(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

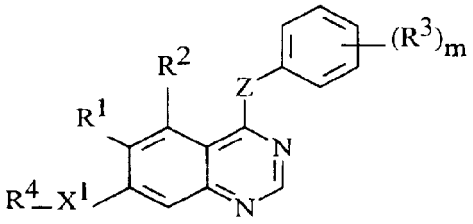
Water for injection to 100%

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

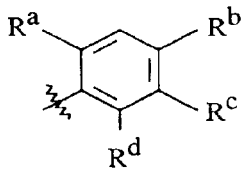
7) C_{1-5}alkylX^4C_{1-5}alkylX^5R^{24} (wherein X^4 and X^5 which may be the same or different are each -O-, -S-, -SO-, $-SO_2$-, $-NR^{25}CO$-, $-CONR^{26}$-, $-SO_2NR^{27}$-, $-NR^{28}SO_2$- or $-NR^{29}$- (wherein R^{25}, R^{26}, R^{27}, R^{28} and R^{29} each independently represents hydrogen, C_{1-3}alkyl 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.

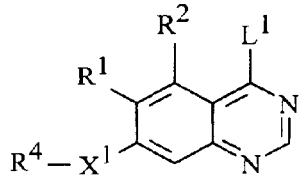
13. A quinazoline derivative as claimed in claim 1 selected from:
4-(4-bromo-2,6-difluoroanilino)-6,7-dimethoxyquinazoline,
4-(4-bromo-2-fluoro-5-hydroxyanilino)-6,7-dimethoxyquinazoline,
4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-thiomorpholinoethoxy)quinazoline,
6,7-dimethoxy-4-(3-hydroxy-4-methylphenoxy)quinazoline,

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.

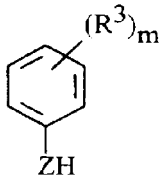
15. A process for the preparation of a quinazoline derivative of formula I or salt thereof (as defined in claim 1) which comprises:

(a) the reaction of a compound of the formula III:



(III)

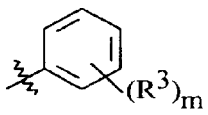
(wherein R^1, R^2, X^1 and R^4 are as defined in claim 1 and L^1 is a displaceable moiety), with a compound of the formula IV:



(IV)

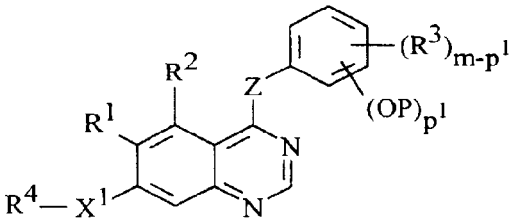
(wherein Z, R^3 and m are as defined in claim 1) whereby to obtain compounds of the formula I and salts thereof;

(b) for the preparation of compounds of formula I and salts thereof in which the group of formula IIa:



(IIa)

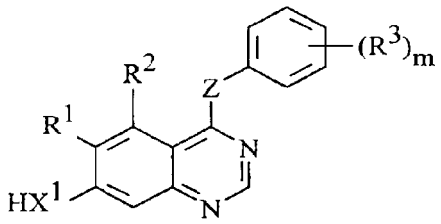
(wherein R^3 and m are as defined in claim 1) represents a phenyl group carrying one or more hydroxy groups, the deprotection of a compound of formula V:



(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:



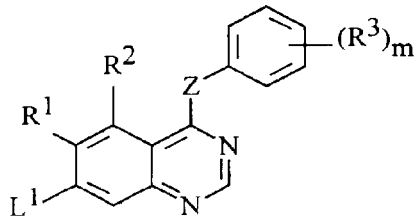
(VI)

(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:

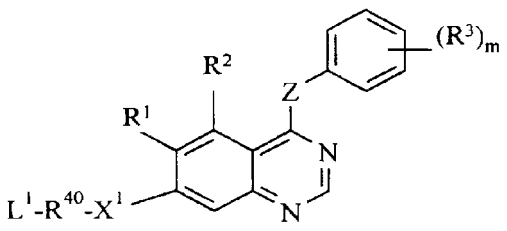
1) X^6C_{1-3}alkyl (wherein X^6 represents -O-, -S-, $-SO_2-$, $-NR^{33}CO-$ or $-NR^{34}SO_2-$ (wherein R^{33} and R^{34} are each independently hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

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.

