_2)_aY^6(CH_2)_bR^{53}$ (wherein R^{53} is as defined hereinbefore, a is 0, or an integer 1-4, b is 0 or an integer 1-4 and Y^6 represents a direct bond, -O-, -C(O)-, -NR^{55}-, -NR^{56}C(O)- or -C(O)NR^{57}- (wherein R^{55}, R^{56}, and R^{57}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl), and wherein one or more of the $(CH_2)_a$ or $(CH_2)_b$ groups may bear one or more substituents selected from hydroxy, amino and halogeno));

with the proviso that R^5 is not alkoxy, substituted alkoxy (wherein R^5 is Y^4R^{35} and Y^4 is -O- and R^{35} is C_{1-7}alkyl bearing one or more substituents selected from the list given hereinbefore), -O-C_{1-7}alkanoyl or benzyloxy.

Preferably R53 is a group selected from morpholino, piperidinyl and piperazinyl which group may be substituted as hereinbefore defined.

Advantageous values for R^5 include:

3-{[(2*R*)-2,6-diaminohexanoyl]amino}propanoyloxy (such as in Example 4),

3-[(2-aminoacetyl)amino]propanoyloxy (such as in Example 5),

2-morpholinoacetylaminomethoxy (such as in Example 38),

2-carboxy-3,4,5-trihydroxytetrahydro-2*H*-pyran-6-yloxy (such as in Example 44),

4-(4-methylpiperazin-1-ylmethyl)phenylcarbonyloxy (such as in Example 16),

4-(morpholinomethyl)phenylcarbonyloxy (such as in Example 17),

3-(4-methylpiperazin-1-ylcarbonyl)propanoyloxy (such as in Example 40),

5-carboxypentanoyloxy (such as in Example 41),

3-(4-carboxyphenyl)propanoyloxy (such as in Example 18) and

(3R)-2-amino-3-hydroxypropanoylamino (such as in Example 28).

Another advantageous value for R^5 is

(2S)-2-amino-5-[(2-nitroethanimidoyl)amino]pentanoylamino (such as in Example 52).

Preferred values for R^5 include

3-{[(2*R*)-2,6-diaminohexanoyl]amino}propanoyloxy (such as in Example 4),

3-[(2-aminoacetyl)amino]propanoyloxy (such as in Example 5),

4-(4-methylpiperazin-1-ylmethyl)phenylcarbonyloxy (such as in Example 16),

4-(morpholinomethyl)phenylcarbonyloxy (such as in Example 17),

3-(4-methylpiperazin-1-ylcarbonyl)propanoyloxy (such as in Example 40),

5-carboxypentanoyloxy (such as in Example 41),

3-(4-carboxyphenyl)propanoyloxy (such as in Example 18) and

(3R)-2-amino-3-hydroxypropanoylamino (such as in Example 28).

More preferred values for R^5 include

4-(4-methylpiperazin-1-ylmethyl)phenylcarbonyloxy (such as in Example 16) and

(3R)-2-amino-3-hydroxypropanoylamino (such as in Example 28).

In another embodiment of the present invention preferred values for R^5 include alanylamino, *N*-(benzyloxycarbonylalanyl)amino, and 4-(piperidino)piperidin-1-ylcarbonyloxy.

A more preferred value for R^5 is alanylamino.

In another embodiment of the present invention particular values of R^5 include amino, C_{1-7}alkylamino and diC_{1-7}alkylamino, especially amino.

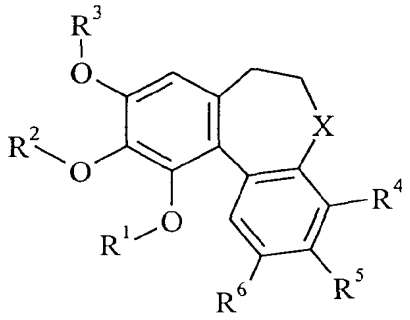
When R^{35} is a sugar moiety it can be, for example a monosaccharide such as a glucuronyl, glucosyl or galactosyl group or a di- or trisaccharide.

When R^{35} is a sugar moiety glucuronyl or a derivative thereof is preferred.

When R^{35} is a mono-, di-, tri- or tetra- peptide it is preferably derived from a natural alpha amino acid for example such as glycine, valine, lysine, alanine or serine.

In another embodiment of the present invention R^{35} is an amino acid group derived from serine, threonine, arginine, glycine, alanine, β-alanine or lysine.

According to another aspect of the present invention there is provided the use of a compound of the formula I:



(I)

wherein

X is

-C(O)-, -C(S)-, -C=NOH, or -CH(R^7)- wherein R^7 is hydrogen, hydroxy, C_{1-7}alkoxy or -NR^8R^9 (wherein R^8 is a group -Y^1R^{10} (wherein Y^1 is a direct bond, -C(O)-, -C(S)-, -S-, -C(O)O-, -C(O)NR^{11}-, -SO_2- or -SO_2NR^{12}- (wherein R^{11} and R^{12}, which may be the same or different, each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{10} is selected from one of the following four groups:

1) hydrogen, C_{1-7}alkyl or C_{3-7}cycloalkyl

(which alkyl or cycloalkyl group may bear one or more substituents selected from: halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, phenyl, nitro, sulphate, phosphate and a group -Y^2R^{13} (wherein Y^2 is -NR^{14}C(O)- or -O-C(O)- (wherein R^{14} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{13} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{15} wherein R^{15} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, -$CONR^{16}R^{17}$ and -$NR^{18}COR^{19}$ (wherein R^{16}, R^{17}, R^{18} and R^{19}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)));

2) R^{15} wherein R^{15} is as defined hereinbefore;

3) C_{2-7}alkenylR^{15} (wherein R^{15} is as defined hereinbefore); and

4) C_{3-7}alkynylR^{15} (wherein R^{15} is as defined hereinbefore));

and R^9 is hydrogen, C_{1-7}alkyl or C_{3-7}cycloalkyl, which alkyl or cycloalkyl group may bear one or more substituents selected from C_{1-4}alkoxy and phenyl);

R^1, R^2 and R^3 are each independently

hydrogen, PO_3H_2, sulphate, C_{3-7}cycloalkyl, C_{2-7}alkenyl, C_{2-7}alkynyl, C_{1-7}alkanoyl, a group $R^{20}C_{1-7}$alkyl (wherein R^{20} is phenyl which may bear one or more substituents selected from C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}aminoalkyl and C_{1-4}hydroxyalkoxy), C_{1-7}alkyl or C_{1-7}alkylsulphonyl

(which alkyl or alkylsulphonyl group may bear one or more substituents selected from: halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-Y^2R^{21}$ (wherein Y^2 is $-NR^{22}C(O)-$ or $-O-C(O)-$ (wherein R^{22} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{21} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{23} wherein R^{23} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{24}R^{25}$ and $-NR^{26}COR^{27}$ (wherein R^{24}, R^{25}, R^{26} and R^{27}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)));

with the proviso that at least two of R^1, R^2 and R^3 are C_{1-7}alkyl;

R^4, R^5 and R^6 are each independently selected from:

hydrogen, $-OPO_3H_2$, cyano, halogeno, nitro, amino, carboxy, hydroxy, C_{1-7}alkoxy, C_{1-7}alkanoyl, C_{1-7}thioalkoxy, C_{1-7}alkyl,

(which alkyl group may bear one or more substituents selected from: halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, sulphate, phosphate and a group $-Y^3R^{28}$ (wherein Y^3 is $-NR^{29}C(O)-$ or $-O-C(O)-$ (wherein R^{29} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{28} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic

heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{31}R^{32}$ and $-NR^{31}COR^{32}$ (wherein R^{31}, R^{32}, R^{33} and R^{34}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))), and

a group $-Y^4R^{35}$

(wherein Y^4 is -C(O)-, -OC(O)-, -O-, -SO-, $-SO_2$-, $-OSO_2$-, $-NR^{36}$-, $-NR^{37}C(O)$-, -OC(O)O-, $-C(O)NR^{38}$- or $-NR^{39}C(O)O$- (wherein R^{36}, R^{37}, R^{38} and R^{39}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and

R^{35} is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate, C_{1-7}alkyl, C_{1-7}alkoxy, C_{1-7}alkanoyl, aminoC_{1-7}alkylamino, C_{1-7}alkylaminoC_{1-7}alkylamino, di(C_{1-7}alkyl)aminoC_{1-7}alkylamino, C_{1-7}alkylphosphate

(which alkyl, alkoxy, alkanoyl, aminoalkylamino, alkylaminoalkylamino, dialkylaminoalkylamino, or alkylphosphate may bear one or more substituents selected from:

halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-Y^5R^{40}$ (wherein Y^5 is $-NR^{41}C(O)$-, $-C(O)NR^{42}$-, -C(O)-O- or -O-C(O)- (wherein R^{41} and R^{42} which may be the same or different each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{40} is C_{1-7}alkyl, C_{3-7}cycloalkyl, carboxyC_{1-7}alkyl or a group R^{43} wherein R^{43} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{44}R^{45}$ and $-NR^{46}COR^{47}$ (wherein R^{44}, R^{45}, R^{46} and R^{47}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))),

R^{48} (wherein R^{48} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected

independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from

hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, phenyl, cyano, -$CONR^{49}R^{50}$ and $-NR^{51}COR^{52}$ (wherein R^{49}, R^{50}, R^{51} and R^{52}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)), or

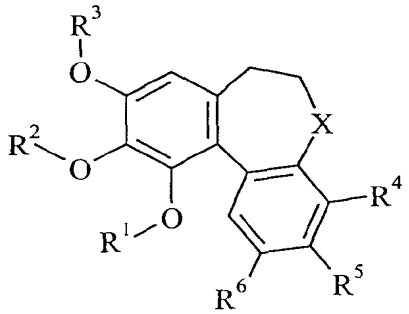
R^{53} (wherein R^{53} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{54} (wherein R^{54} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl)));

with the proviso that R^5 is not hydroxy, alkoxy, substituted alkoxy, $-OPO_3H_2$, -O-C_{1-7}alkanoyl or benzyloxy;

and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof for example esters, amides and sulphides, in the manufacture of a medicament for use in the production of a vascular damaging effect in warm-blooded animals such as humans.

According to another aspect of the present invention there is provided a compound of the formula IIa:



(IIa)

wherein

X is

-C(O)-, -C(S)-, -C=NOH, or -CH(R^7)- wherein R^7 is hydrogen, hydroxy, C_{1-7}alkoxy, -OR^8 or -NR^8R^9 (wherein R^8 is a group -Y^1R^{10} (wherein Y^1 is a direct bond, -C(O)-, -C(S)-, -S-, -C(O)O-, -C(O)NR^{11}-, -SO_2- or -SO_2NR^{12}- (wherein R^{11} and R^{12}, which may be the same or different, each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{10} is selected from one of the following nine groups:

1) hydrogen, C_{1-7}alkyl, C_{3-7}cycloalkyl, C_{1-4}alkylY^8C_{1-4}alkyl wherein Y^8 is as defined hereinafter, or phenyl,

(which alkyl, cycloalkyl, alkylY^8alkyl or phenyl group may bear one or more substituents selected from:

halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, carboxy, carbamoyl, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, phenyl, nitro, sulphate, phosphate,

Z^1 (wherein Z^1 represents a 5-6 membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}aminoalkyl, C_{1-7}alkanoyl, cyanoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and Z^2 (wherein Z^2 is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}aminoalkyl, C_{1-7}alkanoyl, cyanoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl)),

C_{1-4}alkylZ^1 (wherein Z^1 is as defined hereinbefore), and

a group -Y^2R^{13} (wherein Y^2 is -NR^{14}C(O)- or -O-C(O)- (wherein R^{14} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{13} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{15} wherein R^{15} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more

substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{16}R^{17}$ and $-NR^{18}COR^{19}$ (wherein R^{16}, R^{17}, R^{18} and R^{19}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)));

2) R^{15} wherein R^{15} is as defined hereinbefore;

3) C_{2-7}alkenylR^{15} (wherein R^{15} is as defined hereinbefore);

4) C_{3-7}alkynylR^{15} (wherein R^{15} is as defined hereinbefore));

5) Z^1 (wherein Z^1 is as defined hereinbefore);

6) C_{1-7}alkylZ^1 (wherein Z^1 is as defined hereinbefore);

7) C_{1-7}alkylY^8Z^1 (wherein Z^1 is as defined hereinbefore and Y^8 is $-C(O)-$, $-NR^{59}C(O)-$, $-NR^{59}C(O)C_{1-4}$alkyl-, $-C(O)NR^{60}-$ or $-C(O)NR^{60}C_{1-4}$alkyl-, (wherein R^{59} and R^{60}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl, C_{1-3}hydroxyalkyl or C_{1-3}alkoxyC_{2-3}alkyl));

8) $(C_{1-7}alkyl)_cY^9Z^3$ (wherein c is 0 or 1, Z^3 is an amino acid group and Y^9 is a direct bond, $-C(O)-$ or $-NR^{61}-$ (wherein R^{61} is hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)); and

9) C_{1-7}alkylR^{15} (wherein R^{15} is as defined hereinbefore);

and R^9 is hydrogen, C_{1-7}alkyl or C_{3-7}cycloalkyl, which alkyl or cycloalkyl group may bear one or more substituents selected from C_{1-4}alkoxy and phenyl);

R^1, R^2 and R^3 are each independently

hydrogen, PO_3H_2, sulphate, C_{3-7}cycloalkyl, C_{2-7}alkenyl, C_{2-7}alkynyl, C_{1-7}alkanoyl, a group $R^{20}C_{1-7}$alkyl (wherein R^{20} is phenyl which may bear one or more substituents selected from C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}aminoalkyl and C_{1-4}hydroxyalkoxy), C_{1-7}alkyl or C_{1-7}alkylsulphonyl (which alkyl or alkylsulphonyl group may bear one or more substituents selected from: halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-Y^2R^{21}$ (wherein Y^2 is $-NR^{22}C(O)-$ or $-O-C(O)-$ (wherein R^{22} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{21} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{23} wherein R^{23} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-}

$_4$haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, -$CONR^{24}R^{25}$ and -$NR^{26}COR^{27}$ (wherein R^{24}, R^{25}, R^{26} and R^{27}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)));

with the proviso that at least two of R^1, R^2 and R^3 are C_{1-7}alkyl;

R^4 is

hydrogen, cyano, halogeno, nitro, amino, hydroxy, C_{1-7}alkoxy, C_{1-7}thioalkoxy, C_{1-7}alkanoyl or C_{1-7}alkyl,

(which alkyl group may bear one or more substituents selected from:

halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y^3R^{28} (wherein Y^3 is -$NR^{29}C(O)$- or -O-C(O)- (wherein R^{29} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{28} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, -$CONR^{31}R^{32}$ and -$NR^{31}COR^{32}$ (wherein R^{31}, R^{32}, R^{33} and R^{34}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)));

R^5 and R^6 are each independently selected from

hydrogen, -OPO_3H_2, phosphonate, cyano, halogeno, nitro, amino, carboxy, carbamoyl, hydroxy, C_{1-7}alkoxy, C_{1-7}alkanoyl, C_{1-7}thioalkoxy, C_{1-7}alkyl,

(which alkyl group may bear one or more substituents selected from:

halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, sulphate, phosphate and a group -Y^3R^{28} (wherein Y^3 is -$NR^{29}C(O)$- or -O-C(O)- (wherein R^{29} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{28} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear

one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{31}R^{32}$ and $-NR^{31}COR^{32}$ (wherein R^{31}, R^{32}, R^{33} and R^{34}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))), and

a group $-Y^4R^{35}$

(wherein Y^4 is -C(O)-, -OC(O)-, -O-, -SO-, $-SO_2-$, $-OSO_2-$, $-NR^{36}-$, $-C_{1-4}$alkyl$NR^{36}-$, $-C_{1-4}$alkylC(O)-, $-NR^{37}C(O)-$, -OC(O)O-, $-C(O)NR^{38}-$ or $-NR^{39}C(O)O-$ (wherein R^{36}, R^{37}, R^{38} and R^{39}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and

R^{35} is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate, hydroxy, amino, C_{1-7}alkyl, C_{1-7}alkoxy, C_{1-7}alkanoyl, C_{1-7}alkylamino, di(C_{1-7}alkyl)amino, aminoC_{1-7}alkylamino, C_{1-7}alkylaminoC_{1-7}alkylamino, C_{1-7}alkanoylaminoC_{1-7}alkyl, di(C_{1-7}alkyl)aminoC_{1-7}alkylamino, C_{1-7}alkylphosphate, C_{1-7}alkylphosphonate, C_{1-7}alkylcarbamoylC_{1-7}alkyl,

(which alkyl, alkoxy, alkanoyl, alkylamino, dialkylamino, aminoalkylamino, alkylaminoalkylamino, alkanoylaminoalkyl, dialkylaminoalkylamino, alkylphosphate, alkylphosphonate or alkylcarbamoylalkyl, may bear one or more substituents selected from:

halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-Y^5R^{40}$ (wherein Y^5 is $-NR^{41}C(O)-$, $-C(O)NR^{42}-$, -C(O)-O- or -O-C(O)- (wherein R^{41} and R^{42} which may be the same or different each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{40} is C_{1-7}alkyl, C_{3-7}cycloalkyl, carboxyC_{1-7}alkyl or a group R^{43} wherein R^{43} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{44}R^{45}$ and $-NR^{46}COR^{47}$ (wherein R^{44}, R^{45}, R^{46} and R^{47}, which

may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))),

R^{48} (wherein R^{48} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from

hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, di(C_{1-4}alkyl)aminoC_{1-4}alkyl, di(C_{1-4}hydroxyalkyl)aminoC_{1-4}alkyl, di(C_{1-4}aminoalkyl)aminoC_{1-4}alkyl, C_{1-4}hydroxyalkoxy, carboxy, C_{1-4}carboxyalkyl, phenyl, cyano, -CONR^{49}R^{50}, -NR^{51}COR^{52} (wherein R^{49}, R^{50}, R^{51} and R^{52}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and C_{1-4}alkylR^{53} (wherein R^{53} is as defined hereinafter),

C_{1-7}alkylR^{48} (wherein R^{48} is as defined hereinbefore),

R^{53} (wherein R^{53} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}carboxyalkyl, C_{1-4}aminoalkyl, di(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{54} (wherein R^{54} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl)), or

$(CH_2)_aY^6(CH_2)_bR^{53}$ (wherein R^{53} is as defined hereinbefore, a is 0, or an integer 1-4, b is 0 or an integer 1-4 and Y^6 represents a direct bond, -O-, -C(O)-, -NR^{55}-, -NR^{56}C(O)- or -C(O)NR^{57}- (wherein R^{55}, R^{56}, and R^{57}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl), and wherein one or more of the $(CH_2)_a$ or $(CH_2)_b$ groups may bear one or more substituents selected from hydroxy, amino and halogeno));

with the proviso that R^5 is not hydroxy, alkoxy, substituted alkoxy (wherein R^5 is Y^4R^{35} and Y^4 is -O- and R^{35} is C_{1-7}alkyl bearing one or more substituents selected from the list given hereinbefore), -OPO_3H_2, -O-C_{1-7}alkanoyl or benzyloxy;

with the further proviso that at least one of R^5 or R^6 is a group $-Y^4R^{35}$ (wherein Y^4 and R^{35} are as defined hereinbefore) but with the further provisos

that when R^5 is $-Y^4R^{35}$ and R^6 is hydrogen, hydroxy, methoxy or methoxycarbonyl, $-Y^4R^{35}$ is not selected from cases wherein:

Y^4 is -C(O)-, -OC(O)-, -O-, -SO-, $-OSO_2-$, $-NR^{36}-$, $-NR^{37}C(O)-$ or $-C(O)NR^{38}-$ (wherein R^{36}, R^{37} and R^{38}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{35} is

a glycine, valine or lysine group, a dipeptide of glycine and valine groups, C_{1-7}alkyl, C_{1-7}alkoxy, C_{1-7}alkanoyl,

(which alkyl, alkoxy or alkanoyl may bear one or more substituents selected from: halogeno, hydroxy, and a group $-Y^5R^{40}$ (wherein Y^5 is -O-C(O)- and R^{40} is C_{1-7}alkyl)), or

R^{48} (wherein R^{48} is a tetrazolyl group (which may or may not be substituted as hereinbefore defined), a phenyl group or a benzyl group which phenyl or benzyl group may bear one or more substituents selected from C_{1-4}alkyl); and

that when R^6 is $-Y^4R^{35}$ and R^5 is hydrogen, hydroxy, methoxy or methoxycarbonyl, $-Y^4R^{35}$ is not selected from cases wherein:

Y^4 is -C(O)-, -O- or $-OSO_2-$ and R^{35} is

C_{1-7}alkyl, C_{1-7}alkoxy

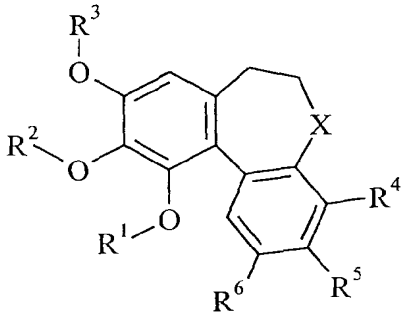
(which alkyl, alkoxy or alkanoyl may bear one or more substituents selected from: halogeno),

R^{48} (wherein R^{48} is a benzyl group which benzyl group may bear one or more substituents selected from C_{1-4}alkyl), or

R^{53} (wherein R^{53} is piperidinyl);

and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof.

According to a further aspect of the present invention there is provided a compound of the formula IIa:



(IIa)

wherein

X is

-C(O)-, -C(S)-, -C=NOH, or -CH(R^7)- wherein R^7 is hydrogen, hydroxy, C_{1-7}alkoxy, -OR^8 or -NR^8R^9 (wherein R^8 is a group -Y^1R^{10} (wherein Y^1 is a direct bond, -C(O)-, -C(S)-, -S-, -C(O)O-, -C(O)NR^{11}-, -SO_2- or -SO_2NR^{12}- (wherein R^{11} and R^{12}, which may be the same or different, each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{10} is selected from one of the following nine groups:

1) hydrogen, C_{1-7}alkyl, C_{3-7}cycloalkyl, C_{1-4}alkylY^8C_{1-4}alkyl wherein Y^8 is as defined hereinafter, or phenyl,

(which alkyl, cycloalkyl, alkylY^8alkyl or phenyl group may bear one or more substituents selected from:

halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, carboxy, carbamoyl, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, phenyl, nitro, sulphate, phosphate,

Z^1 (wherein Z^1 represents a 5-6 membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}aminoalkyl, C_{1-7}alkanoyl, cyanoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and Z^2 (wherein Z^2 is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}aminoalkyl, C_{1-7}alkanoyl, cyanoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl)),

C_{1-4}alkylZ^1 (wherein Z^1 is as defined hereinbefore), and

a group $-Y^2R^{13}$ (wherein Y^2 is $-NR^{14}C(O)-$ or $-O-C(O)-$ (wherein R^{14} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{13} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{15} wherein R^{15} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{16}R^{17}$ and $-NR^{18}COR^{19}$ (wherein R^{16}, R^{17}, R^{18} and R^{19}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)));

2) R^{15} wherein R^{15} is as defined hereinbefore;

3) C_{2-7}alkenylR^{15} (wherein R^{15} is as defined hereinbefore);

4) C_{3-7}alkynylR^{15} (wherein R^{15} is as defined hereinbefore));

5) Z^1 (wherein Z^1 is as defined hereinbefore);

6) C_{1-7}alkylZ^1 (wherein Z^1 is as defined hereinbefore);

7) C_{1-7}alkylY^8Z^1 (wherein Z^1 is as defined hereinbefore and Y^8 is $-C(O)-$, $-NR^{59}C(O)-$, $-NR^{59}C(O)C_{1-4}$alkyl-, $-C(O)NR^{60}-$ or $-C(O)NR^{60}C_{1-4}$alkyl-, (wherein R^{59} and R^{60}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl, C_{1-3}hydroxyalkyl or C_{1-3}alkoxyC_{2-3}alkyl));

8) $(C_{1-7}alkyl)_cY^9Z^3$ (wherein c is 0 or 1, Z^3 is an amino acid group and Y^9 is a direct bond, $-C(O)-$ or $-NR^{61}-$ (wherein R^{61} is hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)); and

9) C_{1-7}alkylR^{15} (wherein R^{15} is as defined hereinbefore);

and R^9 is hydrogen, C_{1-7}alkyl or C_{3-7}cycloalkyl, which alkyl or cycloalkyl group may bear one or more substituents selected from C_{1-4}alkoxy and phenyl);

R^1, R^2 and R^3 are each independently

hydrogen, PO_3H_2, sulphate, C_{3-7}cycloalkyl, C_{2-7}alkenyl, C_{2-7}alkynyl, C_{1-7}alkanoyl, a group $R^{20}C_{1-7}$alkyl (wherein R^{20} is phenyl which may bear one or more substituents selected from C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}aminoalkyl and C_{1-4}hydroxyalkoxy), C_{1-7}alkyl or C_{1-7}alkylsulphonyl

(which alkyl or alkylsulphonyl group may bear one or more substituents selected from: halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-Y^2R^{21}$ (wherein Y^2 is $-NR^{22}C(O)-$ or $-O-C(O)-$ (wherein R^{22} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{21} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{23} wherein R^{23} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{24}R^{25}$ and $-NR^{26}COR^{27}$ (wherein R^{24}, R^{25}, R^{26} and R^{27}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)));

with the proviso that at least two of R^1, R^2 and R^3 are C_{1-7}alkyl;

R^4 is

hydrogen, cyano, halogeno, nitro, amino, hydroxy, C_{1-7}alkoxy, C_{1-7}thioalkoxy, C_{1-7}alkanoyl or C_{1-7}alkyl,

(which alkyl group may bear one or more substituents selected from: halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-Y^3R^{28}$ (wherein Y^3 is $-NR^{29}C(O)-$ or $-O-C(O)-$ (wherein R^{29} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{28} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{31}R^{32}$ and $-NR^{31}COR^{32}$ (wherein R^{31}, R^{32}, R^{33} and R^{34}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)));

R^5 and R^6 are each independently selected from

hydrogen, $-OPO_3H_2$, phosphonate, cyano, halogeno, nitro, amino, carboxy, carbamoyl, hydroxy, C_{1-7}alkoxy, C_{1-7}alkanoyl, C_{1-7}thioalkoxy, C_{1-7}alkyl,

(which alkyl group may bear one or more substituents selected from: halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, sulphate, phosphate and a group $-Y^3R^{28}$ (wherein Y^3 is $-NR^{29}C(O)-$ or $-O-C(O)-$ (wherein R^{29} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{28} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{31}R^{32}$ and $-NR^{31}COR^{32}$ (wherein R^{31}, R^{32}, R^{33} and R^{34}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))), and

a group $-Y^4R^{35}$

(wherein Y^4 is $-C(O)-$, $-OC(O)-$, $-O-$, $-SO-$, $-SO_2-$, $-OSO_2-$, $-NR^{36}-$, $-C_{1-4}$alkyl$NR^{36}-$, $-C_{1-4}$alkyl$C(O)-$, $-NR^{37}C(O)-$, $-OC(O)O-$, $-C(O)NR^{38}-$ or $-NR^{39}C(O)O-$ (wherein R^{36}, R^{37}, R^{38} and R^{39}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and

R^{35} is a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate, hydroxy, amino, C_{1-7}alkyl, C_{1-7}alkoxy, C_{1-7}alkanoyl, C_{1-7}alkylamino, di(C_{1-7}alkyl)amino, aminoC_{1-7}alkylamino, C_{1-7}alkylaminoC_{1-7}alkylamino, C_{1-7}alkanoylaminoC_{1-7}alkyl, di(C_{1-7}alkyl)aminoC_{1-7}alkylamino, C_{1-7}alkylphosphate, C_{1-7}alkylphosphonate, C_{1-7}alkylcarbamoylC_{1-7}alkyl,

(which alkyl, alkoxy, alkanoyl, alkylamino, dialkylamino, aminoalkylamino, alkylaminoalkylamino, alkanoylaminoalkyl, dialkylaminoalkylamino, alkylphosphate, alkylphosphonate or alkylcarbamoylalkyl, may bear one or more substituents selected from:

halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-Y^5R^{40}$ (wherein Y^5

is $-NR^{41}C(O)-$, $-C(O)NR^{42}-$, $-C(O)-O-$ or $-O-C(O)-$ (wherein R^{41} and R^{42} which may be the same or different each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{40} is C_{1-7}alkyl, C_{3-7}cycloalkyl, carboxyC_{1-7}alkyl or a group R^{43} wherein R^{43} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{44}R^{45}$ and $-NR^{46}COR^{47}$ (wherein R^{44}, R^{45}, R^{46} and R^{47}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))),

R^{48} (wherein R^{48} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from

hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, di(C_{1-4}alkyl)aminoC_{1-4}alkyl, di(C_{1-4}hydroxyalkyl)aminoC_{1-4}alkyl, di(C_{1-4}aminoalkyl)aminoC_{1-4}alkyl, C_{1-4}hydroxyalkoxy, carboxy, C_{1-4}carboxyalkyl, phenyl, cyano, $-CONR^{49}R^{50}$, $-NR^{51}COR^{52}$ (wherein R^{49}, R^{50}, R^{51} and R^{52}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and C_{1-4}alkylR^{53} (wherein R^{53} is as defined hereinafter),

C_{1-7}alkylR^{48} (wherein R^{48} is as defined hereinbefore),

R^{53} (wherein R^{53} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}carboxyalkyl, C_{1-4}aminoalkyl, di(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{54} (wherein R^{54} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl)), or

$(CH_2)_aY^6(CH_2)_bR^{53}$ (wherein R^{53} is as defined hereinbefore, a is 0, or an integer 1-4, b is 0 or an integer 1-4 and Y^6 represents a direct bond, -O-, -C(O)-, $-NR^{55}$-, $-NR^{56}C(O)$- or -$C(O)NR^{57}$- (wherein R^{55}, R^{56}, and R^{57}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl), and wherein one or more of the $(CH_2)_a$ or $(CH_2)_b$ groups may bear one or more substituents selected from hydroxy, amino and halogeno));

with the proviso that R^5 is not hydroxy, alkoxy, substituted alkoxy (wherein R^5 is Y^4R^{35} and Y^4 is -O- and R^{35} is C_{1-7}alkyl bearing one or more substituents selected from the list given hereinbefore), $-OPO_3H_2$, -O-C_{1-7}alkanoyl or benzyloxy;

with the further proviso that at least one of R^5 or R^6 is a group $-Y^4R^{35}$ (wherein Y^4 and R^{35} are as defined hereinbefore) but with the further provisos

that when R^5 is $-Y^4R^{35}$ and R^6 is hydrogen, hydroxy, methoxy or methoxycarbonyl, $-Y^4R^{35}$ is not selected from cases wherein:

Y^4 is -C(O)-, -OC(O)-, -O-, -SO-, $-OSO_2$-, $-NR^{36}$-, $-NR^{37}C(O)$- or $-C(O)NR^{38}$- (wherein R^{36}, R^{37} and R^{38}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{35} is

a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, C_{1-7}alkyl, C_{1-7}alkoxy, C_{1-7}alkanoyl,

(which alkyl, alkoxy or alkanoyl may bear one or more substituents selected from: halogeno, hydroxy, and a group $-Y^5R^{40}$ (wherein Y^5 is -O-C(O)- and R^{40} is C_{1-7}alkyl)), or

R^{48} (wherein R^{48} is a tetrazolyl group (which may or may not be substituted as hereinbefore defined), a phenyl group or a benzyl group which phenyl or benzyl group may bear one or more substituents selected from C_{1-4}alkyl); and

that when R^6 is $-Y^4R^{35}$ and R^5 is hydrogen, hydroxy, methoxy or methoxycarbonyl, $-Y^4R^{35}$ is not selected from cases wherein:

Y^4 is -C(O)-, -O- or $-OSO_2$- and R^{35} is

C_{1-7}alkyl, C_{1-7}alkoxy

(which alkyl, alkoxy or alkanoyl may bear one or more substituents selected from: halogeno),

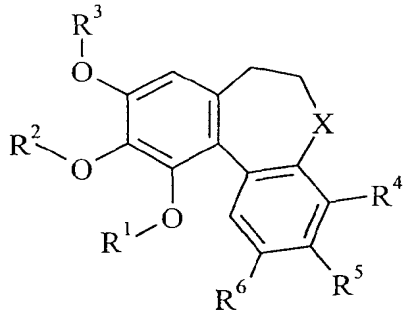
R^{48} (wherein R^{48} is a benzyl group which benzyl group may bear one or more substituents selected from C_{1-4}alkyl), or

R^{53} (wherein R^{53} is piperidinyl);

and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof.

According to a further aspect of the present invention there is provided the use of a compound of the formula IIa as defined hereinbefore, and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof, in the manufacture of a medicament for use in the production of a vascular damaging effect in warm-blooded animals such as humans.

According to another aspect of the present invention there is provided the use of a compound of the formula IIb:



(IIb)

wherein

X is

-C(O)-, -C(S)-, or -CH(R^7)- wherein R^7 is hydrogen, hydroxy or -NR^8R^9 (wherein R^8 is a group -Y^1R^{10} (wherein Y^1 is a direct bond, -C(O)-, -C(S)-, -C(O)O-, -C(O)NR^{11}-, -SO_2- or -SO_2NR^{12}- (wherein R^{11} and R^{12}, which may be the same or different, each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{10} is selected from one of the following two groups:

1) hydrogen, C_{1-7}alkyl or C_{3-7}cycloalkyl

(which alkyl or cycloalkyl group may bear one or more substituents selected from: halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, phenyl, nitro, sulphate, phosphate and a group -Y^2R^{13} (wherein Y^2 is -NR^{14}C(O)- or -O-C(O)- (wherein R^{14} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{13} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{15} wherein R^{15} is a phenyl group or a 5-10-membered aromatic

heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, -$CONR^{16}R^{17}$ and -$NR^{18}COR^{19}$ (wherein R^{16}, R^{17}, R^{18} and R^{19}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))); and

2) R^{15} wherein R^{15} is as defined hereinbefore;

and R^9 is hydrogen, C_{1-7}alkyl or C_{3-7}cycloalkyl, which alkyl or cycloalkyl group may bear one or more substituents selected from C_{1-4}alkoxy and phenyl);

R^1, R^2 and R^3 are each independently

hydrogen, PO_3H_2, sulphate, C_{3-7}cycloalkyl, C_{2-7}alkenyl, C_{2-7}alkynyl, C_{1-7}alkanoyl, a group $R^{20}C_{1-7}$alkyl (wherein R^{20} is phenyl which may bear one or more substituents selected from C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}aminoalkyl and C_{1-4}hydroxyalkoxy), C_{1-7}alkyl or C_{1-7}alkylsulphonyl

(which alkyl or alkylsulphonyl group may bear one or more substituents selected from: halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group -Y^2R^{21} (wherein Y^2 is -$NR^{22}C(O)$- or -O-C(O)- (wherein R^{22} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{21} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{23} wherein R^{23} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, -$CONR^{24}R^{25}$ and -$NR^{26}COR^{27}$ (wherein R^{24}, R^{25}, R^{26} and R^{27}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)));

with the proviso that at least two of R^1, R^2 and R^3 are C_{1-7}alkyl;

R^4 is

hydrogen, cyano, halogeno, nitro, amino, hydroxy, C_{1-7}alkoxy, C_{1-7}thioalkoxy, C_{1-7}alkanoyl or C_{1-7}alkyl,

(which alkyl group may bear one or more substituents selected from:

halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-Y^3R^{28}$ (wherein Y^3 is $-NR^{29}C(O)-$ or $-O-C(O)-$ (wherein R^{29} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{28} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{31}R^{32}$ and $-NR^{31}COR^{32}$ (wherein R^{31}, R^{32}, R^{33} and R^{34}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)));

R^5 and R^6 are each independently selected from

hydrogen, $-OPO_3H_2$, cyano, halogeno, nitro, amino, carboxy, hydroxy, C_{1-7}alkoxy, C_{1-7}alkanoyl, C_{1-7}thioalkoxy, C_{1-7}alkyl,

(which alkyl group may bear one or more substituents selected from:
halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, sulphate, phosphate and a group $-Y^3R^{28}$ (wherein Y^3 is $-NR^{29}C(O)-$ or $-O-C(O)-$ (wherein R^{29} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{28} is C_{1-7}alkyl, C_{3-7}cycloalkyl or a group R^{30} wherein R^{30} is a phenyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{31}R^{32}$ and $-NR^{31}COR^{32}$ (wherein R^{31}, R^{32}, R^{33} and R^{34}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))), and

a group $-Y^4R^{35}$

(wherein Y^4 is $-C(O)-$, $-OC(O)-$, $-O-$, $-SO-$, $-SO_2-$, $-OSO_2-$, $-NR^{36}-$, $-NR^{37}C(O)-$, $-OC(O)O-$, $-C(O)NR^{38}-$ or $-NR^{39}C(O)O-$ (wherein R^{36}, R^{37}, R^{38} and R^{39}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{35} is

a sugar moiety, a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, sulphate, C_{1-7}alkyl, C_{1-7}alkoxy, C_{1-7}alkanoyl, aminoC_{1-7}alkylamino, C_{1-7}alkylaminoC_{1-7}alkylamino, di(C_{1-7}alkyl)aminoC_{1-7}alkylamino, C_{1-7}alkylphosphate

(which alkyl, alkoxy, alkanoyl, aminoalkylamino, alkylaminoalkylamino, dialkylaminoalkylamino, or alkylphosphate may bear one or more substituents selected from:

halogeno, amino, C_{1-4}alkylamino, di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylsulphanyl, C_{1-4}alkylsulphonyl, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, carboxy, phenyl, nitro, sulphate, phosphate and a group $-Y^5R^{40}$ (wherein Y^5 is $-NR^{41}C(O)-$, $-C(O)NR^{42}-$, $-C(O)-O-$ or $-O-C(O)-$ (wherein R^{41} and R^{42} which may be the same or different each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{40} is C_{1-7}alkyl, C_{3-7}cycloalkyl, carboxyC_{1-7}alkyl or a group R^{43} wherein R^{43} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{44}R^{45}$ and $-NR^{46}COR^{47}$ (wherein R^{44}, R^{45}, R^{46} and R^{47}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))),

R^{48} (wherein R^{48} is a phenyl group, a benzyl group or a 5-10-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-4 heteroatoms selected independently from O, N and S, which phenyl, benzyl or aromatic heterocyclic group may bear one or more substituents selected from hydroxy, nitro, halogeno, amino, C_{1-4}alkyl, C_{1-4}haloalkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, phenyl, cyano, $-CONR^{49}R^{50}$ and $-NR^{51}COR^{52}$ (wherein R^{49}, R^{50}, R^{51} and R^{52}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)), or

R^{53} (wherein R^{53} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{54} (wherein R^{54} is a 5-6-membered saturated heterocyclic group (linked via carbon or

nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl)));

with the proviso that R^5 is not hydroxy, alkoxy, substituted alkoxy, $-OPO_3H_2$, -O-C_{1-7}alkanoyl or benzyloxy;

with the further proviso that at least one of R^5 or R^6 is a group $-Y^4R^{35}$ (wherein Y^4 and R^{35} are as defined hereinbefore) but with the further provisos

that when R^5 is $-Y^4R^{35}$ and R^6 is hydrogen, hydroxy or methoxy $-Y^4R^{35}$ is not selected from cases wherein:

Y^4 is -C(O)-, -OC(O)-, -O-, -SO-, $-OSO_2$-, $-NR^{36}$-, $-NR^{37}C(O)$- or $-C(O)NR^{38}$- (wherein R^{36}, R^{37} and R^{38}, which may be the same or different, each represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{35} is

a mono-peptide, a di-peptide, a tri-peptide, a tetra-peptide, C_{1-7}alkyl, C_{1-7}alkoxy, C_{1-7}alkanoyl,

(which alkyl, alkoxy or alkanoyl may bear one or more substituents selected from: halogeno, hydroxy, and a group $-Y^5R^{40}$ (wherein Y^5 is -C(O)-O- or -O-C(O)- and R^{40} is C_{1-7}alkyl)), or

R^{48} (wherein R^{48} is a phenyl group or a benzyl group which phenyl or benzyl group may bear one or more substituents selected from C_{1-7}alkyl); and

that when R^6 is $-Y^4R^{35}$ and R^5 is hydrogen, hydroxy or methoxy $-Y^4R^{35}$ is not selected from cases wherein:

Y^4 is -C(O)-, -O- or $-OSO_2$- and R^{35} is

C_{1-7}alkyl, C_{1-7}alkoxy

(which alkyl, alkoxy or alkanoyl may bear one or more substituents selected from: halogeno),

R^{48} (wherein R^{48} is a benzyl group which phenyl or benzyl group may bear one or more substituents selected from C_{1-7}alkyl), or

R^{53} (wherein R^{53} is piperidinyl);

and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof for example esters, amides and sulphides, in the manufacture of a

medicament for use in the production of a vascular damaging effect in warm-blooded animals such as humans.

According to a further aspect of the present invention there is provided a compound of the formula IIb as defined hereinbefore, and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof for example esters, amides and sulphides.

Preferred compounds of the present invention include:

(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl 3-{[(2*R*)-2,6-diaminohexanoyl]amino}propanoate,

(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl 3-[(2-aminoacetyl)amino]propanoate,

N-([(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]oxymethyl)-2-morpholinoacetamide,

(2*S*,3*S*,4*S*,5*R*,6*R*)-6-{[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]oxy}-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-carboxylic acid,

N-[(5*S*)-3-(4-{4-methylpiperazin-1-ylmethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide,

N-[(5*S*)-3-(4-{morpholinomethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide,

(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl 3-[4-methylpiperazin-1-ylcarbonyl]propanoate,

5-[{(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl}oxycarbonyl]pentanoic acid,

4-(3-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]oxy-3-oxopropyl)benzoic acid and

(2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]-2-amino-3-hydroxypropanamide,

and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof.

More preferred compounds of the present invention include:

(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl 3-{[(2*R*)-2,6-diaminohexanoyl]amino}propanoate,

(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl 3-[(2-aminoacetyl)amino]propanoate,

N-[(5*S*)-3-(4-{4-methylpiperazin-1-ylmethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide,

N-[(5*S*)-3-(4-{morpholinomethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide,

(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl 3-[4-methylpiperazin-1-ylcarbonyl]propanoate,

5-[{(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl}oxycarbonyl]pentanoic acid,

4-(3-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]oxy-3-oxopropyl)benzoic acid and

(2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]-2-amino-3-hydroxypropanamide,

and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof.

Especially preferred compounds of the present invention include:

N-[(5*S*)-3-(4-{4-methylpiperazin-1-ylmethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide and

(2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]-2-amino-3-hydroxypropanamide,

and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof.

In another embodiment of the present invention preferred compounds include (2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]-2-amino-5-[(2-nitroethanimidoyl)amino]pentanamide, and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof.

In one embodiment of the invention preferred compounds include those in which R^1, R^2 and R^3 are each alkyl, Y^4 is NH and R^{35} is an acyl group derived from a natural alpha-amino acid such as glycine, L-alanine or L-serine.

In one embodiment of the invention more preferred compounds include compounds wherein R^1, R^2 and R^3 are each methyl, R^4 is hydrogen and X is $-CH(NHC(O)CH_3)-$.

In another embodiment of the invention particular compounds include compounds wherein R^1, R^2 and R^3 are each alkyl and R^{35} is aminoC_{1-7}alkylamino, C_{1-7}alkylaminoC_{1-7}alkylamino, di(C_{1-7}alkyl)aminoC_{1-7}alkylamino, 1-piperazinyl or 4-(piperidino)piperidin-1-yl.

In another embodiment of the invention further particular compounds include compounds wherein R^1, R^2 and R^3 are each alkyl, R^4 is hydrogen and X is -CH(NHC(O)CH_3)-.

In another embodiment of the invention more particular compounds include compounds wherein R^1, R^2 and R^3 are each alkyl and R^4 and R^6 are each hydrogen.

In another embodiment of the invention especially preferred compounds include compounds wherein R^1, R^2 and R^3 are each methyl and R^{35} is a substituted acyl group.

Preferred compounds of the present invention include:

N-[3-(alanylamino)-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]acetamide and salts thereof, pharmaceutically acceptable salts thereof, solvates and hydrates thereof, and prodrugs thereof for example esters, amides and sulphides.

A preferred compound for use in the manufacture of a medicament for use in the production of a vascular damaging effect in warm-blooded animals such as humans is *N*-[3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide.

For the avoidance of doubt it is to be understood that where in this specification a group is qualified by 'hereinbefore defined' or 'defined hereinbefore', or 'hereinafter defined' or 'defined hereinafter', the said group encompasses the first occurring and broadest definition as well as each and all of the preferred definitions for that group.