* 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 May 1997	13.06.97
Name and mailing address of the ISA 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	Authorized officer Gettins, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/00365

C.(Continuation) 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 LTD) 20 October 1993 see claim 1	1
A	EP 0 520 722 A (ICI PLC) 30 December 1992 see claim 1	1
A	DE 29 36 705 A (SANKYO CO ;UBE INDUSTRIES (JP)) 20 March 1980	1

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 97/00365

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9515758 A	15-06-95	US 5480883 A	02-01-96
		AU 1305095 A	27-06-95
EP 0635498 A	25-01-95	AU 7191694 A	20-02-95
		WO 9503283 A	02-02-95
		JP 9500636 T	21-01-97
		US 5475001 A	12-12-95
		ZA 9405156 A	19-01-95
WO 9220642 A	26-11-92	AU 658646 B	27-04-95
		AU 1993492 A	30-12-92
		CA 2102780 A	11-11-92
		EP 0584222 A	02-03-94
		JP 6507643 T	01-09-94
		US 5409930 A	25-04-95
		US 5480883 A	02-01-96
EP 0326330 A	02-08-89	AU 2872889 A	03-08-89
		CN 1034925 A,B	23-08-89
		EG 18859 A	29-09-94
		FI 94523 B	15-06-95
		HU 208611 B	28-12-93
		JP 1246263 A	02-10-89
		JP 2559485 B	04-12-96
		US 5145843 A	08-09-92
		US 5240940 A	31-08-93
EP 0566226 A	20-10-93	AT 130000 T	15-11-95
		AU 661533 B	27-07-95
		AU 3101093 A	22-07-93
		CA 2086968 A	21-07-93
		DE 69300754 D	14-12-95
		DE 69300754 T	28-03-96
		ES 2078798 T	16-12-95
		HU 9500185 A	28-07-95
		NZ 245662 A	26-09-95
		SK 1693 A	09-09-93
		US 5457105 A	10-10-95
		US 5616582 A	01-04-97
		ZA 9300015 A	20-07-93

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 97/00365

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0566226 A		JP 6073025 A	15-03-94
EP 0520722 A	30-12-92	AT 146781 T	15-01-97
		AU 651215 B	14-07-94
		AU 1842292 A	07-01-93
		CA 2071087 A	29-12-92
		DE 69216158 D	06-02-97
		DE 69216158 T	10-04-97
		JP 5208911 A	20-08-93
		NO 180105 B	11-11-96
		NZ 243082 A	24-02-95
		SK 182792 A	09-08-95
DE 2936705 A	20-03-80	JP 1400162 C	28-09-87
		JP 55038325 A	17-03-80
		JP 62006546 B	12-02-87
		BE 878723 A	11-03-80
		CA 1151168 A	02-08-83
		CH 642361 A	13-04-84
		FR 2435248 A	04-04-80
		GB 2033894 A,B	29-05-80
		NL 7906761 A	13-03-80
		SE 446337 B	01-09-86
		SE 7907493 A	12-03-80
		US 4322420 A	30-03-82
		US 4464375 A	07-08-84

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K — A2

(11) International Publication Number: **WO 00/41669**

(43) International Publication Date: 20 July 2000 (20.07.00)

(21) International Application Number: PCT/GB00/00099

(22) International Filing Date: 14 January 2000 (14.01.00)

(30) Priority Data:
9900752.8 15 January 1999 (15.01.99) GB

(71) Applicant *(for all designated States except US)*: ANGIOGENE PHARMACEUTICALS LTD. [GB/GB]; 14 Plowden Park, Aston Rowant, Watlington, Oxfordshire OX9 5SW (GB).

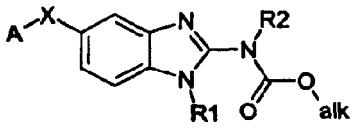
(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: DAVIS, Peter, David [GB/GB]; 10 Aston Park, Aston Rowant, Watlington OX9 5SW (GB).

(74) Agents: BAILLIE, Iain, C. et al.; Langner Parry, 52–54 High Holborn, London WC1V 6RR (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, 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, 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, ARIPO patent (GH, GM, KE, LS, MW, 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), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: BENZIMIDAZOLE VASCULAR DAMAGING AGENTS



(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.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

BENZIMIDAZOLE VASCULAR DAMAGING AGENTS

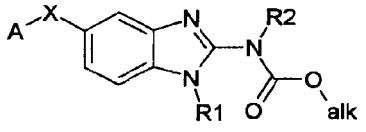
This invention relates to vascular damaging agents and particularly to the use of new and known substituted benzimidazoles in the preparation of medicaments for the treatment of diseases involving neovascularisation.

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

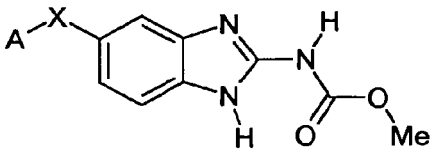
A is an optionally substituted aromatic, optionally substituted heteroaromatic, optionally substituted heterocycloalkyl, optionally substituted alkyl or optionally substituted cycloalkyl group

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.

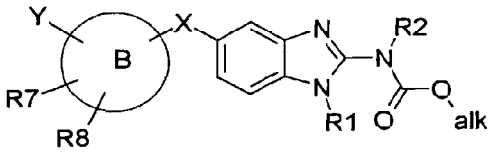
Some of the compounds usable in the invention are known, for example the following compounds within the following formula:



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.

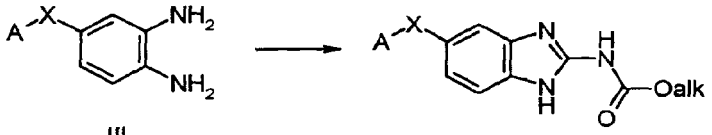
Where one or more functional groups in compounds of formula I or II are sufficiently basic or acidic the formation of salts is possible. Suitable salts include pharmaceutically acceptable salts for example acid addition salts including hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, 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

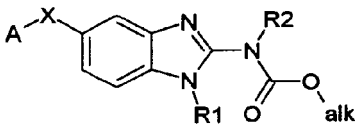
Example 19: [5(6)-(4-hydroxybenzoyl)-1*H*-benzimidazol-2yl]carbamate

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

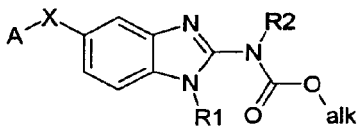
A is an optionally substituted aromatic, optionally substituted heteroaromatic, optionally substituted heterocycloalkyl, optionally substituted alkyl or optionally substituted cycloalkyl group

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

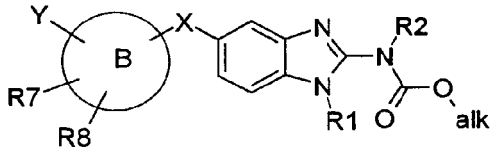
R^2 is hydrogen, alkoxycarbonyl, cyanomethyl, cyanoethyl, alkoxymethyl or acetoxymethyl.

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.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 January 2002 (17.01.2002)

PCT

(10) International Publication Number
WO 02/04434 A1

(51) International Patent Classification[7]: C07D 295/16, 295/18, 295/14, 211/62, C07C 235/74, A61K 31/495, 31/16, 31/44

(21) International Application Number: PCT/GB01/02966

(22) International Filing Date: 4 July 2001 (04.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
00401978.2 7 July 2000 (07.07.2000) EP

(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).

(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*
— *with amended claims*

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 02/04434 A1

(54) Title: COLCHINOL DERIVATIVES AS VASCULAR DAMAGING AGENTS

(57) Abstract: The invention relates to colchinol derivatives of the formula I: 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.