In this specification unless stated otherwise the term "alkyl" includes both straight and branched chain alkyl groups but references to individual alkyl groups such as "propyl" are specific for the straight chain version only. An analogous convention applies to other generic terms. Unless otherwise stated the term "alkyl" advantageously refers to chains with 1-7 carbon atoms, preferably 1-4 carbon atoms. The term "alkoxy" as used herein, unless stated otherwise includes "alkyl"-O- groups in which "alkyl" is as hereinbefore defined. The term "aryl" as used herein unless stated otherwise includes reference to a C_{6-10}aryl group which may, if desired, carry one or more substituents selected from halogeno, alkyl, haloalkyl, alkoxy, hydroxy, amino, nitro and cyano, (wherein alkyl, haloalkyl and alkoxy are as hereinbefore and hereinafter defined). The term "aryloxy" as used herein unless otherwise stated includes "aryl"-O-groups in which "aryl" is as hereinbefore defined. The term "sulphonyloxy" as used herein refers to alkylsulphonyloxy and arylsulphonyloxy groups in

which "alkyl" and "aryl" are as hereinbefore defined. The term "heteroaryl" as used herein unless stated otherwise includes reference to a C_{6-10}aryl group containing one or more ring heteroatoms selected from O, N and S which heteroaryl group may, if desired, carry one or more substituents selected from halogeno, alkyl, haloalkyl, alkoxy, hydroxy, amino, nitro and cyano, (wherein alkyl, haloalkyl and alkoxy are as hereinbefore and hereinafter defined). The term "alkanoyl" as used herein unless otherwise stated includes formyl and alkylC=O groups in which "alkyl" is as defined hereinbefore, for example C_2alkanoyl is ethanoyl and refers to CH_3C=O, C_1alkanoyl is formyl and refers to CHO. In this specification unless stated otherwise the term "alkenyl" includes both straight and branched chain alkenyl groups but references to individual alkenyl groups such as 2-butenyl are specific for the straight chain version only. Unless otherwise stated the term "alkenyl" advantageously refers to chains with 2-7 carbon atoms, preferably 2-4 carbon atoms. In this specification unless stated otherwise the term "alkynyl" includes both straight and branched chain alkynyl groups but references to individual alkynyl groups such as 2-butynyl are specific for the straight chain version only. Unless otherwise stated the term "alkynyl" advantageously refers to chains with 2-7 carbon atoms, preferably 2-4 carbon atoms. The term "halogeno" means fluoro, chloro, bromo or iodo unless otherwise stated. A haloalkyl group is an alkyl group as defined hereinbefore substituted with one or more halogeno groups for example trifluoromethyl and dichloromethyl. A hydroxyalkyl group is an alkyl group as defined hereinbefore substituted with one or more hydroxy groups.

In this specification unless stated otherwise the term "acyl" refers to a group linked via a carbonyl group. "Acyl" includes a group -C(O)-R^{58} wherein R^{58} is an alkyl, aryl or heteroaryl group as hereinbefore defined, or -C(O)-R^{58} is derived from an amino acid.

In this specification mono-peptide means an amino acid including α-amino acids β-amino acids and γ-amino acids. The amino acids may be L-isomers or D-isomers, preferably L-isomers. Preferred amino acids include glycine, alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tryptophan, serine, threonine, cysteine, tyrosine, asparaginine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, β-alanine and ornithine. More preferred amino acids include serine, threonine, arginine, glycine, alanine, β-alanine and lysine. Especially preferred amino acids include serine, threonine, arginine, alanine and β-alanine.

An aromatic heterocyclic group includes those selected from pyridyl, pyrimidyl, furyl, thienyl, pyrrolyl, pyrazolyl, indolyl, benzofuryl, benzothienyl, benzothiazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, imidazolyl, triazolyl, quinolyl and isoquinolyl.

For the avoidance of any doubt, it is to be understood that when Y^1 is, for example, a group of formula $-C(O)NR^{11}-$, it is the nitrogen atom bearing the R^{11} group which is attached to the group R^{10} and the carbonyl group (C(O)) is attached to the nitrogen atom which is linked to the hepten ring. A similar convention applies to the other two atom Y^1 linking groups such as $-SO_2NR^{12}-$. An analogous convention applies to other groups. It is further to be understood that when Y^1 represents $-C(O)NR^{11}-$ and R^{11} is $C_{1-3}alkoxyC_{2-3}alkyl$ it is the $C_{2-3}alkyl$ moiety which is linked to the nitrogen atom of Y^1 and an analogous convention applies to other groups.

For the avoidance of any doubt it is to be understood that when Y^2 is, for example, a group of formula -OC(O)- it is the oxygen atom which is bound to the substituted group and the carbonyl group (C(O)) which is bound to R^{13} and an analogous convention applies to other groups.

For the avoidance of any doubt it is to be understood that when Y^4 is, for example, a group of formula $-NR^{39}C(O)O-$ it is the nitrogen atom which is bound to the benz ring and the oxygen atom which is bound to R^{35} and an analogous convention applies to other groups.

For the avoidance of any doubt it is to be understood that when R^{35} is a group $C_{1-7}alkylR^{48}$ it is the alkyl chain which is linked to Y^4, similarly when R^{35} is a group $(CH_2)_aY^6(CH_2)_bR^{53}$ it is the $(CH_2)_a$ group which is linked to Y^4 and a similar convention applies to other groups.

For the avoidance of any doubt, it is to be understood that when a group carries a $C_{1-4}alkylamino$ substituent it is the amino moiety which is attached to the group whereas when a group carries a $C_{1-4}aminoalkyl$ substituent it is the $C_{1-4}alkyl$ moiety which is attached to the group and an analogous convention applies to other substituents.

Within the present invention it is to be understood that a compound of the formula I or a salt thereof may exhibit the phenomenon of tautomerism and that the formulae drawings within this specification can represent only one of the possible tautomeric forms. It is to be understood that the invention encompasses any tautomeric form which has vascular damaging activity and is not to be limited merely to any one tautomeric form utilised within the formulae drawings. The formulae drawings within this specification can represent only one of

the possible tautomeric forms and it is to be understood that the specification encompasses all possible tautomeric forms of the compounds drawn not just those forms which it has been possible to show graphically herein.

It will be appreciated that compounds of the formula I or a salt thereof may possess an asymmetric carbon atom. Such an asymmetric carbon atom is also involved in the tautomerism described above, and it is to be understood that the present invention encompasses any chiral form (including both pure enantiomers and racemic mixtures), as well as any tautomeric form, which has vascular damaging activity, and is not to be limited merely to any one tautomeric form or chiral form utilised within the formulae drawings. It is to be understood that the invention encompasses all optical and diastereomers which have vascular damaging activity.

It is also to be understood that certain compounds of the formula I and salts thereof can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms which have vascular damaging activity.

The present invention relates to the compounds of formula I as hereinbefore defined as well as to the salts thereof. Salts for use in pharmaceutical compositions will be pharmaceutically acceptable salts, but other salts may be useful in the production of the compounds of formula I and their pharmaceutically acceptable salts. Pharmaceutically acceptable salts of the invention may, for example, include acid addition salts of the compounds of formula I as hereinbefore defined which are sufficiently basic to form such salts. Such acid addition salts include for example salts with inorganic or organic acids affording pharmaceutically acceptable anions such as with hydrogen halides (especially hydrochloric or hydrobromic acid of which hydrochloric acid is particularly preferred) or with sulphuric or phosphoric acid, or with trifluoroacetic, citric or maleic acid. Suitable salts include hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates and tartrates. In addition where the compounds of formula I are sufficiently acidic, pharmaceutically acceptable salts may be formed with an inorganic or organic base which affords a pharmaceutically acceptable cation. Such salts with inorganic or organic bases include for example an alkali metal salt, such as a sodium or potassium salt, an alkaline earth metal salt such as a calcium or magnesium salt, an ammonium salt or for

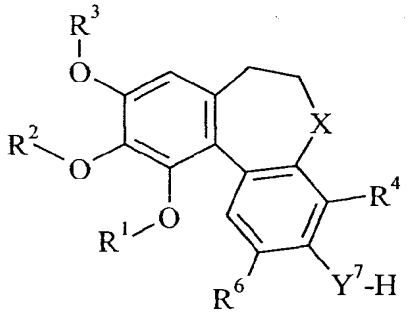
example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

Compounds of Formula I may be prepared by any process known to a person skilled in the art. Such processes include, for example, solid phase synthesis. Compounds of Formula I may be prepared by a number of processes as generally described hereinbelow and more specifically in the Examples hereinafter. In the general preparations described hereinafter it may be necessary to employ protecting groups which are then removed during the final stages of the synthesis. The appropriate use of such protecting groups and processes for their removal will be readily apparent to those skilled in the art. Processes for the preparation of novel compounds of formula I, are provided as a further feature of the invention and are as described hereinafter. Necessary starting materials may be obtained by standard procedures of organic chemistry. The preparation of such starting materials is described within the accompanying non-limiting Examples. Alternatively necessary starting materials are obtainable by analogous procedures to those illustrated which are within the ordinary skill of an organic chemist.

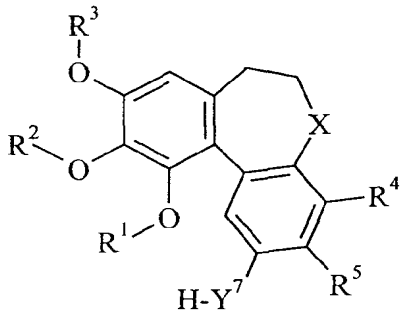
Thus, the following processes (a) to (i) and (i) to (iii) constitute further features of the present invention.

Synthesis of Compounds of Formula I

(a) Thus according to a further aspect of the invention a compound of formula I, in which R^5 or R^6 is a group Y^4R^{35} (wherein R^{35} is as defined hereinbefore and Y^4 is a group -OC(O)- or -NHC(O)-), can be prepared from a compound of formula III or IV:



(III)



(IV)

(wherein X, R^1, R^2, R^3, R^4, R^5, R^6 are as defined hereinbefore and Y^7 is -O- or -NH-), as appropriate, by standard acylation or coupling conditions including, for example, treatment of a compound of formula III or IV with a substituted carboxylic acid in the presence of a

coupling agent such as dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and, optionally, a base such as an organic base for example triethylamine in a solvent such as an aprotic solvent for example dimethylformamide or in a chlorinated solvent for example trichloromethane or dichloromethane at a temperature in the range from about -30°C to about 60°C, conveniently at or near ambient temperature.

(b) In another general example a compound of formula I, in which R^5 or R^6 is a group Y^4R^{35} (wherein R^{35} is C_{1-7}alkoxy which may be substituted as defined hereinbefore and Y^4 is a group -OC(O)- or -NHC(O)-), can be prepared from a compound of formula III and IV, as appropriate, by standard acylation reactions including, for example, treatment of a compound of formula III or IV with a substituted alkylchloroformate in the presence of a base such as an organic base for example, triethylamine or *N*-methylmorpholine in a solvent such as an ether solvent for example tetrahydrofuran or in a chlorinated solvent for example dichloromethane at a temperature in the range from about -20°C to the reflux temperature of the solvent.

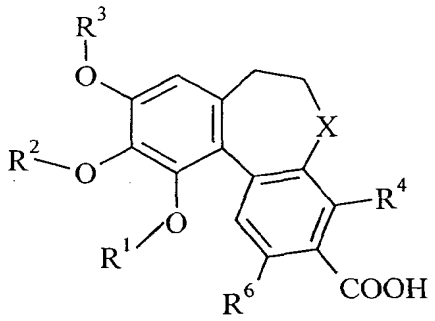
(c) In a further general example a compound of formula I, in which R^5 or R^6 is a group Y^4R^{35} (wherein R^{35} is aminoC_{1-7}alkylamino, C_{1-7}alkylaminoC_{1-7}alkylamino, di(C_{1-7}alkyl)aminoC_{1-7}alkylamino and may be substituted as defined hereinbefore, or is R^{53} (wherein R^{53} is as defined hereinbefore) and Y^4 is a group -OC(O)- or -NHC(O)-), can be prepared from a compound of formula III or IV, as appropriate, by standard acylation reactions including, for example, treatment of a compound of formula III or IV with a substituted alkylisocyanate or a carbamoyl chloride in the presence of a base such as an organic base for example triethylamine, pyridine or *N*-methylmorpholine in a solvent such as an ether solvent for example tetrahydrofuran or in a chlorinated solvent for example dichloromethane at a temperature in the range from about -20°C to the reflux temperature of the solvent.

(d) In a further example a compound of formula I, in which R^5 or R^6 is a group Y^4R^{35} (wherein R^{35} is a sugar moiety and Y^4 is a group -O- or -NH-), can be prepared from a compound of formula III or IV, as appropriate, by standard glycosylation reactions including for example treatment of a compound of formula III or IV with a suitably protected 1-bromo sugar in a solvent such as a chlorinated solvent for example trichloromethane or an aromatic solvent for example toluene at a temperature in the range from about 0°C to the reflux temperature of the solvent, followed by deprotection. Suitable protecting groups include acetyl groups for the sugar hydroxyl groups and esters for sugar carboxylic acids.

(e) In a further example a compound of formula I in which R^5 or R^6 is a group Y^4R^{35} (wherein R^{35} is sulphate and Y^4 is a group -O- or -NH-), can be prepared from a compound of formula III or IV, as appropriate, by standard sulphonylation reactions including for example treatment of a compound of formula III or IV with chlorosulphonic acid in the presence of a base such as dimethylaniline in a chlorinated solvent such as trichloromethane at a temperature in the range from about -20°C to about 60°C, or more preferably with chlorosulphonic acid in pyridine at a temperature in the range from about -20°C to about 60°C.

(f) In a further example a compound of formula I in which R^5 or R^6 is a group Y^4R^{35} (wherein R^{35} is C_{1-7}alkylphosphate and may be substituted as defined hereinbefore and Y^4 is a group -O- or -NH-), can be prepared from a compound of formula III or IV, as appropriate, by standard phosphorylation reactions including for example treatment of a compound of formula III or IV with phosphorus oxychloride in the presence of a base such as triethylamine in a chlorinated solvent such as trichloromethane at a temperature in the range from about -20°C to about 60°C, followed by treatment with an alcohol, or more preferably with alkyl dichlorophosphate in the presence of a base such as lithiumHMDS in THF at a temperature in the range from about -20°C to about 60°C, followed by treatment with water.

(g) Compounds of formula I in which R^5 is amino can be prepared from carboxylic acids of formula V:



(V)

(wherein X, R^1, R^2, R^3, R^4 and R^6 are as defined hereinbefore) via Curtius rearrangement and hydrolysis (V. Fernholz Justus Liebigs Ann., 1950, 568, 63-72).

(h) Compounds of formula I may also be prepared from other compounds of formula I by chemical modification. Examples of such chemical modifications that may be applied are standard alkylation, arylation, heteroarylation, acylation, thioacylation, sulphonylation, sulphation, phosphorylation, aromatic halogenation and coupling reactions. These reactions

may be used to add new substituents or to modify existing substituents. Alternatively, existing substituents in compounds of formula I may be modified by, for example, oxidation, reduction, elimination, hydrolysis or other cleavage reaction to yield other compounds of formula I.

(i) A compound of formula I in which R^5 or R^6 is chloro may be prepared from a compound of formula III or IV by standard processes such as the Sandmeyer reaction.

Thus for example a compound of formula I containing an amino group may be acylated on the amino group by treatment with, for example, an acyl halide or anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for example, a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In another general example of an interconversion process an amino group in a compound of formula I may be sulphonylated by treatment with, for example, an alkyl or aryl sulphonyl chloride or an alkyl or aryl sulphonic anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for example a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In a further general example a compound of formula I containing a hydroxy group can be converted into the corresponding dihydrogenphosphate ester by treatment with for example di-tert-butyl diisopropylphosphoramidite or di-tert-butyl diethylphosphoramidite in the presence of a suitable catalyst for example tetrazole in a solvent such as an ether solvent for example tetrahydrofuran at a temperature in the range -40°C to 40°C, conveniently at or near ambient temperature, followed by treatment with an oxidising agent for example 3-chloroperoxy benzoic acid at a temperature in the range -78°C to 40°C preferably -40°C to 10°C. The resulting intermediate phosphate triester is treated with an acid for example trifluoroacetic acid in a solvent such as a chlorinated solvent e.g. dichloromethane at a temperature in the range -30°C to 40°C conveniently at or near 0°C to give the compound of formula I containing a dihydrogenphosphate ester.

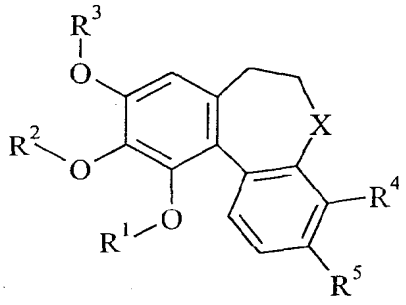
In a further general example a compound of formula I containing an amide can be hydrolysed by treatment with for example an acid such as hydrochloric acid in a solvent such as an alcohol, for example methanol at an elevated temperature conveniently at the reflux temperature.

In another general example an O-alkyl group may be cleaved to the corresponding alcohol (OH) by reaction with boron tribromide in a solvent such as a chlorinated solvent e.g. dichloromethane at a low temperature e.g. around -78°C.

In a further general example compounds of formula I may be alkylated by reaction with a suitable alkylating agent such as an alkyl halide, an alkyl toluenesulphonate, an alkyl methanesulphonate or an alkyl triflate. The alkylation reaction can be carried out in the presence of a base for example an inorganic base such as a carbonate e.g. caesium or potassium carbonate, a hydride such as sodium hydride or an alkoxide such as potassium t-butoxide in a suitable solvent such as an aprotic solvent e.g. dimethylformamide or an ether solvent such as tetrahydrofuran at a temperature of around -10°C to 80°C.

Synthesis of Intermediates

(i) Compounds of formula III or IV, used as starting materials for the preparation of compounds of the invention are either known or can be prepared from known compounds by the application of standard procedures of organic synthesis known in the art. For example a compound of formula VI:



(VI)

(wherein X, R^1, R^2, R^3, R^4 and R^5 are as defined hereinbefore), can be converted into a compound of formula IV where Y^7 is NH by the sequential application of standard nitration conditions followed by reduction of the incorporated nitro group under standard reduction conditions. Suitable nitration conditions include, for example, treatment with concentrated nitric acid in a solvent such as glacial acetic acid at a temperature from about -40°C to about 40°C. Suitable reduction conditions include, for example, treatment with tin(II) chloride in a solvent such as hydrochloric acid, with or without an alcoholic cosolvent, at a temperature between ambient temperature and about 100°C.

(ii) Compounds of formulae III or IV can also be prepared from other compounds of formulae III or IV by chemical modification. For example a compound of formula IV in which Y^7 is NH can be converted into the corresponding compound in which Y^7 is O by treatment with sodium nitrite in sulphuric acid at around 0°C followed by heating to around 100°C.

Preparation of a compound of formula I as a single enantiomer or, where appropriate, diastereomer may be effected by synthesis from an enantiomerically pure starting material or intermediate or by resolution of the final product in a conventional manner.

Acid addition salts of the compounds of formula I are prepared in a conventional manner by treating a solution or suspension of the free base I with about one equivalent of a pharmaceutically acceptable acid. Salts of compounds of formula I derived from inorganic or organic bases are prepared in a conventional manner by treating a solution or suspension of the free acid I with about one equivalent of a pharmaceutically acceptable organic or inorganic base. Alternatively both acid addition salts and salts derived from bases may be prepared by treatment of the parent compound with the appropriate ion-exchange resin in a standard fashion. Conventional concentration and recrystallistion techniques are employed in isolating the salts.

(iii) Compounds of formula V may be prepared from the corresponding colchicine derivatives by treatment with sodium methoxide in methanol followed by ester hydrolysis with aqueous acid or aqueous base (V. Fernholz Justus Liebigs Ann., 1950, 568, 63-72). Compounds of formula VI may be prepared by any of the methods described for compounds of formula I.

Many of the intermediates defined herein, for example, those of the formulae III, IV, V, and VI are novel and these are provided as a further feature of the invention. The preparation of these compounds is as described herein and/or is by methods well known to persons skilled in the art of organic chemistry.

Compounds according to the invention are able to destroy vasculature that has been newly formed such as tumour vasculature while leaving unaffected normal, mature vasculature. The identification of compounds which selectively, and preferably potently, damage newly-formed vasculature is desirable and is the subject of the present invention. The ability of the compounds to act in this way may be assessed, for example, using one or more of the procedures set out below:

(a) Activity against tumour vasculature measured by radioactive tracer

This assay demonstrates the ability of compounds to damage selectively tumour vasculature.

Subcutaneous CaNT tumours were initiated by injecting 0.05ml of a crude tumour cell suspension, approximately 10^6 cells, under the skin overlying the rear dorsum of 12-16 week-old mice. The animals were selected for treatment after approximately 3-4 weeks, when their tumours reached a geometric mean diameter of 5.5-6.5 mm. Compounds were dissolved in sterile saline and injected intraperitoneally in a volume of 0.1 ml per 10g body weight. Tumour perfusion was measured 6 hours after intraperitoneal administration in tumour, kidney, liver, skin, muscle, gut and brain by the ^{86}RbCl extraction technique (Sapirstein, Amer. Jnl. Physiol., 1958, 193, 161-168). Tissue radioactivity measured 1 minute after an intravenous injection of ^{86}RbCl was used to calculate relative blood flow as a proportion of cardiac output (Hill and Denekamp, Brit. Jnl. Radiol., 1982, 55, 905-913). Five animals were used in control and treated groups. Results were expressed as a percentage of the blood flow in the corresponding tissues in vehicle treated animals.

(b) Activity against tumour vasculature measured by fluorescent dye

This assay demonstrates the ability of compounds to damage tumour vasculature.

Tumour functional vascular volume in CaNT tumour-bearing mice was measured using the fluorescent dye Hoechst 33342 according to the method of Smith et al (Brit. Jnl. Cancer 1988, 57, 247-253). Five animals were used in control and treated groups. The fluorescent dye was dissolved in saline at 6.25mg/ml and injected intravenously at 10mg/kg 24 hours after intraperitoneal drug treatment. One minute later, animals were killed and tumours excised and frozen; 10μm sections were cut at 3 different levels and observed under UV illumination using an Olympus microscope equipped with epifluorescence. Blood vessels were identified by their fluorescent outlines and vascular volume was quantified using a point scoring system based on that described by Chalkley, (Jnl. Natl. Cancer Inst., 1943, 4, 47-53). All estimates were based on counting a minimum of 100 fields from sections cut at the 3 different levels.

The ability of the compounds to bind to preparations of mammalian tubulin can be evaluated by a number of methods available in the literature, for example by following temperature initiated tubulin polymerisation by turbidity in the absence and presence of the compound (for example O.Boye *et al* Med. Chem. Res., 1991, 1, 142-150).

The activity of *N*-[3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide, (V. Fernholz Justus Liebigs Ann., 1950, 568, 63-72), against tumour vasculature was measured by the fluorescent dye method described above. This compound decreased perfused vascular volume by 88% relative to control when dosed at 50mg/kg intraperitoneally. The IC_{50} of this compound in a tubulin polymerisation assay was 58 micromolar (O.Boye *et al* Med. Chem. Res., 1991, 1, 142-150).