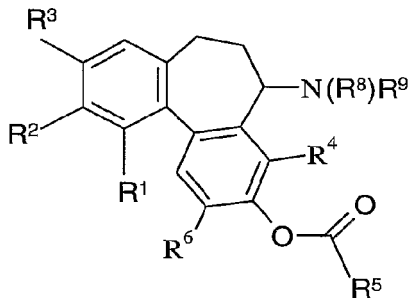
Reversal of neovascularisation by damaging the newly-formed vascular endothelium is expected to have a beneficial therapeutic effect. The present invention is based on the discovery of tricyclic compounds that surprisingly specifically damage newly formed vasculature without affecting the normal, established 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;

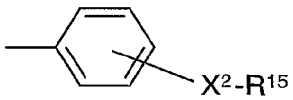
(wherein $\mathbf{R^{12}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from:

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 $\mathbf{R^{12}}$ 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:

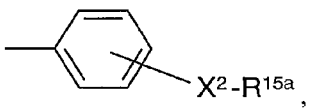
A is ethylene or phenylene;

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:

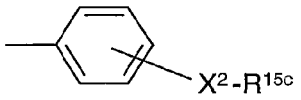
A is ethylene or phenylene;

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

$\mathbf{R^8}$ 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);

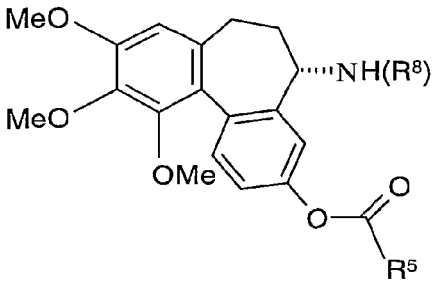
3) R^{22b} -C_{1-4}alkyl- (wherein R^{22b} is as hereinabove defined); or

4) $R^{12b}Y^7C_{1-4}$alkyl- (wherein R^{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 $\mathbf{R^9}$ is hydrogen;

or a pharmaceutically-aceeptable salt, solvate or pro-drug thereof.

A preferred compound of the present invention is of the formula (II):

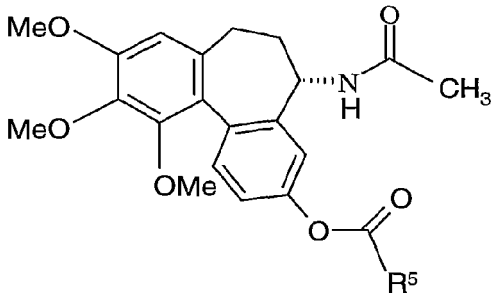


(II)

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:

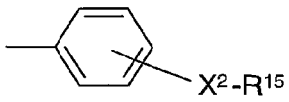
A is ethylene or phenylene;

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:

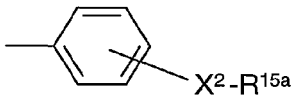
A is ethylene or phenylene;

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:

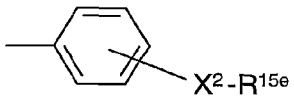
A is phenylene;

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.

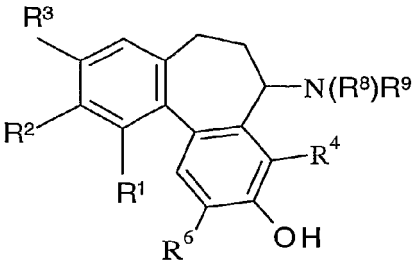
A suitable protecting group for an amino, imino or alkylamino group is, for example, an acyl group, for example a (2-4C)alkanoyl group (especially acetyl), a (1-4C)alkoxycarbonyl group (especially methoxycarbonyl, ethoxycarbonyl or tert-butoxycarbonyl), an arylmethoxycarbonyl group (especially benzyloxycarbonyl) 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:

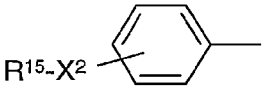
(a) reacting a compound of the formula (X):



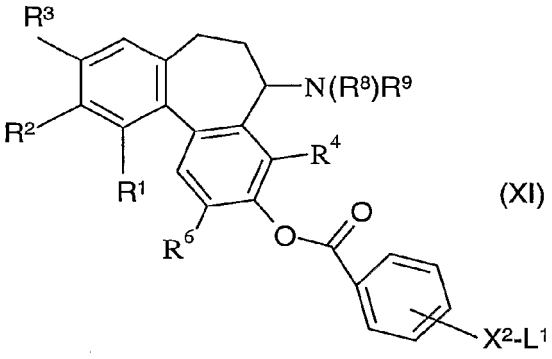
(X)

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.