The activity of compounds Examples 2 and 3 (described hereinafter) against tumour vasculature was measured by the fluorescent dye method described hereinbefore.

Compound of Example	% Decrease in vascular volume
2	95
3	45

(c) HUVEC detachment assay

This assay examined the effects of compounds on the adherence of HUVECs to tissue culture plasticware.

HUVECs were plated in 0.2% gelatin-coated 12 well tissue culture plates at a concentration of $3x10^4$ cells per well in 1ml TCS medium. After 24 hours, when the cells were at ~30% confluency, the cells were dosed with compound for 40 minutes at 37°C, 5% CO_2. After this incubation the medium containing drug was pipetted off, and the cells were then gently washed in 2mls of HBSS (Hanks' Balanced Salt Solution purchased from Life Technologies Ltd, Paisley UK; Catalogue # 24020-083) to remove any detached cells. The washing solution was then removed, and the adherent cells remaining were trypsinised using 300µl of 1x Trypsin-EDTA solution (Life Technologies Ltd, Paisley, UK; Catalogue # 43500-019) at ambient temperature for 2 minutes. The trypsinised cells were then made up to 1ml with TCS Biologicals medium, then centrifuged at 2000rpm for 2 minutes. The cell pellet was then resuspended in a volume of 50µl of TCS Biologicals medium. Total cell counts were obtained by counting the cells on a haemocytometer. The amount of cell detachment was calculated by comparing the number of cells remaining attached following treatment with the number in undosed control wells.

(d) Hras5 necrosis model

NIH 3T3 fibroblasts transfected with Harvey ras, clone 5, (Hras5 cells) were kept in continual passage in Dulbecco's modifed Eagles medium (DMEM) containing 10% foetal bovine serum (FBS) and 1% glutamine, at 37°C in a humidified incubator gassed with 7.5% carbon dioxide and 92.5% oxygen. Cells were implanted subcutaneously into the left flank of male nude mice (8-10weeks of age) at an inoculum of 2 x 10^5 cells/mouse. Tumours were measured using calipers and randomised into groups of 2-4 mice between days 9-14 after implant. Mice were dosed with compounds, either intravenously or intraperitoneally, once on day of randomisation and culled 24 hours after dosing. Compounds were dissolved in 20% hydroxypropyl beta cyclodextrin in physiological saline at pH 7 and dosed in a volume of 0.1ml per 10g body weight. Tumours were excised, weighed and placed in buffered formalin. Area of necrosis in individual tumours was assessed from a haematoxylin/eosin stained-slide by a pathologist and scored from 0, meaning no significant change, to 10, meaning 91-100% necrosis.

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises a compound of the formula I as defined hereinbefore or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable excipient or carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for nasal administration or administration by inhalation, for example as a powder or solution, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) for example as a sterile solution, suspension or emulsion, for topical administration for example as an ointment or cream or for rectal administration for example as a suppository. In general the above compositions may be prepared in a conventional manner using conventional excipients.

The compositions of the present invention are advantageously presented in unit dosage form. The compound will normally be administered to a warm-blooded animal at a unit dose within the range 5-5000mg per square metre body area of the animal, i.e. approximately 0.1-100mg/kg. A unit dose in the range, for example, 1-100mg/kg, preferably 1-50mg/kg is envisaged and this normally provides a therapeutically-effective dose. A unit dose form such as a tablet or capsule will usually contain, for example 1-250mg of active ingredient.

As stated above the size of the dose required for the therapeutic or prophylactic treatment of a particular disease state will necessarily be varied depending on the host treated, the route of administration and the severity of the illness being treated. Preferably a daily dose in the range of 1-50mg/kg is employed. However the daily dose will necessarily be varied depending upon the host treated, the particular route of administration, and the severity of the illness being treated. Accordingly the optimum dosage may be determined by the practitioner who is treating any particular patient.

According to a further aspect of the present invention there is provided a compound of the formula I or a pharmaceutically acceptable salt thereof as defined hereinbefore for use in a method of treatment of the human or animal body by therapy.

A further feature of the present invention is a compound of formula I, or a pharmaceutically acceptable salt thereof, for use as a medicament, conveniently a compound of formula I, or a pharmaceutically acceptable salt thereof, for use as a medicament for producing a vascular damaging effect in a warm-blooded animal such as a human being.

Thus according to a further aspect of the invention there is provided the use of a compound of the formula I, or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm-blooded animal such as a human being.

According to a further feature of the invention there is provided a method for producing a vascular damaging effect in a warm-blooded animal, such as a human being, in need of such treatment which comprises administering to said animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof as defined hereinbefore.

The antiangiogenic treatment defined hereinbefore may be applied as a sole therapy or may involve, in addition to a compound of the invention, one or more other substances and/or treatments. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate administration of the individual components of the treatment. In the field of medical oncology it is normal practice to use a combination of different forms of treatment to treat each patient with cancer. In medical oncology the other component(s) of such conjoint treatment in addition to the antiangiogenic treatment defined hereinbefore may be: surgery, radiotherapy or chemotherapy. Such chemotherapy may include the following categories of therapeutic agent:

(i) other antiangiogenic agents that work by different mechanisms from those defined hereinbefore (for example linomide, inhibitors of integrin αvβ3 function, angiostatin, endostatin, razoxin, thalidomide) and including vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitors (RTKIs) (for example those described in International Patent Applications Publication Nos. WO 97/22596, WO 97/30035, WO 97/32856 and WO 98/13354 the entire disclosure of which documents is incorporated herein by reference);

(ii) cytostatic agents such as antioestrogens (for example tamoxifen,toremifene, raloxifene, droloxifene, iodoxyfene), progestogens (for example megestrol acetate), aromatase inhibitors (for example anastrozole, letrazole, vorazole, exemestane), antiprogestogens, antiandrogens (for example flutamide, nilutamide, bicalutamide, cyproterone acetate), LHRH agonists and antagonists (for example goserelin acetate, luprolide), inhibitors of testosterone 5α-dihydroreductase (for example finasteride), anti-invasion agents (for example metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator receptor function) and inhibitors of growth factor function, (such growth factors include for example epidermal growth factor (EGF), platelet derived growth factor and hepatocyte growth factor such inhibitors include growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors and serine/threonine kinase inhibitors);

(iii) biological response modifiers (for example interferon);

(iv) antibodies (for example edrecolomab); and

(v) antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as antimetabolites (for example antifolates like methotrexate, fluoropyrimidines like 5-fluorouracil, purine and adenosine analogues, cytosine arabinoside); antitumour antibiotics (for example anthracyclines like doxorubicin, daunomycin, epirubicin and idarubicin, mitomycin-C, dactinomycin, mithramycin); platinum derivatives (for example cisplatin, carboplatin); alkylating agents (for example nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide, nitrosoureas, thiotepa); antimitotic agents (for example vinca alkaloids like vincristine and taxoids like taxol, taxotere); enzymes (for example asparaginase); thymidylate synthase inhibitors (for example raltitrexed); topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan, irinotecan).

As stated above the compounds defined in the present invention are of interest for their vascular damaging effects. Such compounds of the invention are expected to be useful

in the prophylaxis and treatment of a wide range of disease states where inappropriate angiogenesis occurs including cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation, endometriosis, dysfunctional uterine bleeding and ocular diseases with retinal vessel proliferation. In particular such compounds of the invention are expected to slow advantageously the growth of primary and recurrent solid tumours of, for example, the colon, breast, prostate, lungs and skin.

In addition to their use in therapeutic medicine, the compounds of formula I and their pharmaceutically acceptable salts are also useful as pharmacological tools in the development and standardisation of in vitro and in vivo test systems for the evaluation of the effects of vascular damaging agents in laboratory animals such as cats, dogs, rabbits, monkeys, rats and mice, as part of the search for new therapeutic agents.

It is to be understood that where the term "ether" is used anywhere in this specification it refers to diethyl ether.

The invention will now be illustrated in the following non-limiting Examples in which, unless otherwise stated:

(i) evaporations were carried out by rotary evaporation in vacuo and work-up procedures were carried out after removal of residual solids such as drying agents by filtration;

(ii) operations were carried out at ambient temperature, that is in the range 18-25°C and under an atmosphere of an inert gas such as argon or nitrogen;

(iii) yields are given for illustration only and are not necessarily the maximum attainable;

(iv) the structures of the end-products of the formula I were confirmed by nuclear (generally proton) magnetic resonance (NMR) and mass spectral techniques; proton magnetic resonance chemical shift values were measured on the delta scale and peak multiplicities are shown as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; q, quartet, quin, quintet;

(v) intermediates were not generally fully characterised and purity was assessed by thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), infra-red (IR) or NMR analysis;

Abbreviations

1,3-Dicyclohexylcarbodiimide	DCCI
4-Dimethylaminopyridine	DMAP
Tetrahydrofuran	THF
Diethyl azodicarboxylate	DEAD
N,N-Dimethylformamide	DMF
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride	EDCI
Dimethyl sulphoxide	DMSO
Trifluoroacetic acid	TFA
1,1,1,3,3,3-hexamethyldisilazane	HMDS

Example 1

N-[3-((*N*-benzyloxycarbonylalanyl)amino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide

A solution of *N*-benzyloxycarbonyl-(L)-alanine (63mg, 0.28mmol) in dichloromethane (4ml) at -20°C was treated with *N*-[3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohpeten-5yl]acetamide (100mg, 0.28mmol), (V. Fernholz Justus Liebigs Ann., 1950, 568, 63-72), and 1,3-dicyclohexylcarbodiimide (134mg, 0.31mmol) and the solution stirred for 16 hours at ambient temperature. Solvent was evaporated under reduced pressure and the residue chromatographed on silica gel, eluting with ethyl acetate to give a white solid which was triturated with diethyl ether. The title compound (85mg) was obtained as a white solid.

m.p. 140-141°C

m/e 561

Example 2

N-[3-(alanylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide

A solution of *N*-[3-((*N*-benzyloxycarbonylalanyl)amino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (70mg, 0.125mmol), (prepared as described in Example 1), in ethanol (2ml) was hydrogenated at atmospheric pressure over 5% palladium on carbon (10mg) for 2 hours. Ethanol (3ml) was added and the solution was

filtered through diatomaceous earth and the filtrate concentrated under reduced pressure. Trituration with ethyl acetate/diethyl ether gave the title compound (35mg) as a white solid.

m.p. 170-173°C

m/e 427

Example 3

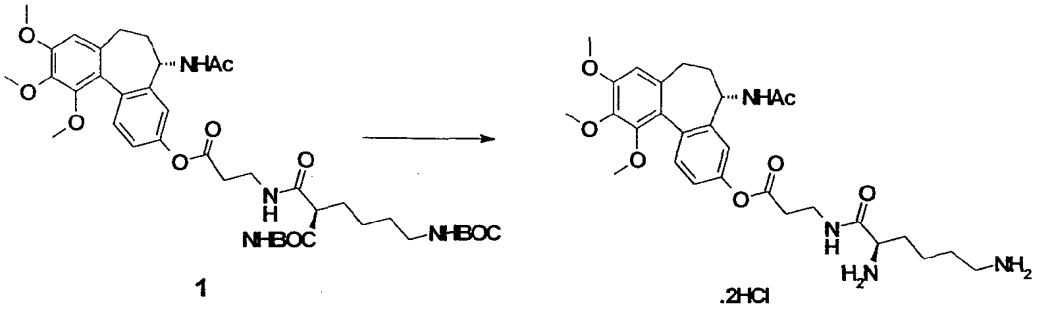
N-[3-(4-(1-piperidinyl)piperidinylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5yl]acetamide

A solution of *N*-acetyl-colchinol (300mg, 0.84mmol), (J. Cech F. Santacy Collect. Czech Comm 1949, 4, 532-539), in pyridine (5ml) was treated with 4-piperidinopiperidine carbamoyl chloride (346mg, 1.5mmol), (K.H.Henegar et al. J.Org. Chem., 1997, 62, 6588-6597) and the solution heated at reflux for 1 hour. The cooled mixture was filtered and the filtrate concentrated under reduced pressure. The residue was purified on silica gel eluting with methanol to give the title compound (180mg) as a white solid.

m.p. 168-175°C

m/e 551

Example 4



A solution of (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl 3-{[(2*R*)-2,6-di(*tert*butoxycarbonylamino)hexanoyl]amino}propanoate (1) (0.123 g ; 0.162 mmol) in dichloromethane (10 ml) was treated with a 4.8M solution of hydrogen chloride in ether (170 µl ; 0.81 mmol). The mixture was stirred at ambient temperature for 1 hour and the resulting precipitate was filtered, washed with ether and dried to give **(5*S*)-5-(acetylamino)-9,10,11-**

trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl 3-{[(2*R*)-2,6-diaminohexanoyl]amino}propanoate as a white solid.

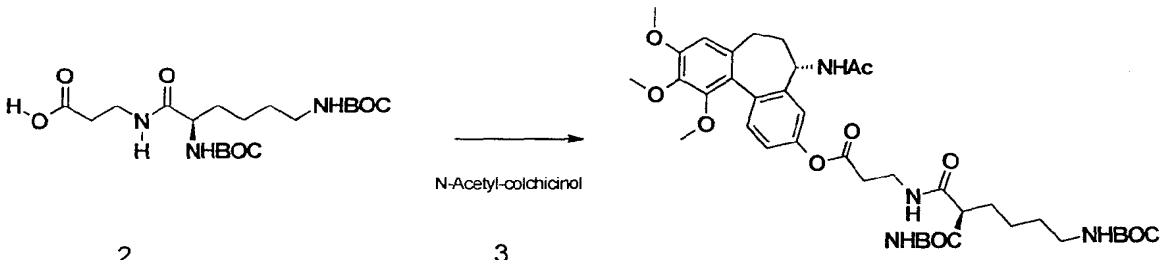
Yield : 84%

^{1}H NMR spectrum ($DMSOd_6$) : 1.39 (m, 2H) ; 1.58 (m, 2H) ; 1.74 (m, 2H) ; 1.89 (s, 3H) ; 1.89 (m, 1H) ; 2.02 (m, 1H) ; 2.15 (m, 1H) ; 2.5 (m, 1H, signal obscured partially by DMSO peak) ; 2.74 (m, 2H) ; 2.84 (t, 2H) ; 3.52 (m, 5H) ; 3.78 (s, 3H) ; 3.78 (m, 1H) ; 3.84 (s, 3H) ; 4.55 (m, 1H) ; 6.80 (s, 1H) ; 7.1-7.15 (m, 2H) ; 7.35 (dd, 1H) ; 8.01 (br s, 2H) ; 8.32 (m, 2H) ; 8.53 (d, 1H) ; 8.96 (t, 1H).

MS-ESI : 557 $[MH]^+$

Elemental analysis :	Found	C 53.8	H 6.8	N 8.7
$C_{29}H_{40}N_4O_7$; 0.8 H_2O, 2 HCl	Requires	C 53.9	H 7.2	N 8.1%

The starting material was prepared as follows:



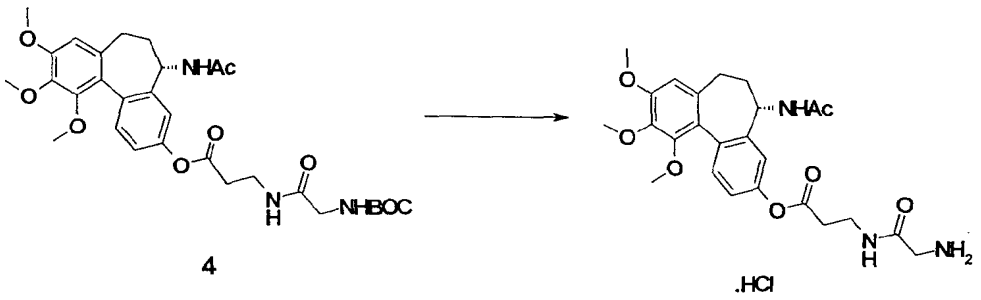
2 3 1

A mixture of 3-{[(2*R*)-2,6-di(*tert*butoxycarbonylamino)hexanoyl]amino}propanoic acid (2) (0.178 g ; 0.5 mmol), DCCI (0.124 g ; 0.6 mmol), DMAP (0.013 g ; 0.1mmol) and *N*-acetyl-colchicinol (0.25 g ; 1.2 mmol) in dichloromethane was stirred under argon atmosphere at ambient temperature for 5 hours. After filtration of the insoluble material the residue was purified by flash chromatography eluting with dichloromethane/ethanol (95/5) to give (1).

Yield : 32%

^{1}H NMR spectrum ($DMSOd_6$) : 1.15-1.6 (m, 6H) ; 1.36 (s, 18H) ; 1.87 (s, 3H) ; 1.87 (m, 1H) ; 2.05 (m, 1H) ; 2.15 (m, 1H) ; 2.50 (m, 1H, signal obscured partially by DMSO peak) ; 2.75 (t, 2H) ; 2.86 (m, 2H) ; 3.85 (m, 2H) ; 3.51 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 3.85 (m, 1H) ; 4.55 (m, 1H) ; 3.7-3.8 (m, 2H) ; 6.8 (s, 1H) ; 7.08 (s, 1H) ; 7.1 (m, 1H) ; 7.32 (dd, 1H) ; 8.01 (t, 1H) ; 8.35 (d, 1H).

Example 5



A solution of (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl 3-[(2-*tert*butoxycarbonylaminoacetyl)amino]propanoate (4) (0.36 g ; 0.61 mmol) in dichloromethane (5 ml) was treated with a 4.8M solution of hydrogen chloride in ether (1 ml). The mixture was stirred at ambient temperature for 1 hour. After dilution with ether, the resulting precipitate was filtered, washed with ether and dried to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl 3-[(2-aminoacetyl)amino]propanoate.**

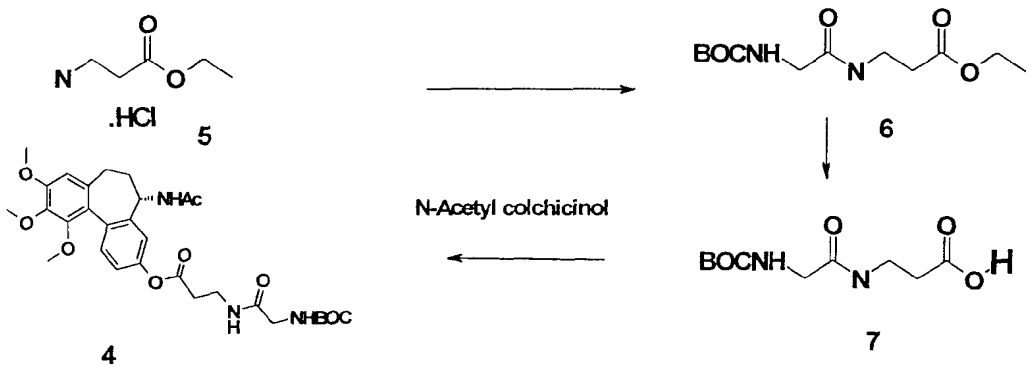
Yield : 98%

^{1}H NMR spectrum ($DMSOd_6$) : 1.90 (m, 1H) ; 1.90 (s, 3H) ; 2.05 (m, 1H) ; 2.3 (m, 1H) ; 2.9 (m, 1H, signal obscured partially by DMSO peak) ; 2.84 (t, 2H) ; 3.52 (s, 3H) ; 3.52 (m, 2H) ; 3.6 (m, 2H, signal obscured partially by H_2O peak) ; 3.8 (s, 3H) ; 3.86 (s, 3H) ; 4.55 (m, 1H) ; 6.82 (s, 1H) ; 7.13 (m, 2H) ; 7.37 (dd, 1H) ; 8.1 (br s, 2H) ; 8.46 (d, 1H) ; 8.67 (t, 1H).

MS-ESI : 486 $[MH]^+$

Elemental analysis :	Found	C 54.8	H 6.2	N 7.7
$C_{25}H_{31}N_3O_7$; 0.8 H_2O, 1.3 HCl	Requires	C 54.8	H 6.3	N 7.6%

The starting material was prepared as follows:



A solution of β-alanine ethyl ester hydrochloride salt (5) (3.07 g ; 0.02 mmol), *N*-(*tert*butoxycarbonyl)glycine (3.5 g ; 0.02 mmol), DCCI (4.12 g ; 0.02mmol) and 4-methylmorpholine (2.2 ml) in dichloromethane (60 ml) was stirred overnight under argon atmosphere at ambient temperature. After filtration the residue was purified by flash chromatography eluting with dichloromethane/ethanol (96/4) to give ethyl 3-[(2-*tert*butoxycarbonylaminoacetyl)amino]propanoate (6).

Yield : 62%

^{1}H NMR spectrum ($CDCl_3$) : 1.27 (t, 3H) ; 1.45 (s, 9H) ; 3.55 (m, 2H) ; 3.77 (d, 2H) ; 4.15 (q, 2H) ; 5.3 (br s, 2H) ; 6.56 (br s, 2H).

A solution of (6) at 0°C (3.43 g ; 0.012 mmol) in methanol (40 ml) was treated with 2N sodium hydroxide (6.9 ml ; 0.013 mmol). The mixture was stirred at ambient temperature for 90 minutes. After evaporation of the methanol and removal of the insoluble material by filtration, the solution was adjusted to pH5 with 6N hydrochloric acid. The mixture was extracted with dichloromethane and the organic layer evaporated to dryness to give 3-[(2-*tert*butoxycarbonylaminoacetyl)amino]propanoic acid (7).

Yield : 16%

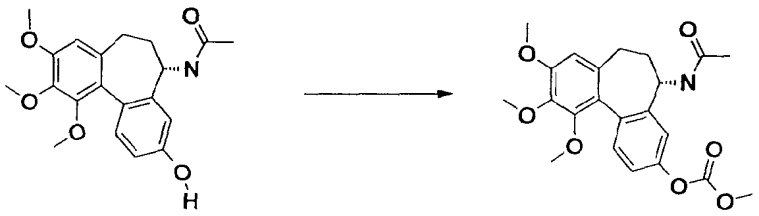
^{1}H NMR spectrum ($CDCl_3$ + CD_3CO_2D) : 1.44 (s, 9H) ; 2.61 (t, 2H) ; 3.5 (m, 2H) ; 3.80 (m, 2H).

A mixture of *N*-acetyl-colchicinol (0.357 g ; 1 mmol), DCCI (0.248 g ; 1.2 mmol), DMAP (0.025 g ; 0.2 mmol) and (7) (0.246 g ; 1 mmol) in dichloromethane (7 ml) was stirred under argon atmosphere for 5 hours. After removal of the insoluble material by filtration the residue was purified by flash chromatography eluting with dichloromethane/ethanol (92/8) to give (4) as a foam.

Yield : 61%

^{1}H NMR spectrum ($DMSOd_6$) : 1.4 (s, 9H) ; 1.89 (s, 3H) ; 1.89 (m, 1H) ; 2.07 (m, 1H) ; 2.18 (m, 1H) ; 2.5 (m, 1H, signal obscured partially by DMSO peak) ; 2.77 (t, 2H) ; 3.45 (m, 2H) ; 3.52 (s, 3H) ; 3.5 (m, 2H) ; 3.8 (s, 3H) ; 3.85 (s, 3H) ; 4.55 (m, 1H) ; 6.81 (s, 1H) ; 6.97 (t, 1H) ; 7.11 (m, 2H) ; 7.35 (dd, 1H) ; 8.01 (t, 1H) ; 8.38 (d, 1H).

Example 6



Triethylamine (70 µl ; 0.5 mmol) and methyl chloroformate (39 µl ; 0.5 mmol)were added to a solution of *N*-acetyl-colchicinol (0.178 g ; 0.5 mmol) in THF (3 ml). The mixture was stirred at ambient temperature for 90 minutes. After removal of the insoluble material by filtration the residue was purified by flash chromatography eluting with dichloromethane/ethanol (98/2) to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl methyl carbonate**.

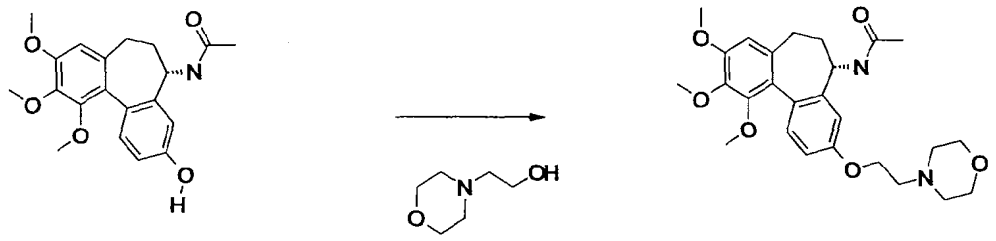
Yield : 75%

^{1}H NMR spectrum ($DMSOd_6$) : 1.87 (s, 3H) ; 1.87 (m, 1H) ; 2.05 (m, 1H) ; 2.17 (m, 1H) ; 2.5 (m, 1H, signal obscured partially by DMSO peak) ; 3.52 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 3.85 (s, 3H) ; 4.52 (m, 1H) ; 6.8 (s, 1H) ; 7.16-7.18 (m, 2H) ; 7.36 (dd, 1H) ; 8.38 (d, 1H).

MS-ESI : 438 $[MH]^+$

Elemental analysis :	Found	C 61.7	H 6.2	N 3.3
$C_{22}H_{25}NO_7$; 0.7 H_2O	Requires	C 61.6	H 6.2	N 3.4%

Example 7



DEAD (0.118 g ; 0.75 mmol), triphenylphosphine (0.196 g ; 0.75 mmol) and 4-(2-hydroxyethyl)morpholine (61 µl ; 0.5 mmol) were added to a solution of *N*-acetyl-colchicinol (0.178 g ; 0.5 mmol) in dichloromethane (5 ml) under argon atmosphere. The mixture was stirred at ambient temperature for 6 hours. After evaporation the residue was purified by flash chromatography eluting with a gradient of 2-10% ethanol/dichloromethane to give ***N*-[(5*S*)-3-(2-morpholinoethoxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide.**

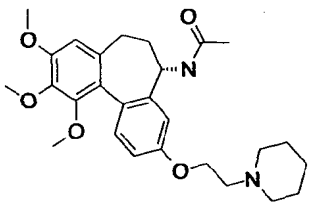
Yield : 37%

^{1}H NMR spectrum ($DMSOd_6$) : 1.85 (m, 1H) ; 1.87 (s, 3H) ; 2.05 (m, 1H) ; 2.15 (m, 1H) ; 2.39 (m, 2H) ; 2.5 (m, 3H, signal obscured partially by DMSO peak) ; 2.72 (t, 2H) ; 3.46 (s, 3H) ; 3.54-3.6 (s, 4H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.09-4.12 (m, 2H) ; 4.55 (m, 1H) ; 6.76 (s, 1H) ; 6.86-6.90 (m, 2H) ; 7.23 (dd, 2H) ; 8.35 (d, 1H).

MS-ESI : 471 $[MH]^+$

Elemental analysis :	Found	C 66.4	H 7.3	N 6.0
$C_{26}H_{34}N_2O_6$	Requires	C 66.6	H 7.3	N 6.3%

<u>Example 8</u>



Using an analogous procedure to that described for Example 7, *N*-acetyl-colchicinol was reacted with 4-(2-hydroxyethyl)piperidine to give ***N*-[(5*S*)-3-(2-piperidinoethoxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide.**

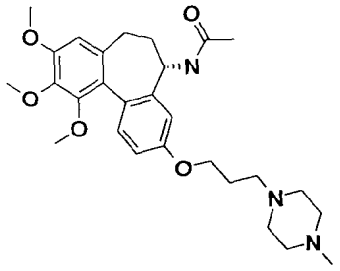
Yield : 20%

^{1}H NMR spectrum ($DMSOd_6$) : 1.39 (m, 2H) ; 1.49 (m, 4H) ; 1.80 (m, 1H) ; 1.88 (s, 3H) ; 2.05 (m, 1H) ; 2.15 (m, 1H) ; 2.45 (m, 4H) ; 2.5 (m, 1H, signal obscured partially by DMSO peak) ; 2.67 (m, 2H) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.08 (t, 2H) ; 4.55 (m, 1H) ; 6.76 (s, 1H) ; 6.86-6.9 (m, 2H) ; 7.22 (dd, 1H) ; 8.35 (d, 1H).

MS-ESI : 469 $[MH]^+$

Elemental analysis :	Found	C 68.7	H 7.8	N 5.9
$C_{27}H_{36}N_2O_5$; 0.2 H_2O	Requires	C 68.5	H 8.0	N 6.1%

Example 9



Using an analogous procedure to that described for Example 7, *N*-acetyl-colchicinol was reacted with 4-(3-hydroxypropyl)-1-methylpiperazine to give ***N*-[(5*S*)-3-(3-(4-methylpiperazin-1-yl)propoxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide.**

Yield : 22%

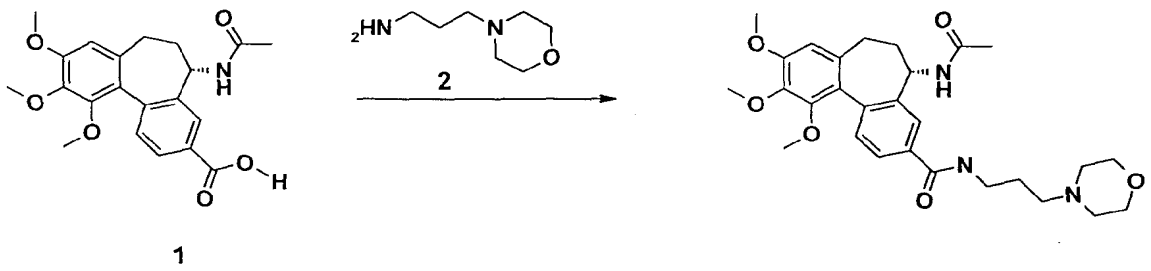
^{1}H NMR spectrum (DMSO d_6) : 1.88 (s, 3H) ; 1.85-1.9 (m, 3H) ; 2.04-2.16 (m, 5H) ; 2.32-2.53 (m, 11H, signals obscured partially by DMSO peak) ; 3.47 (s, 3H) ; 3.78 (s, 3H) ; 3.83 (s, 3H) ; 4.03 (t, 2H) ; 4.55 (m, 1H) ; 6.72 (s, 1H) ; 6.85 (dd, 1H) ; 6.9 (m, 1H) ; 7.23 (d, 1H) ; 8.23 (d, 1H).

MS-ESI : 498 $[MH]^+$

Elemental analysis :	Found	C 64.8	H 8.0	N 8.1
$C_{28}H_{39}N_3O_5$; 0.8 H_2O,	Requires	C 64.7	H 7.7	N 8.2%

0.1 dichloromethane

Example 10



1

A solution of *N*-[(5*S*)-3-carboxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (0.3 g ; 0.779 mmol), (Med. Chem. Res. 1991, 142), DCCI (0.322 g ; 1.55 mmol), DMAP (0.069 g ; 0.389mmol) and 4-(3-aminopropyl)morpholine (170 µl ; 1.17 mmol) in dichloromethane (6 ml) was stirred under argon atmosphere overnight. After removal of the insoluble material by filtration, the residue was purified on reverse phase silica eluting with a gradient of 40-50% methanol/ammonium carbonate buffer (2 g/l, pH7). The appropriate fractions were evaporated to dryness and triturated in ether to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-*N*-(3-morpholinopropyl)-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cycloheptene-3-carboxamide** as a white solid.

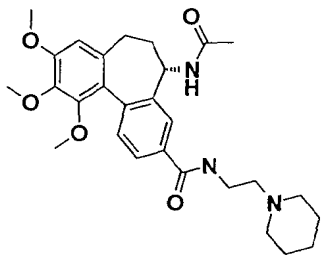
Yield : 30%

^{1}H NMR spectrum ($DMSOd_6$) : 1.7 (m, 2H) ; 1.91 (s, 3H) ; 1.91 (m, 1H) ; 2.05 (m, 1H) ; 2.2 (m, 1H) ; 2.37 (m, 6H) ; 2.5 (m, 3H, signal obscured partially by DMSO peak) ; 3.51 (s, 3H) ; 3.58 (m, 4H) ; 3.8 (s, 3H) ; 3.86 (s, 3H) ; 4.58 (m, 1H) ; 6.83 (s, 1H) ; 7.39 (dd, 1H) ; 7.74 (m, 1H) ; 7.84 (s, 1H) ; 8.51 (m, 2H).

MS-ESI : 512 $[MH]^+$

Elemental analysis :	Found	C 64.8	H 7.3	N 8.1
$C_{28}H_{37}N_3O_6$; 0.4 H_2O	Requires	C 64.5	H 7.3	N 8.0%

Example 11



Using an analogous procedure to that described for Example 10, *N*-[(5*S*)-3-carboxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide was reacted with 1-(2-aminoethyl)piperidine to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-*N*-(2-piperidinoethyl)-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cycloheptene-3-carboxamide**.

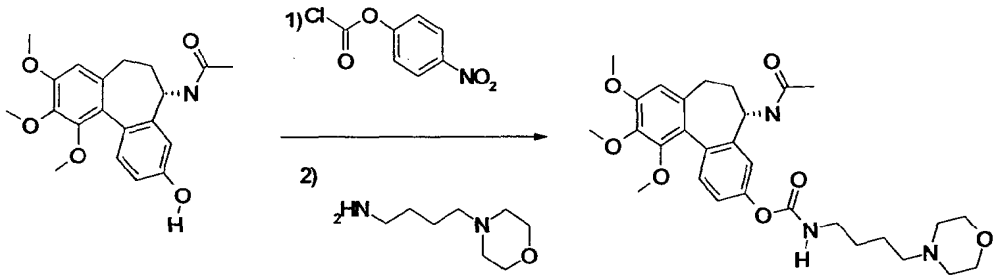
Yield : 43%

^{1}H NMR spectrum ($DMSOd_6$) : 1.38 (m, 2H) ; 1.49 (m, 4H) ; 1.89 (s, 3H) ; 1.89 (m, 1H) ; 2.05 (m, 1H) ; 2.18 (m, 1H) ; 2.4-2.5 (m, 4H) ; 2.5 (m, 1H, signal obscured partially by DMSO peak) ; 3.38 (m, 4H) ; 3.49 (s, 3H) ; 3.79 (s, 3H) ; 3.84 (s, 3H) ; 4.58 (m, 1H) ; 6.81 (s, 1H) ; 7.37 (d, 1H) ; 7.71 (m, 1H) ; 7.81 (s, 1H) ; 8.35 (t, 1H) ; 8.49 (d, 1H).

MS-ESI : 496 $[MH]^+$

Elemental analysis :	Found	C 66.4	H 7.6	N 8.3
$C_{28}H_{37}N_3O_5$; 0.6 H_2O	Requires	C 66.1	H 7.7	N 8.3%

Example 12



A solution of *N*-acetyl-colchicinol (0.357 g ; 1 mmol), 4-nitrophenyl chloroformate (0.262g; 1.3 mmol) and triethylamine (182 µl ; 1.3 mmol) in dichloromethane (10 ml) was stirred, under argon atmosphere, at ambient temperature for 90 minutes. 4-(4-Aminobutyl)morpholine (0.237g ; 1.5 mmol) was then added and the mixture was further

stirred for 4 hours. After evaporation to dryness the residue was purified by flash chromatography eluting with a gradient of 0-12% ethanol/dichloromethane to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl *N*-(4-morpholinobutyl)carbamate** as a white foam.

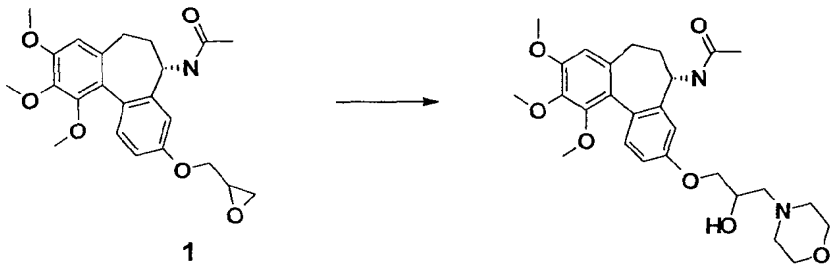
Yield : 24%

^{1}H NMR spectrum ($DMSOd_6$) : 1.49 (m, 4H) ; 1.86 (s, 3H) ; 1.86 (m, 1H) ; 2.05 (m, 1H) ; 2.18 (m, 1H) ; 2.26-2.34 (m, 6H) ; 2.5 (m, 1H, signal obscured partially by DMSO peak) ; 3.08 (m, 2H) ; 3.51 (s, 3H) ; 3.57 (m, 4H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.55 (m, 1H) ; 6.79 (s, 1H) ; 7.02 (m, 2H) ; 7.29 (d, 1H) ; 7.78 (t, 1H) ; 8.39 (d, 1H).

MS-ESI : 542 $[MH]^+$

Elemental analysis :	Found	C 63.5	H 7.3	N 7.7
$C_{29}H_{39}N_3O_7$; 0.4 H_2O	Requires	C 63.4	H 7.3	N 7.7%

Example 13



A solution of *N*-[(5*S*)-3-(2,3-epoxypropoxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (1) (0.092 g ; 0.22 mmol) and morpholine (40 µl ; 0.44 mmol) in methanol was heated at reflux for 4 hours. After evaporation to dryness the residue was purified by flash chromatography eluting with dichloromethane/ethanol (90/10) to give ***N*-[(5*S*)-3-(2-hydroxy-3-morpholinopropoxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide** as a yellow foam.

Yield : 55%

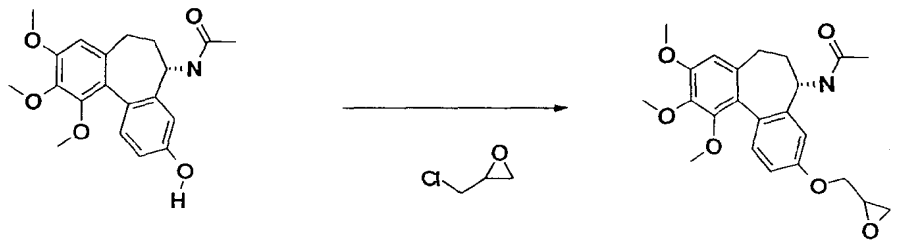
^{1}H NMR spectrum ($DMSOd_6$) : 1.88 (s, 3H) ; 1.88 (m, 1H) ; 2.05 (m, 1H) ; 2.15 (m, 1H) ; 2.42-2.5 (m, 4H) ; 2.5 (m, 1H, signal obscured partially by DMSO peak) ; 3.33-3.4 (m, 4H) ;

3.46 (s, 3H) ; 3.57 (m, 2H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 3.90 (m, 1H) ; 3.99 (m, 2H) ; 4.52 (m, 1H) ; 4.9 (t, 1H) ; 6.76 (s, 1H) ; 6.86-6.9 (m, 2H) ; 7.23 (d, 1H) ; 8.37 (d, 1H).

MS-ESI : 501 $[MH]^+$

Elemental analysis :	Found	C 64.8	H 7.3	N 5.6
$C_{27}H_{36}N_2O_7$	Requires	C 64.5	H 7.5	N 5.5%

The starting material was prepared as follows:



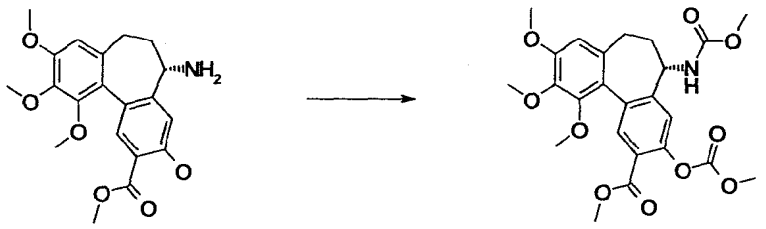
1

A solution of *N*-acetyl-colchicinol (0.179 g ; 0.5 mmol), potassium carbonate (0.083g; 0.6 mmol) and epichlorohydrin (0.059 g ; 0.75 mmol) in DMF (2 ml) was heated at 80°C for 5 hours. The mixture was poured into saturated sodium hydrogen carbonate and extracted with ethyl acetate. The organic phase was washed with brine, dried ($MgSO_4$) and evaporated to give an oil which was purified by flash chromatography eluting with a 2-4% gradient of ethanol/dichloromethane to give (1).

Yield : 46%

^1H NMR spectrum ($DMSOd_6$) : 1.88 (s, 3H) ; 1.84 'm, 1H) ; 2.05 (m, 1H) ; 2.15 (m, 1H) ; 2.5 (m, 1H partially obscured by DMSO peak) ; 2.75 (m, 1H) ; 2.88 (m, 1H) ; 3.42 (m, 1H) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 3.87 (m, 1H) ; 4.35 (m, 1H) ; 4.52 (m, 1H) ; 6.76 (s, 1H) ; 6.88-6.94 (m, 2H) ; 7.24 (d, 1H) ; 8.35 (d, 1H).

Example 14



1

A solution of methyl (5*S*)-9,10,11-trimethoxy-5-amino-3-hydroxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cycloheptene-2-carboxylate (1) (0.373 g; 1 mmol) methyl chloroformate (0.17 ml ; 2.2 mmol) and triethylamine was stirred at ambient temperature overnight. After evaporation to dryness, the residue was purified by flash chromatography, eluting with ethanol/dichloromethane (2/98) and further purified by preparative HPLC on reverse phase silica eluting with methanol/ammonium carbonate buffer (2 g/l pH7) (50/50) to give **methyl (5*S*)-9,10,11-trimethoxy-5-[(methoxycarbonyl)amino]-3-[(methoxycarbonyl)oxy]-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cycloheptene-2-carboxylate**.

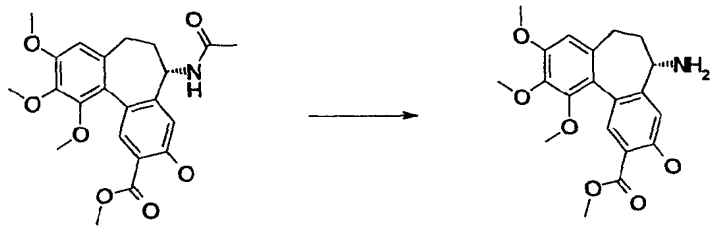
Yield : 48 %

^{1}H NMR spectrum ($DMSOd_6$) : 1.84-2.09 (m, 2H) ; 2.19-2.31 (m, 1H) ; 2.57 (m,1H, partially obscured by DMSO peak) ; 3.50 (s, 3H) ; 3.55 (s, 3H) ; 3.81 (s, 3H) ; 3.82 (s, 3H) ; 3.86 (s, 3H) ; 3.87 (s, 3H) ; 4.28-4.389 (m, 1H) ; 6.85 (s, 1H) ; 7.24 (s, 1H) ; 7.88-7.97 (m, 1H) ; 7.91 (s, 1H).

MS-ESI : 512 $[MNa]^+$

Elemental analysis	Found	C 58.7	H 5.7	N 3.0	
$C_{24}H_{27}NO_{10}$	Requires	C 58.9	H 5.6	N 2.9%	

The starting material was prepared as follows:

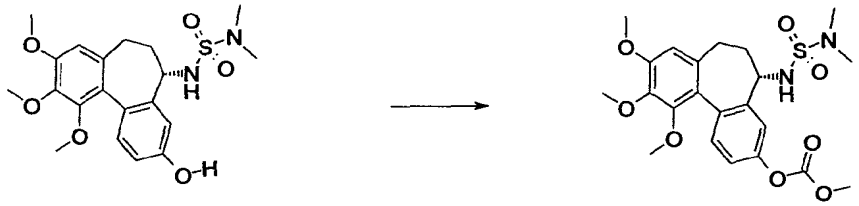


2 1

A solution of methyl (5*S*)-9,10,11-trimethoxy-5-acetylamino-3-hydroxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cycloheptene-2-carboxylate (2) (Collect. Czech. Chem. Communi. 64, 217 (1999)) in a mixture of 6N hydrochloric acid and methanol (30/70) was heated at reflux for 8 hours. The mixture was adjusted to pH8 by addition of sodium carbonate. Extraction with dichloromethane and purification by flash chromatography (elution with dichloromethane /methanol (94/6)) gave (1) as a foam.

^{1}H NMR spectrum (DMSOd_6) : 1.61 (m, 1H) ; 2.04 (m, 1H) ; 2.28 (m, 1H) ; 2.45 (m, 1H) ; 3.50 (s, 3H) ; 3.54 (m, 1H) ; 3.77 (s, 3H) ; 3.83 (s, 3H) ; 3.90 (s, 3H) ; 6.77 (s, 1H) ; 7.30 (s, 1H) ; 7.73 (s, 1H) ; 1.57 (br s, 1H).

Example 15



1

A solution of (5*S*)-5-{[(dimethylamino)sulphonyl]amino}-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-ol (1) (0.9 ; 0.71 mmol), methyl chloroformate (0.061 ml ; 0.782 mmol) and triethylamine (0.109 ml ; 0.782 mmol) in acetonitrile (8 ml) was stirred under argon atmosphere at 40°C for 4 hours. After evaporation to dryness, the residue was purified by flash chromatography eluting with ethanol/dichloromethane (2/98) to give **(5*S*)-5-{[(dimethylamino)sulphonyl]amino}-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl methyl carbonate**.

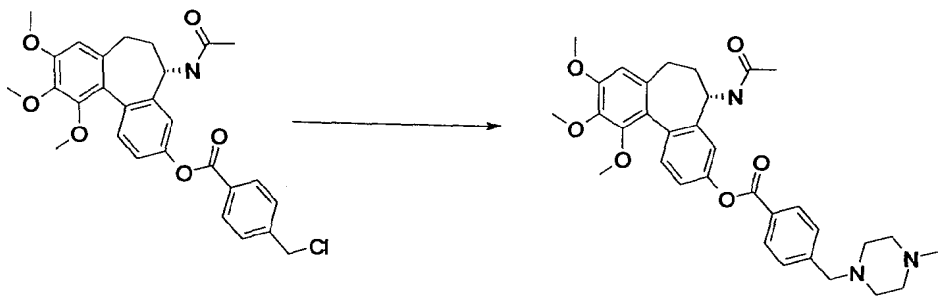
Yield : 32 %

^{1}H NMR spectrum (DMSOd_6) : 1.93-2.03 (m, 2H) ; 2.12-2.17 (m, 1H) ; 2.46 (s, 6H) ; 2.45-2.55 (m, 1H) ; 3.46 (s, 3H) ; 3.79 (s, 3H) ; 3.85 (s, 3H) ; 3.87 (s, 3H) ; 3.94-4.10 (m, 1H) ; 6.82 (s, 1H) ; 7.21 (d, 1H) ; 7.37 (d, 1H) ; 7.43 (s, 1H) ; 7.93 (br s, 1H).

MS-ESI : 503 $[MNa]^+$

Elemental analysis	Found	C 55.2	H 6.1	N 5.8	S 6.3	
$C_{22}H_{28}N_2O_8S$	Requires	C 55.0	H 5.9	N 5.8	S 6.7%	

Example 16



1

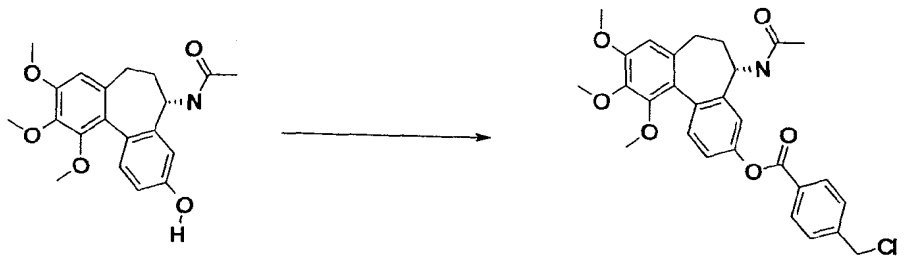
A solution of *N*-[(5*S*)-3-(4-chloromethylphenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (1) (0.308 g ; 0.604 mmol), 1-methylpiperazine (0.088 ml ; 0.785 mmol) and sodium iodide (0.02 g ; 0.121 mmol) in acetonitrile (10 ml) was stirred under argon atmosphere overnight. After evaporation to dryness, the residue was purified by flash chromatography eluting with a 5-12 % gradient of methanol/dichloromethane. After evaporation of the appropriate fractions, the solid was triturated in ether/pentane to give ***N*-[(5*S*)-3-(4-{4-methylpiperazin-1-ylmethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide** as a solid.

Yield : 75 %

^{1}H NMR spectrum ($DMSOd_6$) : 1.86 (s, 3H) ; 1.83-1.98 (m, 1H) ; 2.00-2.26 (m, 2H) ; 2.31 (br s, 3H) ; 2.4-2.6 (m, 8H) ; 2.53-2.59 (m, 1H) ; 3.54 (s, 3H) ; 3.62 (s, 2H) ; 3.80 (s, 3H) ; 3.85 (s, 3H) ; 4.53-4.64 (m, 1H) ; 6.82 (s, 1H) ; 7.20-7.25 (m, 2H) ; 7.41 (d, 1H) ; 7.56 (d, 2H) ; 8.13 (d, 2H) ; 8.39 (d, 1H).

MS-ESI : 574 $[MH]^+$

The starting material was prepared as follows

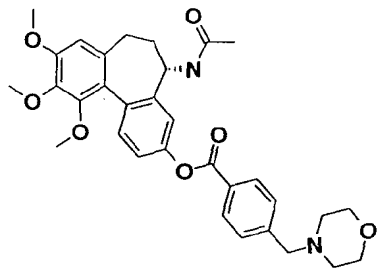


A solution of *N*-acetyl-colchicinol (0.357 g ; 1 mmol), EDCI (0.23 g ; 1.2 mmol), DMAP (0.025 g ; 0.2 mmol) and 4-chloromethylbenzoic acid (0.205 g ; 1.2 mmol) in dichloromethane (8 ml) was stirred under argon atmosphere overnight. After evaporation to dryness, the residue was purified by flash chromatography eluting with dichloromethane/ethanol (98/2) to give (1).

Yield : 72 %

^{1}H NMR spectrum ($DMSOd_6$) : 1.86 (s, 3H) ; 1.91 (m, 1H) ; 1.04-2.14 (m, 1H) ; 2.14-2.67 (m, 1H) ; 2.57 (m ,1H;, partially obscured by DMSO peak) ; 3.54 (s, 3H) ; 3.80 (s, 3H) ; 3.86 (s, 3H) ; 4.54-4.64 (m, 1H) ; 4.91 (s, 2H) ; 6.83 (s, 1H) ; 7.21-7.28 (m, 2H) ; 7.42 (d, 1H) ; 7.70 (d, 2H) ; 8.14 (d, 2H) ; 8.40 (d, 1H).

Example 17



Using an analogous procedure to that described for Example 16, *N*-[(5*S*)-3-(4-chloromethylphenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide was reacted with morpholine to give ***N*-[(5*S*)-3-(4-{morpholinomethyl}phenylcarbonyloxy)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide.**

Yield : 86 %

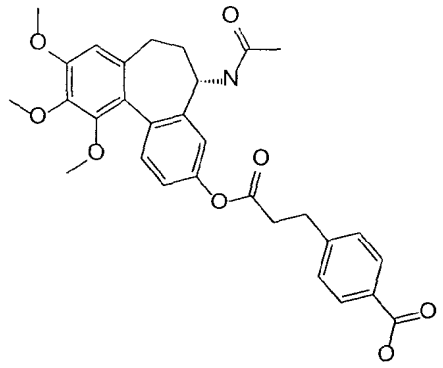
^{1}H NMR spectrum ($DMSOd_6$) : 1.90 (s, 3H) ; 1.88-2.01 (m, 1H) ; 2.06-2.30 (m, 2H) ; 2.43 (br s, 4H) ; 2.54-2.63 (m, 1H) ; 3.30 (m, 2H) ;3.58 (s, 3H) ; 3.62-3.67 (m, 6H) ; 3.84 (s, 3H) ; 3.89 (s, 3H) ; 4.57-4.67 (m, 1H) ; 3.86 (s, 1H) ; 7.23-7.30 (m, 2H) ; 7.45 (d, 1H) ; 7.61 (d, 1H) ; 8.17 (d, 1H) ; 8.43 (d, 1H).

MS-ESI : 561 $[MH]^+$

Elemental analysis	Found	C 66.1	H 6.5	N 4.9
$C_{32}H_{36}N_2O_7$; 0.3 dichloromethane	Requires	C 66.2	H 6.3	N 4.8%

Example 18

CHIRAL



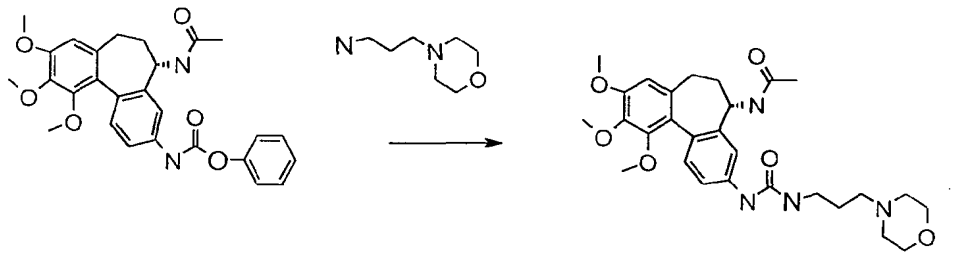
A solution of *N*-acetyl-colchicinol (0.357 g ; 1 mmol), EDCI (0.23 g ; 1.2 mmol), DMAP (0.025 g ; 0.2 mmol) and 3-(4-carboxyphenyl)propionic acid (0.233 g ; 1.2 mmol) was stirred at ambient temperature overnight. After removal of the solvent by evaporation, the residue was purified by preparative HPLC on reverse phase silica eluting with methanol/ammonium carbonate buffer (2 g/l pH7) (50/50) to give **4-(3-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]oxy-3-oxopropyl)benzoic acid**.

Yield :70%

^{1}H NMR spectrum ($DMSOd_6$) : 1.82-1.93 (m, 4H) ; 1.97-2.22 (m, 2H) ; 2.39-2.63 (m, 1H) ; 2.93-2.99 (m, 2H) ; 2.99-3.06 (m, 2H) ; 3.51 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 3.47-3.56 (m, 1H) ; 6.89 (s, 1H) ; 6.94 (d, 1H) ; 6.99 (dd, 1H) ; 7.30-7.37 (m, 3H) ; 7.85 (d, 2H) ; 8.46 (d, 1H).

MS-ESI : 534 $[MH]^+$

Example 19



1

A solution of *N*-[(5*S*)-3-phenoxycarbonylamino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (1) (0.1 g ; 0.21 mmol) and 4-(3-aminopropyl)morpholine (0.095 g ; 0.66 mmol) in DMSO (1 ml) was stirred at ambient temperature for 1 hour. The mixture was purified by preparative HPLC on reverse phase silica eluting with methanol/ammonium carbonate buffer (2 g/l, pH7) (40/60) to give, after evaporation, ***N*-[(5*S*)-9,10,11-trimethoxy-3-([(3-morpholinopropyl)amino]carbonylamino)-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide** as a foam.

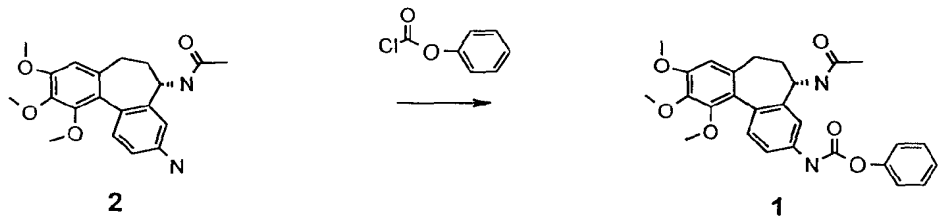
Yield : 84%

^{1}H NMR spectrum ($DMSOd_6$) : 1.6 (m, 2H) ; 1.88 (s, 3H) ; 1.90 (m, 1H) ; 2.0-2.2 (m, 2H) ; 2.3-2.4 (m, 6H) ; 2.45 (m, 1H, signal obscured by DMSO peak) ; 3.15 (m, 2H) ; 3.47 (s, 3H) ; 3.6 (m, 4H) ; 3.78 (s, 3H) ; 3.83 (d, 3H) ; 4.47 (m, 1H) ; 6.13 (t, 1H) ; 6.76 (s, 1H) ; 7.16 (d, 1H) ; 7.29 (d, 1H) ; 7.37 (dd, 1H) ; 8.37 (d, 1H) ; 8.47 (s, 1H).

MS-ESI : 527 $[MH]^+$

Elemental analysis :	Found	C 63.4	H 7.4	N 10.6
$C_{28}H_{38}N_4O_6$; 0.1 H_2O	Requires	C 63.6	H 7.3	N 10.6%

The starting material was prepared as follows:



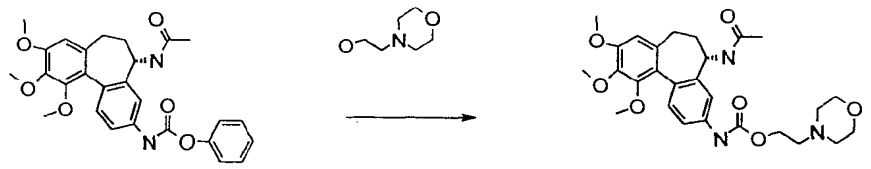
2 1

Pyridine (570 µl ; 7 mmol) and phenyl chloroformate (720 µl ; 10.3 mmol) were added to a solution of *N*-[(5*S*)-3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (2) cooled at 0°C (2 g ; 5.6 mmol) in THF (40 ml), under argon atmosphere. The mixture was stirred at 0°C for 5 minutes and then at ambient temperature for 1 hour. The mixture was extracted with ethyl acetate. The organic phase was washed with 1M hydrochloric acid, saturated sodium hydrogen carbonate and brine. After evaporation to dryness the solid was triturated with ether and hexane to give (1) as a solid.

Yield : 89%

^{1}H NMR spectrum ($DMSOd_6$) : 1.82 (s, 3H) ; 1.85 (m, 1H) ; 2.10 (m, 2H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 3.47 (s, 3H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.48 (m, 1H) ; 6.77 (s, 1H) ; 7.20-7.50 (m, 7H) ; 7.55 (s, 1H) ; 8.38 (d, 1H) ; 9.31 (s, 1H).

Example 20



1

Using an analogous procedure to that described for Example 19, *N*-[(5*S*)-3-phenoxycarbonylamino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (1) was reacted with 4-(2-hydroxyethyl)morpholine and the mixture was heated at 60°C for 2 hours to give ***N*-[(5*S*)-3-(2-morpholinoethoxycarbonylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide**.

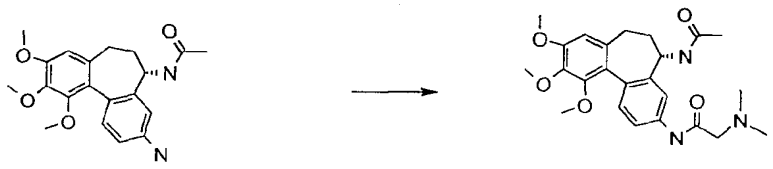
Yield : 84%

^{1}H NMR spectrum ($DMSOd_6$) : 1.89 (s, 3H) ; 1.90 (m, 1H) ; 2.0-2.2 (m, 2H) ; 2.4-2.45 (m, 4H) ; 2.46 (m, 1H, signal obscured by DMSO peak) ; 2.6 (t, 2H) ; 3.42 (s, 3H) ; 3.59 (m, 4H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.22 (m, 2H) ; 4.44 (m, 1H) ; 6.78 (s, 1H) ; 7.22 (d, 1H) ; 7.39 (dd, 1H) ; 7.50 (s, 1H) ; 8.39 (d, 1H) ; 9.73 (s, 1H).

MS-ESI : 514 $[MH]^+$

Elemental analysis :	Found	C 60.4	H 6.6	N 8.0
$C_{27}H_{35}N_3O_7$; 1.1 H_2O	Required	C 60.8	H 7.0	N 7.9%

Example 21



1

A solution of *N*-[(5*S*)-3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (1) (0.2 g ; 0.56 mmol), *N*,*N*-dimethylglycine (0.058 g ; 0.56 mmol), EDCI (0.14g ; 0.73 mmol) and DMAP (0.014 g ; 0.11 mmol) in dichloromethane (8 ml) was stirred at ambient temperature overnight. The mixture was washed with water and the organic phase was evaporated and purified by preparative HPLC on reverse phase silica eluting with methanol/ammonium carbonate buffer (2 g/l, pH7) (40/60) to give, after evaporation, ***N*-[(5*S*)-3-(*N*,*N*-dimethylaminoacetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide** as a white foam.

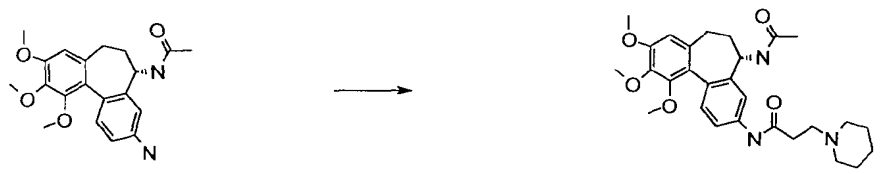
Yield : 57%

^{1}H NMR spectrum ($DMSOd_6$) : 1.90 (s, 3H) ; 1.93 (m, 1H) ; 2.0-2.2 (m, 2H) ; 2.31 (s, 6H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 3.09 (d, 1H) ; 3.10 (d, 1H) ; 3.48 (s, 3H) ; 3.79 (s, 3H) ; 3.84 (s, 3H) ; 4.5 (m, 1H) ; 6.78 (s, 1H) ; 7.25 (d, 1H) ; 7.6 (m, 2H) ; 8.4 (d, 1H) ; 9.73 (s, 1H).

MS-ESI : 442 $[MH]^+$

Elemental analysis :	Found	C 63.0	H 7.0	N 9.2
$C_{24}H_{31}N_3O_5$; 0.8 H_2O	Required	C 63.2	H 7.2	N 9.2%

Example 22



Using an analogous procedure to that described for Example 21, *N*-[(5*S*)-3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide was reacted with 1-piperidinepropionic acid to give ***N*-[(5*S*)-3-(3-piperidinopropanoylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide.**

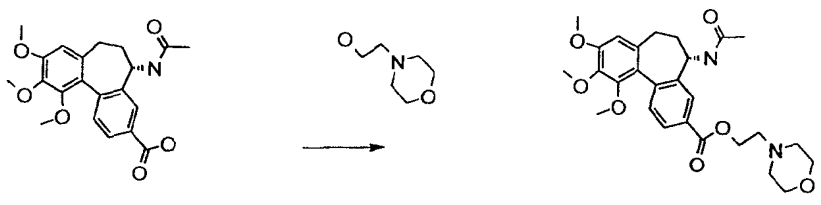
Yield : 61%

^{1}H NMR spectrum ($DMSOd_6$) : 1.40 (m, 2H) ; 1.55 (m, 4H) ; 1.89 (s, 3H) ; 1.90 (m, 1H) ; 2.0-2.2 (m, 2H) ; 2.4 (br s, 4H) ; 2.45 (m, 2H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 2.62 (m, 2H) ; 3.47 (s, 3H) ; 3.79 (s, 3H) ; 3.84 (s, 3H) ; 4.45 (m, 1H) ; 6.78 (s, 1H) ; 7.24 (d, 1H) ; 7.5 (s, 1H) ; 7.58 (dd, 1H) ; 8.40 (d, 1H).

MS-ESI : 496 $[MH]^+$

Elemental analysis :	Found	C 65.7	H 7.4	N 8.2
$C_{28}H_{37}N_3O_5$	Required	C 65.7	H 7.6	N 8.2%

Example 23



1

A solution of *N*-[(5*S*)-3-carboxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (1) (0.385 g ; 1 mmol), EDCI (0.248 g ; 1.3 mmol), DMAP (0.248 g ; 0.2 mmol) and 4-(2-hydroxyethyl)morpholine (127 µl ; 1.05 mmol) was stirred at ambient temperature overnight. After evaporation to dryness the residue was

purified by preparative HPLC on reverse phase silica eluting with a 40-60% gradient of methanol/ammonium carbonate buffer (2 g/l, pH7) to give, after evaporation, ***N*-[(5*S*)-3-(2-morpholinoethoxycarbonyl)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide** as a solid.

Yield : 47%

^{1}H NMR spectrum ($DMSOd_6$) : 1.88 (s, 3H) ; 1.88-2.05 (m, 2H) ; 2.2 (m, 1H) ; 2.5 (m, 5H, signal obscured by DMSO peak) ; 2.7 (m, 2H) ; 3.5 (s, 3H) ; 3.6 (m, 4H) ; 3.79 (s, 3H) ; 3.85 (s, 3H) ; 4.4 (m, 2H) ; 4.55 (m, 1H) ; 6.82 (s, 1H) ; 7.47 (d, 1H) ; 7.89 (dd, 1H) ; 7.95 (d, 1H) ; 8.58 (d, 1H).

MS-ESI : 499 $[MH]^+$

Elemental analysis :	Found	C 63.2	H 6.7	N 5.5
$C_{27}H_{34}N_2O_7$; 0.6 H_2O	Required	C 65.0	H 6.9	N 5.6%

Example 24

1

A solution of methyllithium in ether (1.6 M ; 2.14 ml ; 3.4 mmol) was added at -78°C under argon atmosphere to dry THF (5 ml). After 5 minutes a solution of *N*-[(5*S*)-3-(methoxycarbonyl)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (1) (0.274 g ; 0.68 mmol) in THF (11 ml) was added. The mixture was stirred at -78°C for 30 minutes, allowed to warm up and further stirred at ambient temperature for 90 minutes. After removal of the solvents by evaporation, the residue was taken up in an aqueous ammonium chloride/ethyl acetate mixture and extracted. The organic phase was evaporated and purified by flash chromatography eluting with ethyl acetate to give ***N*-[(5*S*)-3-(1-hydroxy-1-methylethyl)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide** as a white foam.

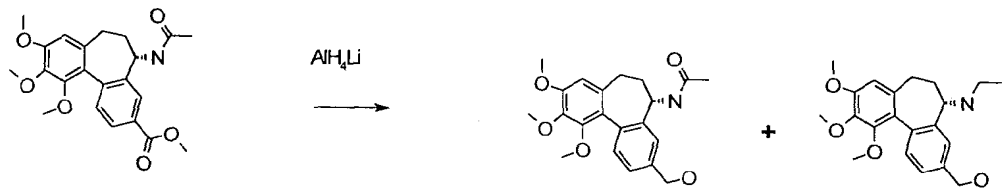
Yield : 45%

^{1}H NMR spectrum (DMSOd_6) : 1.45 (s, 3H) ; 1.48 (s, 3H) ; 1.86 (m, 1H) ; 1.88 (s, 3H) ; 2.03 (m, 1H) ; 2.15 (m, 1H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 3.5 (s, 3H) ; 3.77 (s, 3H) ; 3.83 (s, 3H) ; 6.77 (s, 1H) ; 7.23 (d, 1H) ; 7.35 (dd, 1H) ; 7.5 (d, 1H) ; 8.43 (d, 1H).

MS-ESI : 422.1 $[MNa]^+$

Elemental analysis :	Found	C 66.5	H 7.3	N 3.5
$C_{23}H_{29}NO_5$; 0.8 H_2O	Required	C 66.7	H 7.5	N 3.4%

Examples 25 and 26



A suspension of (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cycloheptene-3-carboxylate (2.28 ml ; 5.7 mmol) and lithium aluminium hydride (0.216 g ; 22.8 mmol) in a mixture of THF (10 ml) and ether (60 ml) was stirred at reflux under argon atmosphere overnight. After addition of water (60 ml), the mixture was stirred for 2 hours. The resulting solid was filtered and the filtrate was evaporated and purified by flash chromatography eluting with a 5-10% gradient of methanol/dichloromethane to give, after evaporation, ***N*-[(5*S*)-3-hydroxymethyl-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide** (yield : 33%) and **[(5*S*)-5-(ethylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]methanol** (yield : 47%).

Example 25

^{1}H NMR spectrum (DMSOd_6) : 1.86 (m, 1H) ; 1.87 (s, 3H) ; 2.01 (m, 1H) ; 2.14 (m, 1H) ; 2.48 (m, 1H, signal obscured by DMSO peak) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.83 (s, 3H) ; 4.54 (m, 3H) ; 5.21 (t, 1H) ; 6.78 (s, 1H) ; 7.27 (m, 2H) ; 7.32 (s, 1H) ; 8.42 (d, 1H).

MS-ESI : 394.1 $[MH]^+$

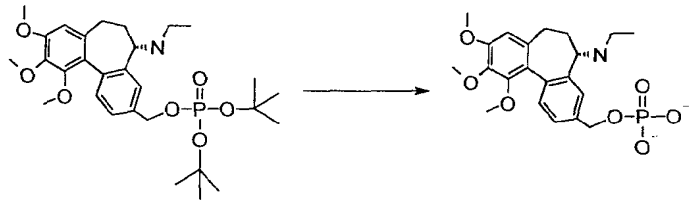
Elemental analysis :	Found	C 66.0	H 6.8	N 3.7
$C_{21}H_{25}NO_5$; 0.5 H_2O	Requires	C 66.3	H 6.9	N 3.7%

Example 26

^{1}H NMR spectrum ($DMSOd_6$) : 1.90 (s, 3H) ; 1.90 (m, 1H) ; 2.01 (m, 1H) ; 2.20 (m, 1H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 3.53 (s, 3H) ; 3.78 (s, 3H) ; 3.85 (s, 3H) ; 4.53 (m, 1H) ; 6.84 (s, 1H) ; 7.50 (d, 1H) ; 7.72 (d, 1H) ; 7.77 (dd, 1H) ; 8.45 (d, 1H).

MS-ESI : 389 $[MNa]^+$

Example 27



1

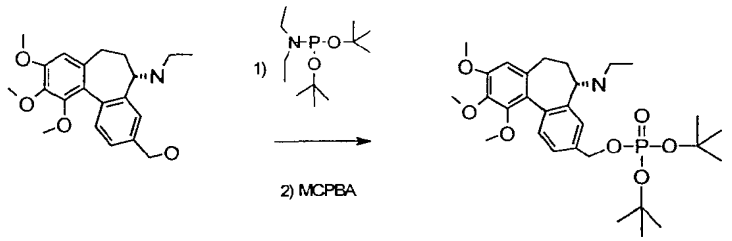
A solution of (5*S*)-5-(ethylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl di*tert*butyl phosphate (1) (0.215 g ; 0.391 mmol) in 1M hydrogen chloride solution in 1,4-dioxane (2 ml) was stirred at ambient temperature overnight. After addition of ether (20 ml) the resulting precipitate was filtered, washed with ether and dried to give **(5*S*)-5-(ethylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl dihydrogen phosphate.**

Yield : 88%

^{1}H NMR spectrum ($DMSOd_6$) : 1.04 (t, 3H) ; 1.78 (m, 1H) ; 2.0 (m, 1H) ; 2.32 (m, 1H) ; 2.5 (m, 3H, signals obscured by DMSO peak) ; 3.48 (s, 3H) ; 3.5 (m, 1H,) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.79 (m, 3H) ; 6.78 (s, 1H) ; 7.27 (s, 2H) ; 7.66 (s, 1H).

MS-ESI : 460 $[MH]^+$

The starting material was prepared as follows:



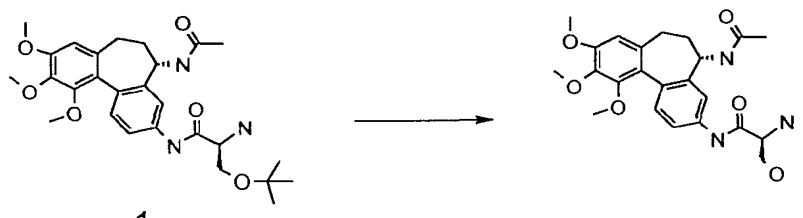
1

1*H*-Tetrazole (0.182 g ; 2.6 mmol) was added, under argon atmosphere, to a solution of [(5*S*)-5-(ethylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]methanol, (pepared as described in Example 26), (0.3 g ; 0.84 mmol) and di-*tert*-butyl diethylphosphoramidite (0.33 g ; 1.34 mmol) in dry THF (5.5 ml). After 5 minutes, the solution was cooled to -78°C and a solution of m-chloroperbenzoic acid (0.375 g ; 1.68 mmol) in dichoromethane (3 ml) was added in portions. The mixture was allowed to warm to ambient temperature and further stirred for 5 minutes. After addition of aqueous ammonium hydrogen carbonate and aqueous sodium sulphite, the organic solvent was removed by evaporation and the residue was taken up in dichloromethane. The organic phase was washed with water, dried and evaporated. The residue was purified by flash chromatography eluting with ethyl acetate/methanol (95/5) to give (1) as a foam.

Yield : 54 %.

^{1}H NMR spectrum (DMSOd_6) : 1.03 (t, 3H) ; 1.43 (s, 18H) ; 1.72 (m, 1H) ; 2.0 (m, 1H) ; 2.35 (m, 1H) ; 2.50 (m, 3H, signal obscured by DMSO peak) ; 3.2-3.6 (m, 1H signal obscured by H_2O peak) ; 3.50 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.99 (m, 2H) ; 6.80 (s, 1H) ; 7.35 (m, 2H).

Example 28



1

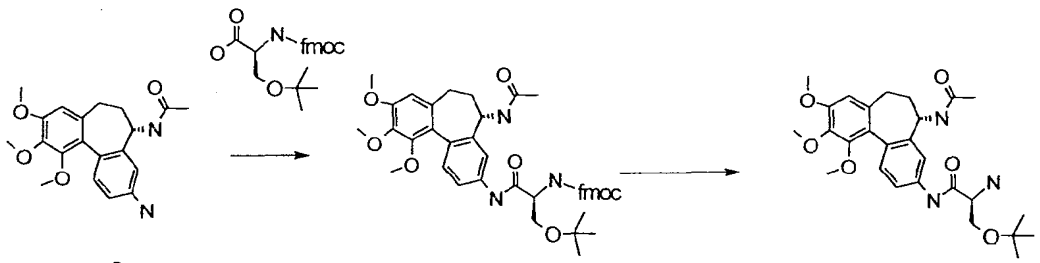
12N Hydrochloric acid (5ml) was added to a solution of (2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]-2-amino-3-*tert*butoxypropanamide (1) (0.35 g ; 0.7 mmol) in 1,4-dioxane (5 ml). The mixture was heated at 60°C under argon atmosphere for 1 hour. After dilution with ether, the resulting precipitate was filtered, washed with ether and dried to give **(2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]-2-amino-3-hydroxypropanamide** as a white solid.

Yield : 65%

^{1}H NMR spectrum (DMSOd_6) : 1.90 (s, 3H) ; 1.95 (m, 1H) ; 2.05 (m, 1H) ; 2.18 (m, 1H) ; 2.50 (m, 1H, signal obscured by DMSO peak) ; 3.48 (s, 3H) ; 3.79 (s, 3H) ; 3.84 (s, 3H) ; 3.88 (m, 2H) ; 4.05 (m, 1H) ; 4.45 (m, 1H) ; 6.8 (s, 1H) ; 7.29 (d, 1H) ; 7.58 (d, 1H) ; 7.65 (dd, 1H) ; 8.32 (br s, 3H) ; 8.47 (d, 1H).

MS-ESI : 444 $[MH]^+$

The starting material was prepared as follows:



2 3 1

O-(7-(Azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (0.468 g ; 1.28 mmol) and *N*-[(5*S*)-3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (2) (0.398 g ;1.12 mmol) were added to a solution of *N*-fmoc-O-*tert*-butyl-l-serine (0.428 g ; 1.12 mmol) in dichloromethane (18ml) and *N*,*N*-diisopropylethylamine (0.222 ml ; 1.28 mmol). The mixture was stirred overnight under argon atmosphere at ambient temperature. After addition of water, the organic phase was dried over $MgSO_4$ and evaporated to give **(2*S*)-*N*-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]-2-(fmoc-amino)-3-(*tert*butoxy)propanamide** (3).

Yield : 95%

^{1}H NMR spectrum ($DMSOd_6$) : 1.14 (s, 9H) ; 1.87 (s, 3H) ; 1.80 (m, 1H) ; 2.0-2.2 (m, 2H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 3.46 (s, 3H) ; 3.50-3.60 (m, 2H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.20-4.35 (m, 4H) ; 4.45 (m, 1H) ; 6.77 (s, 1H) ; 7.25 (d, 1H) ; 7.32 (m, 2H) ; 7.42 (m, 2H) ; 7.54 (m, 2H) ; 7.61 (s, 1H) ; 7.76 (m, 2H) ; 7.89 (m, 2H) ; 8.40 (d, 1H).

MS-ESI : 744 $[MNa]^+$

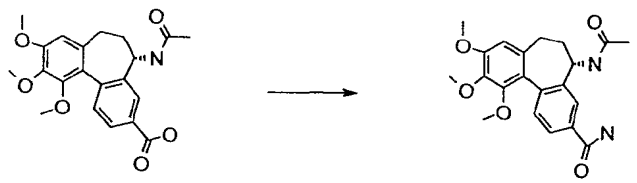
A solution of (3) (0.75 g ; 1.04 mmol) and piperidine (1 ml) in dichloromethane (1.5 ml) was stirred at ambient temperature for 45 minutes. After evaporation to dryness the residue was purified by flash chromatography eluting with ethyl acetate/methanol (95/5) to give (1) as a foam.

Yield : 70%

^{1}H NMR spectrum ($DMSOd_6$) : 1.14 (s, 9H) ; 1.87 (s, 3H) ; 1.89 (m, 1H) ; 1.90-2.15 (m, 2H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 3.46 (s, 3H) ; 3.40-3.50 (m, 3H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.49 (m, 1H) ; 6.77 (s, 1H) ; 7.25 (d, 1H) ; 7.58 (m, 2H) ; 8.39 (d, 1H).

MS-ESI : 500.2 $[MH]^+$

Example 29



Oxalyl chloride (0.44 g ; 3.4 mmol) and DMF (50 µl) were added to a suspension of *N*-[(5*S*)-3-carboxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (1) under argon atmosphere (1.15 g ; 3 mmol) in dichloromethane (10 ml). The mixture was stirred at ambient temperature for 2 hours, evaporated to dryness and redissolved in dichloromethane (20 ml). The solution was cooled at -78° C and ammonia gas was allowed to bubble through the solution for 5 minutes. The mixture was allowed to warm up and further stirred at ambient temperature for 15 minutes. After evaporation to dryness, the residue was taken up in aqueous sodium hydrogen carbonate/ethyl acetate. The organic phase was separated, evaporated and purified by flash chromatography eluting with ethyl

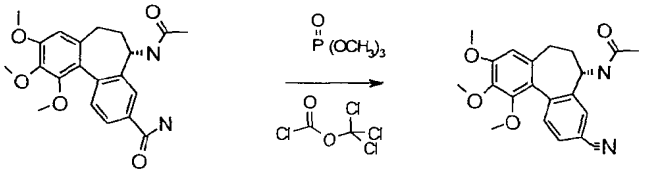
acetate/methanol (95/5) to give ***N*-[(5*S*)-3-carbamoyl-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide.**

Yield : 20 %

^{1}H NMR spectrum ($DMSOd_6$) : 1.89 (s, 3H) ; 1.89 (m, 1H) ; 1.96 (m, 1H) ; 2.5 (m, 1H, signal obscured by DMSO peak) ; 3.49 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.54 (m, 1H) ; 6.81 (s, 1H) ; 7.36 (d, 1H) ; 7.78 (dd, 1H) ; 7.87 (d, 1H) ; 7.96 (s, 1H) ; 8.47 (d, 1H).

MS-ESI : 407 $[MNa]^+$

Example 30



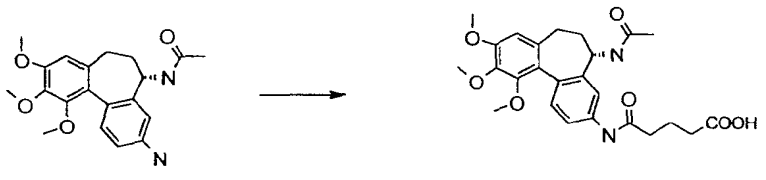
Trichloromethyl chloroformate (0.094 ml ; 0.77 mmol) was added in portions at 0°C to a solution of *N*-[(5*S*)-3-carbamoyl-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (0.2 g ; 0.484 mmol), (prepared as described for Example 29) and trimethyl phosphate (0.306 ml ; 2.6 mmol). The mixture was allowed to warm up and then heated at 60°C for 5 minutes. The reaction mixture was poured onto ice and stirred. The resulting precipitate was filtered, dried and purified by flash chromatography, eluting with dichloromethane/ethyl acetate (1/1) to give ***N*-[(5*S*)-3-cyano-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide.**

Yield : 61 %.

^{1}H NMR spectrum ($DMSOd_6$) : 0.97 (t, 3H) ; 1.60 (m, 1H) ; 1.95 (m, 2H) ; 2.30 (m, 2H) ; 2.45 (m, 1H, signal partially obscured by DMSO peak) ; 3.46 (s, 3H) ; 3.76 (s, 3H) ; 3.82 (s, 3H) ; 4.54 (m, 2H) ; 5.16 (t, 1H) ; 6.75 (s, 1H) ; 7.15-7.30 (m, 2H) ; 7.53 (s, 1H).

MS-ESI : 358 $[MH]^+$

Example 31



1

A solution of *N*-[(5*S*)-3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (1) (0.3 g ; 84 mmol) and glutaric anhydride (0.199 g ; 84 mmol) in dichloromethane (20 ml) was stirred at ambient temperature for 90 minutes. After removal of the solvent by evaporation, the residue was purified by flash chromatography, eluting with dichloromethane /methanol (80/20) to give, after trituration in ether, ***N*-[(5*S*)-3-(4-carboxybutanoylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide** as a white solid.

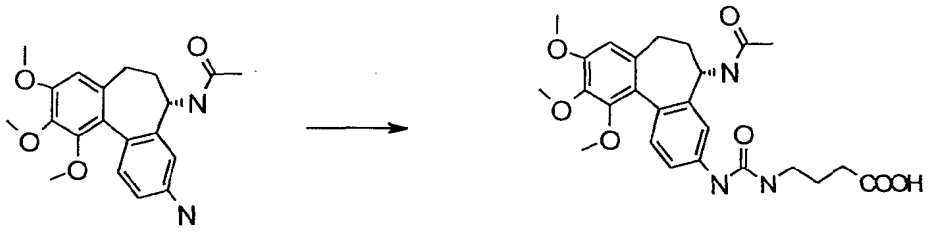
Yield : 63 %

^{1}H NMR spectrum ($DMSOd_6$) : 1.8-2 (m, 3H) ; 1.88 (s, 3H) ; 2-2.25 (m, 2H) ; 2.26 (t, 2H) ; 2.36 (t, 2H) ; 2.5 (s, 1H, signal partially observed by DMSO peak) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.48 (m, 1H) ; 6.76 (s, 1H) ; 7.22 (d, 1H) ; 7.53 (d, 1H) ; 7.56 (s, 1H) ; 8.4 (d, 1H) ; 9.97 (s, 1H).

MS-ESI / 471 $[MH]^+$

Elemental analysis	Found	C 59.2	H 6.2	N 5.4
$C_{25}H_{30}N_2O_7$	Requires	C 63.8	H 6.4	N 6.0%

Example 32



A suspension of 4-aminobutyric acid (0.111 g ; 1.08 mmol) and *N,O*-bis(trimethylsilyl)acetamide (1.8 ml ; 7.3 mmol) in dichloromethane (10 ml) was stirred at ambient temperature for 2 hours. The mixture was evaporated to dryness and redissolved in dichloromethane (10 ml) under argon atmosphere. A solution of *N*-[(5*S*)-3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (0.35 g ; 0.98 mmol), phenyl chloroformate (135 µl ; 1.08 mmol) and triethylamine (151 µl ; 1.08 mmol) in

dichloromethane (10 ml) was stirred for 1 hour under argon atmosphere and added to the above solution. The mixture was stirred overnight, evaporated and purified by preparative HPLC on reverse phase silica eluting with a 0-30 % gradient of methanol/ammonium carbonate buffer pH7 to give, after evaporation, **4-[([(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]aminocarbonyl)amino]butanoic acid** as a solid

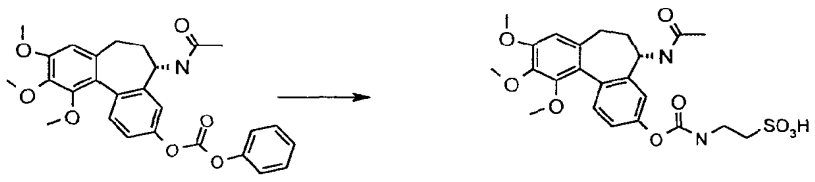
Yield : 50 %

^{1}H NMR spectrum ($DMSOd_6$, CF_3CO_2D) : 1.68 (m, 2H) ; 1.88 (s, 3H) ; 1.85-2 (m, 1H) ; 2-2.2 (m, 2H) ; 2.27 (m, 2H) ; 2.5 (m, 1H, signal partially observed by DMSO peak) ; 3.12 (m, 2H) ; 3.47 (s, 3H) ; 3.78 (s, 3H) ; 3.83 (s, 3H) ; 4.48 (m, 1H) ; 6.75 (s, 1H) ; 7.17 (d, 1H) ; 7.3 (s, 1H) ; 7.39 (d, 1H).

MS-ESI : 486 $[MH]^+$

Elemental analysis	Found	C 58.3	H 6.5	N 8.7
$C_{25}H_{31}N_3O_7$	Requires	C 61.8	H 6.4	N 8.7%

Example 33



1

A solution of *N*-[(5*S*)-3-phenoxycarbonyloxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (1) (0.35 g ; 0.73 mmol), 2-aminomethanesulphonic acid (0.156 g ; 1.25 mmol) and triethylamine (174 µl ; 1.25 mmol) in DMSO (2.5 ml) was heated at 70°C for 2 days. The mixture was taken up in water and purified by preparative HPLC eluting with a 0-30 % gradient of methanol/ammonium carbonate buffer (2 g/l pH7). The appropriate fractions were evaporated and the resulting solid triturated in ether and dried

to give **2-[([(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]oxycarbonyl)amino]ethane-1-sulphonic acid**.

Yield : 22 %

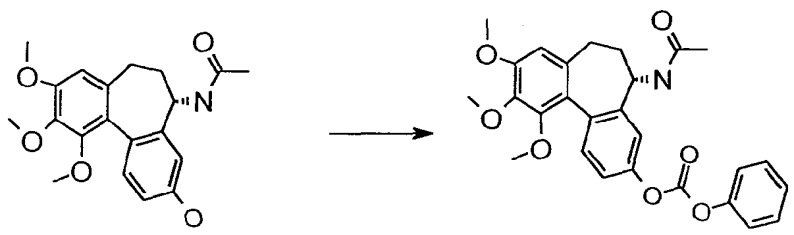
^{1}H NMR spectrum ($DMSOd_6$; CF_3CO_2D) : 1.89 (s, 3H) ; 1.8-1.95 (m, 1H) ; 2-2.5 (m, 2H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 2.71 (t, 2H) ; 3.4 (m, 2H) ; 3.54 (s, 3H) ; 3.8 (s, 3H) ; 3.85 (s, 3H) ; 4.58 (m, 1H) ; 6.8 (s, 1H) ; 7.08 (d, 1H) ; 7.1 (s, 1H) ; 7.32 (d, 1H).

MS-ESI : 509 $[MH]^+$

Elemental analysis: Found C 48.1 H 6.0 N 7.3 S 5.2

$C_{23}H_{28}N_2O_9S$; 1 NH_3, 2.5 H_2O Requires C 48.4 H 6.4 N 7.4 S 5.6%

The starting material was prepared as follows:

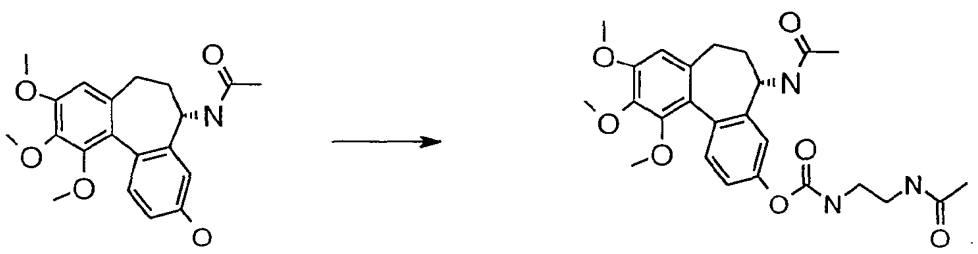


A solution of *N*-acetyl colchicinol (0.35 g ; 0.98 mmol), phenyl chloroformate (145 µl ; 1.08 mmol) and triethylamine (150 µl ; 1.08 mmol) in dichloromethane (20 ml) was stirred at ambient temperature for 1 hour. The mixture was washed with water and the organic phase evaporated. The residue was purified by flash chromatography, eluting with ethyl acetate/petroleum ether (80/20) to give *N*-[(5*S*)-3-phenoxycarbonyloxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide as a foam.

Yield : 79 %

^{1}H NMR spectrum ($DMSOd_6$) : 1.89 (s, 3H) ; 1.8-1.95 (m, 1H) ; 2-2.3 (m, 2H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 3.52 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.58 (m, 1H) ; 6.8 (s, 1H) ; 7.2 - 7.6 (m, 8H) ; 8.41 (d, 1H).

Example 34



A solution of *N*-acetyl-cochicinol (0.25 g ; 0.7 mmol), 4-nitrophenyl chloroformate (0.169 g ; 0.84 mmol) and triethylamine (117 µl ; 0.84 mmol) in dichloromethane (10 ml) was stirred under argon atmosphere for 1 hour. *N*-Acetylethylenediamine (0.086 g ; 0.84 mmol) was then added and the mixture was stirred further for 3 hours. After evaporation to dryness, the residue was purified by flash chromatography, eluting with methanol/acetonitrile/dichloromethane (4/48/48) to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[a,c]cyclohepten-3-yl *N*-[2-(acetylamino)ethyl]carbamate.**

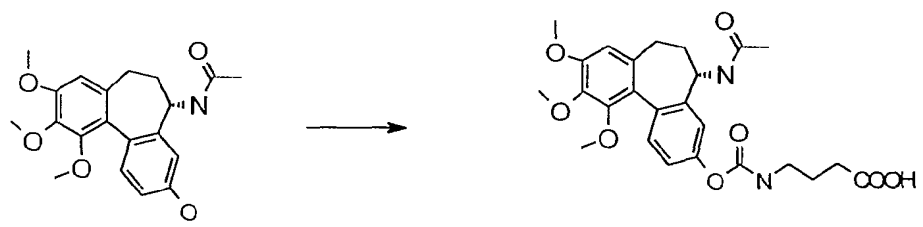
Yield : 49 %

^{1}H NMR spectrum ($DMSOd_6$) : 1.84 (s, 3H) ; 1.88 (s, 3H) ; 1.85-1.95 (m, 1H) ; 2-2.25 (m, 2H) ; 2.5 (m, 1H, signal partially observed by H_2O peak) ; 3.53 (s, 3H) ; 3.79 (s, 3H) ; 3.85 (s, 3H) ; 4.55 (m, 1H) ; 6.81 (s, 1H) ; 7.06 (dd, 1H) ; 7.07 (d, 1H) ; 7.32 (d, 1H) ; 7.79 (m, 1H) ; 7.8 (m, 1H) ; 8.41 (d, 1H).

MS-ESI : 486.1 $[MH]^+$

Elemental analysis	Found	C 60.3	H 6.6	N 8.3
$C_{25}H_{31}N_3O_7$ 0.6 H_2O	Requires	C 60.5	H 6.5	N 8.5%

Example 35



A suspension of 4-aminobutyric acid (0.087 g ; 0.84 mmol) and *N,O*-bis(trimethylsilyl)acetamide (0.865 ml ; 3.5 mmol) in dichloromethane (10 ml) was stirred under argon atmosphere for 3 hours and evaporated to dryness. The residue was then redissolved in dichloromethane (10 ml). 4-Nitrophenyl chloroformate (0.17 g ; 0.84 mmol) and triethylamine (0.117 ml ; 0.84 mmol) were added to a solution of *N*-acetyl-colchicinol (0.25 g ; 0.7 mmol) in dichloromethane (10 ml). The solution was stirred for 1 hour and added to the above solution. The resulting mixture was stirred further for 3 hours. After evaporation to dryness the residue was purified by preparative HPLC on reverse phase silica eluting with methanol/ammonium carbonate buffer (2 g/l pH7) (30/70) to give, after evaporation of the appropriate fractions, **4-[([(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[a,c]cyclohepten-3-yl]oxycarbonyl)amino]butanoic acid.**

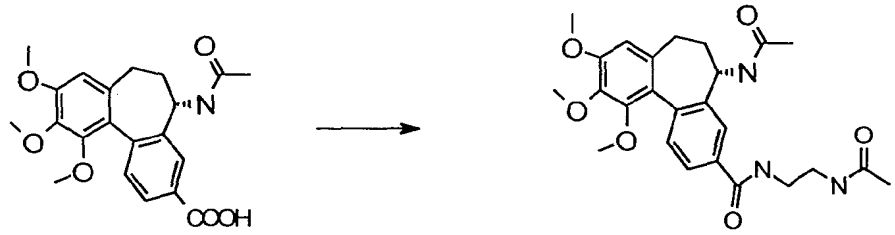
Yield : 44 %

^{1}H NMR spectrum (DMSOd_6, CF_3CO_2D) : 1.73 (m, 2H) ; 1.88 (s, 3H) ; 1.8-1.95 (m, 1H) ; 2-2.25 (m, 2H) ; 2.3 (m, 2H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 3.11 (m, 2H) ; 3.53 (s, 3H) ; 3.79 (s, 3H) ; 3.84 (s, 3H) ; 4.45 (m, 1H) ; 6.8 (s, 1H) ; 7.05 (dd, 1H) ; 7.07 (d, 1H) ; 7.31 (d, 1H) ; 7.87 (m, 1H) ; 8.42 (d, 1H).

MS-ESI : 487.1 $[MH]^+$

Elemental analysis	Found	C 60.2	H 6.3	N 6.0
$C_{25}H_{30}N_2O_8$ 0.5 H_2O	Requires	C 60.6	H 6.3	N 5.7%

Example 36



1

A mixture of *N*-[(5*S*)-3-carboxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (1) (0.3 g ; 0.78 mmol), DCCI (0.193 g ; 0.93 mmol),

DMAP (0.019 g ; 0.15 mmol) and *N*-acetylethylenediamine (0.096 g ; 0.985 mmol) in dichloromethane was stirred at ambient temperature overnight. After evaporation of the solvent, the residue was purified by flash chromatography and eluted with methanol/dichloromethane (10/90) to give ***N*-[(5*S*)-3-(2-acetylaminoethylcarbamoyl)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide.**

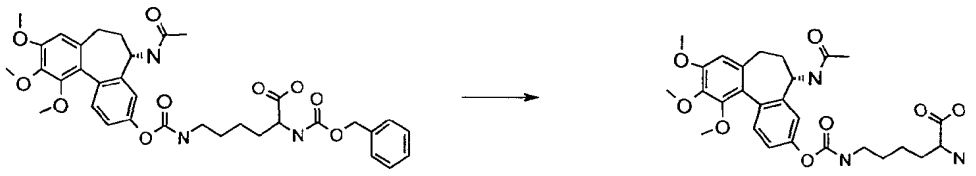
Yield : 71 %

^{1}H NMR spectrum ($DMSOd_6$) : 1.82 (s, 3H) ; 1.89 (s, 3H) ; 1.85-2.05 (m, 2H) ; 2.17 (m, 1H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 3.22 (m, 2H) ; 3.32 (m, 2H, signal partially observed by H_2O peak) ; 3.49 (s, 3H) ; 3.79 (s, 3H) ; 3.84 (s, 3H) ; 4.55 (m, 1H) ; 6.81 (s, 1H) ; 7.38 (d, 1H) ; 7.75 (dd, 1H) ; 7.84 (d, 1H) ; 8 (m, 1H) ; 8.52 (m, 2H).

MS-ESI : 470.2 $[MH]^+$

Elemental analysis:	Found	C 60.3	H 6.6	N 8.2
$C_{25}H_{31}N_3O_6$ 0.4 dichloromethane	Requires	C 60.6	H 6.4	N 8.4%

Example 37



1

A solution of 6-[({[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[a,c]cyclohepten-3-yl]oxy}carbonyl)amino]-2-(benzyloxycarbonylamino)-hexanoic acid (1) (0.4 g ; 0.6 mmol) in ethanol (80 ml) was hydrogenated in the presence of 10% palladium on carbon (0.08 g). After filtration of the catalyst and evaporation to dryness, the residue was purified by preparative HPLC eluting with a 0-40% gradient of methanol/ammonium carbonate buffer (2 g/l pH7) to give, after evaporation and trituration in ether, **6-[({[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]oxy}carbonyl)amino]-2-aminohexanoic acid** as a solid.

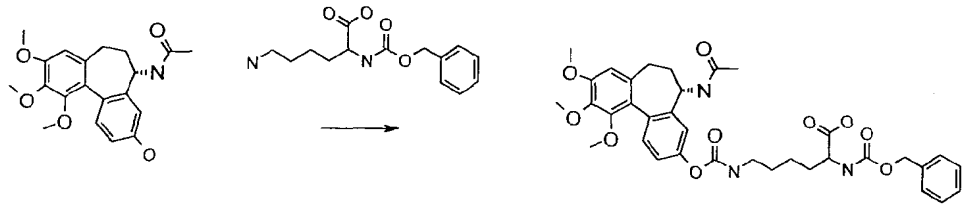
Yield : 75 %

^{1}H NMR spectrum ($DMSOd_6$) : 1.35-1.55 -m, 4H) ; 1.62 (m, 1H) ; 1.72 (m, 1H) ; 1.9 (s, 3H) ; 1.9 (m, 1H) ; 2.05 (m, 1H) ; 2.15 (m, 1H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 3-3.2 (m, 3H) ; 3.51 (s, 3H) ; 3.78 (s, 3H) ; 3.83 (s, 3H) ; 4.55 (m, 1H) ; 6.79 (s, 1H) ; 7.02 (dd, 1H) ; 7.12 (d, 1H) ; 7.29 (d, 1H) ; 7.74 (m, 1H) ; 8.7 (d, 1H).

MS-ESI : 530.1 $[MH]^+$

Elemental analysis:	Found	C 60.2	H 7.0	N 7.8	
$C_{27}H_{35}N_3O_9$ 0.5 H_2O	Requires	C 60.2	H 6.7	N 7.8%	

The starting material was prepared as follows:



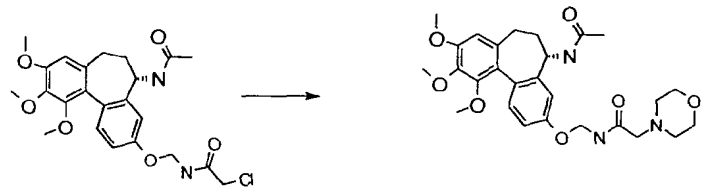
1

A suspension of *N*-(carboxybenzyloxy)-L-lysine (0.141 g ; 0.5 mmol) and *N,O*-bis(trimethylsilyl)acetamide (0.519 ml ; 2 mmol) in dichloromethane (10 ml) was stirred at ambient temperature under argon atmosphere for 3 hours. The mixture was evaporated to dryness and the residue redissolved in dichloromethane (10 ml). A solution of *N*-acetyl-colchicinol (0.15 g ; 0.42 mmol), and 4-nitrophenyl chloroformate (0.102g; 0.5mmol) was stirred at ambient temperature for 1 hour and then added to the above solution. The resulting mixture was stirred overnight, evaporated to dryness and purified by preparative HPLC eluting with a 0-55% gradient of methanol/ammonium carbonate buffer (2 g/l pH7) to give (1).

Yield : 63 %

^{1}H NMR spectrum ($DMSOd_6$) : 1.35 (m, 2H) ; 1.49 (m, 2H) ; 1.62 (m, 1H) ; 1.72 (m, 1H) ; 1.89 (s, 3H) ; 1.9 (m, 1H) ; 2. 07 (m, 1H) ; 2.15 (m, 1H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 3.07 (m, 2H) ; 3.52 (s, 3H) ; 3.8 (s, 3H) ; 3.85 (s, 3H) ; 4.57 (m, 1H) ; 5.05 (m, 2H) ; 6.81 (s, 1H) ; 7 (m, 1H) ; 7.05 (dd, 1H) ; 7.1 (d, 1H) ; 7.32 (d, 1H) ; 7.3-7.4 (m, 5H) ; 7.75 (m, 1H ; 8.58 (d, 1H).

Example 38



1

A solution of *N*-([(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]oxymethyl)-2-chloroacetamide (1) (0.25 g ; 0.55 mmol) in morpholine (2 ml)) was stirred at ambient temperature for 2 hours. After addition of dichloromethane and removal of the insoluble material by filtration, the filtrate was evaporated to dryness and the residue was purified by preparative HPLC eluting with a 0-45% gradient of ethanol/ammonium carbonate buffer (2 g/l pH7) to give, after evaporation and trituration in ether, ***N*-([(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl]oxymethyl)-2-morpholinoacetamide.**

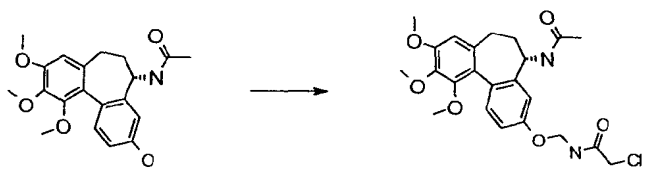
Yield : 60 %

^{1}H NMR spectrum ($DMSOd_6$) : 1.85 (m, 1H) ; 1.88 (s, 3H) ; 1.95-2.2 (m, 2H) ; 2.4 (m, 4H) ; 2.5 (m, 1H, signal partially observed by DMSO peak) ; 3 (s, 2H) ; 3.47 (s, 3H) ; 3.57 (m, 4H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.5 (m, 1H) ; 5.18 (m, 2H) ; 6.76 (s, 1H) ; 6.91 (d, 1H) ; 6.98 (dd, 1H) ; 7.22 (d, 1H) ; 8.32 (m, 1H) ; 8.85 (m, 1H).

MS-ESI : 514.1 $[MH]^+$

Elemental analysis	Found	C 61.2	H 6.9	N 7.9
$C_{27}H_{35}N_3O_7$	Requires	C 61.4	H 7.0	N 8.0%

The starting material was prepared as follows:



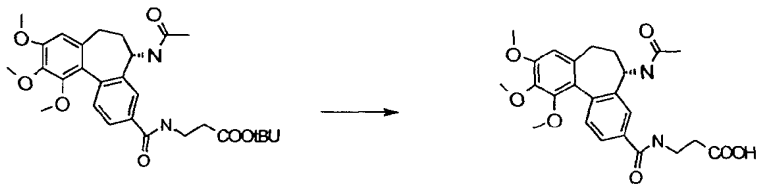
1

2-Chloro-*N*-(hydroxymethyl)-acetamide (0.342 g ; 2.7 mmol), triphenylphosphine (1.1 g ; 4.19 mmol) and DEAD (0.6 ml ; 4.19 mmol) were added to a solution of *N*-acetyl-colchicinol (0.3 g ; 0.84 mmol) in dichloromethane (20 ml) under argon atmosphere. The mixture was stirred at ambient temperature for 2 hours, evaporated and purified by flash chromatography eluting with ethyl acetate/dichloromethane (50/50) and dichloromethane/methanol (98/2) to give (1).

Yield : 76 %

MS-ESI : 485.1 $[MH]^+$

Example 39



1

N-[(5*S*)-3-(2-*tert*Butoxycarbonylethylcarbamoyl)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (1) (0.26 g ; 0.5 mmol) in solution in dichloromethane (10 ml) was treated with TFA (10 ml) at ambient temperature for 1 hour. After evaporation to dryness, the residue was purified by preparative HPLC eluting with methanol/ammonium carbonate buffer (2 g/l pH7) (35/65) to give, after evaporation and trituration in ether, **3-[(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-ylcarbonylamino]propanoic acid** as a white solid.

Yield : 56%

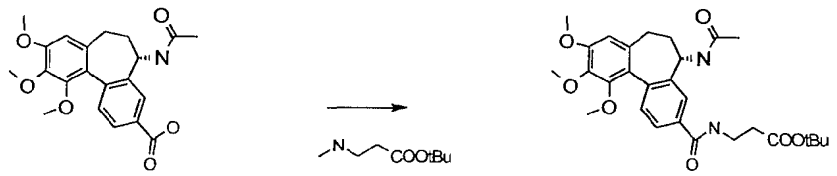
^1H NMR spectrum ($DMSOd_6$) : 1.91 (s, 3H) ; 1.85-2.1 (m, 2H) ; 2.2 (m, 1H) ; 2.5 (m, 1H, signal partially observed by DMSO peak) ; 3.2-3.6 (m, 4H, signal partially obscured by H_2O

peak) ; 3.5 (s, 3H) ; 3.8 (s, 3H) ; 3.86 (s, 3H) ; 4.6 (m, 1H) ; 6.82 (s, 1H) ; 7.39 (d, 1H) ; 7.74 (dd, 1H) ; 7.85 (d, 1H) ; 8.54 (d, 1H) ; 8.62 (m, 1H).

MS-ESI : 457.1 $[MH]^+$

Elemental analysis:	Found	C 60.9	H 6.7	N 6.7	
$C_{24}H_{28}N_2O_7$	Requires	C 63.2	H 6.2	N 6.1%	

The starting material was prepared as follows:



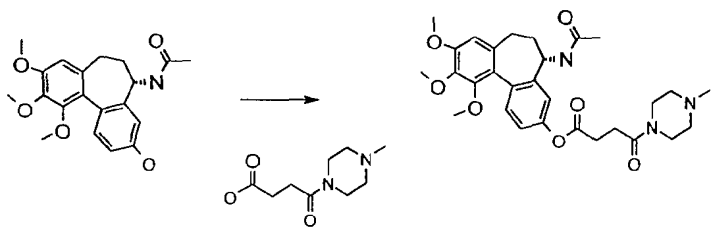
2 1

A mixture of *N*-[(5*S*)-3-carboxy-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide (1) (0.3 g ; 0.78 mmol), EDCI (0.179 g ; 0.93 mmol), DMAP (0.019 g ; 0.15 mmol), triethylamine (0.13 ml; 0.985 mmol) and *tert*butyl 3-methylaminopropanoate (0.17 g ; 0.985 mmol) in dichloromethane was stirred at ambient temperature overnight. After removal of the solvent by evaporation, the residue was purified by flash chromatography and eluted with ethyl acetate to give (1).

Yield : 84%

^{1}H NMR spectrum ($DMSOd_6$) : 1.4 (s, 9H) ; 1.89 (s, 3H) ; 1.9 (m, 1H) ; 2 (m, 1H) ; 2.17 (m, 1H) ; 2.5 (m, 1H, signal partially obscured by DMSO peak) ; 3.25-3.55 (m, 4H) ; 3.49 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.55 (m, 1H) ; 6.81 (s, 1H) ; 7.37 (d, 1H) ; 7.7 (dd, 1H) ; 7.81 (d, 1H) ; 8.5 (m, 2H).

Example 40



2 1

A suspension of 3-(4-methylpiperazin-1-ylcarbonyl)propanoic acid (2) (0.219 g ; 1.1 mmol), DCCI (0.226 ml ; 1.1 mmol) and DMAP (0.052 ml ; 0.42 mmol) in dichloromethane (20 ml) was stirred under argon atmosphere for 1 hour. *N*-Acetyl-colchicinol (0.3 g ; 0.84 mmol) was then added and the mixture was stirred overnight. After removal of the insoluble material by filtration, the filtrate was evaporated and purified by preparative HPLC eluting with a 0-50% gradient of methanol/ammonium carbonate buffer (2 g/l pH7) to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl 3-[4-methylpiperazin-1-ylcarbonyl]propanoate.**

Yield : 48 %

^{1}H NMR spectrum ($DMSOd_6$) : 1.80-1.96 (m, 1H) ; 1.88 (s, 3H) ; 2.07 (m, 1H) ; 2.18 (m, 1H) ; 2.20 (s, 3H) ; 2.26 (m, 2H) ; 2.33 (m, 2H) ; 2.59 (m, 1H, signal partially obscured by DMSO peak) ; 2.73 (m, 2H) ; 2.79 (m, 2H) ; 3.48 (m, 4H) ; 3.53 (s, 3H) ; 3.80 (s, 3H) ; 3.86 (s, 3H) ; 4.55 (m, 1H) ; 6.82 (s, 1H) ; 7.03-7.12 (m, 2H) ; 7.36 (d, 1H) ; 8.43 (d, 1H).

MS-ESI : 540 $[MH]^+$

Elemental analysis	Found	C 63.9	H 7.1	N 7.5	
$C_{29}H_{37}N_3O_7$; 0.3 H_2O	Requires	C 63.9	H 7.0	N 7.7%	

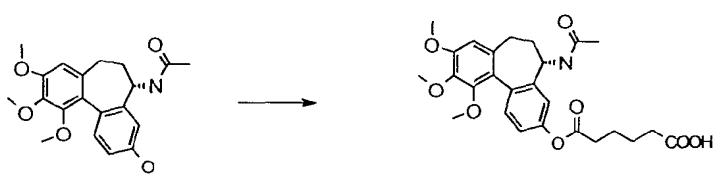
The starting material was prepared as follows:

A suspension of *N*-methylpiperazine (1.1 ml ; 10 mmol) and succinic anhydride (1.2 g ; 12 mmol) in dichloromethane (20 ml) was stirred under argon atmosphere for 24 hours. After evaporation to dryness, the residue was triturated in ether/pentane to give (2) as a solid.

Yield : 91 %

^{1}H NMR Spectrum ($DMSOd_6$) : 2.37 (s, 3H) ; 2.53 (m, 2H) ; 2.58 (m, 2H) ; 2.64 (m, 4H) ; 3.59 (m, 2H) ; 3.69 (m, 2H) ; 5.70 (br s, 1H).

Example 41



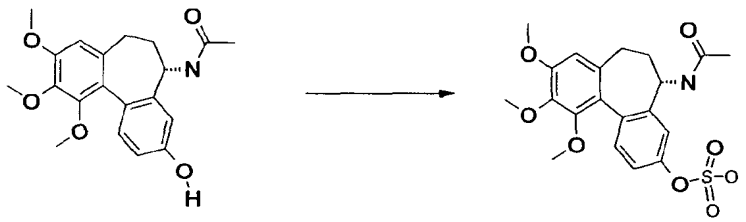
N-Acetyl-colchicinol (0.3 g ; 0.84 mmol) was added under argon atmosphere to a solution of adipic acid (0.147 g ; 1 mmol, *O*-(7-(azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (0.383 g ; 1 mmol) and diisopropylethylamine (0.352 ml ; 2 mmol) in acetonitrile (20 ml). The reaction mixture was stirred at ambient temperature overnight and evaporated to dryness. The residue was taken up in water (4 ml), the pH was adjusted to 6.5 with 0.1M hydrochloric acid. The solution was purified by preparative HPLC eluting with a 0-40% gradient of methanol/ammonium carbonate buffer (2 g/l pH7) to give **5-[{(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl}oxycarbonyl]pentanoic acid.**

Yield : 31 %

^{1}H NMR spectrum ($DMSOd_6$) : 1.54-1.75 (m, 4H) ; 1.85-1.90 (m, 1H) ; 1.87 (s, 3H) ; 1.98-2.28 (m, 4H) ; 2.57 (m, 1H, signal partially obscured by DMSO peak) ; 2.61 (t, 2H) ; 3.51 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.55 (m, 1H) ; 6.80 (s, 1H) ; 7.04-7.11 (m, 2H) ; 7.34 (d, 1H) ; 8.43 (d, 1H).

MS-ESI : 508 $[MNa]^+$

Example 42



Chlorosulphonic acid (1 ml) was added at 0°C in portions to a solution of pyridine (10 ml). After 15 minutes at 0°C, a solution of *N*-acetyl-colchicinol (1 g, 2.8 mmol) in pyridine (10 ml) was added. The solution was stirred overnight at ambient temperature. Water (30 ml) was added and the mixture was adjusted to pH8 by addition of sodium hydrogen carbonate. The aqueous layer was extracted with ether (3 x 20 ml) and purified on HP20SS resin, eluted with a 0-40 % gradient of methanol/water. The volatiles were removed by evaporation to give **(5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-3-yl hydrogen sulphate** as a white solid.

Yield = 71 %