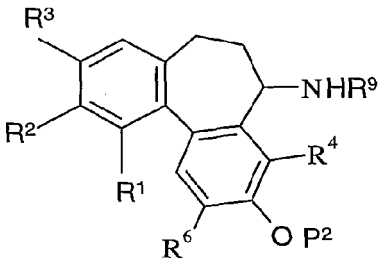
A amino group can be alkylated or acylated using similar reaction conditions to those described above for alkylation or acylation of a ring nitrogen atom in R^{12} or R^{15}.

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=N-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}-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-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-X^2-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-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-COOH.

When R^5 is of the formula -A-X^1Y^1-B, and X^1 is -$N(R^{10})CO$- or -$CON(R^{10})$- then a compound of the formula R^5-COOH can be formed by reacting a compound of the formula P^1OOC-A-NHR^{19} , wherein P^1 is a carboxy-protecting group, with a compound of the formula HOOC-Y^1-B, or of the formula P^1OOC-A-COOH with a compound of the formula $NH(R^{10})$-Y^1-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;

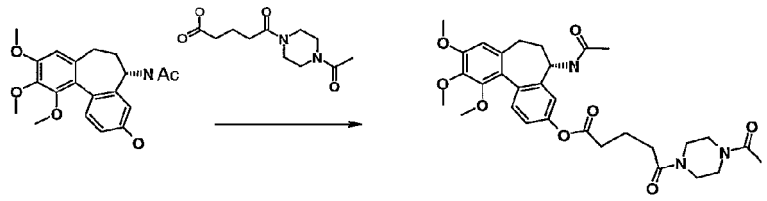
(v) intermediates were not generally fully characterised and purity was assessed by thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), infra-red (IR) or NMR analysis;

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.

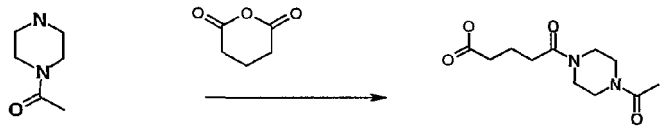
Yield : 82 %

^{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 :



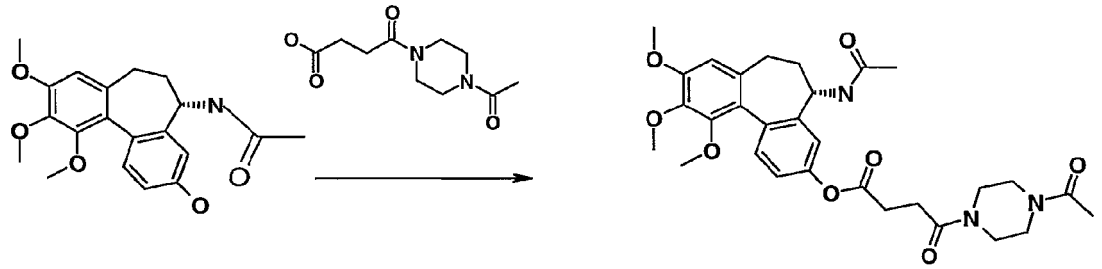
A solution of glutaric anhydride (1.6 g ; 14 mmol) and N-acetylpiperazine (1.5 g ; 12 mmol) in dichloromethane (20 ml) was stirred overnight. The resulting precipitate was filtered, washed with ether and dried to give 5-(4-acetylpiperazin-1-yl)-5-oxopentanoic acid as a white solid.

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



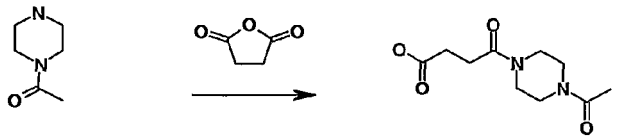
The compound was prepared using a similar method to that described in Example 1, but using 4-(4-acetylpiperazin-1-yl)-4-oxobutanoic acid in place of 5-(4-acetylpiperazin-1-yl)-5-oxopentanoic acid.

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:



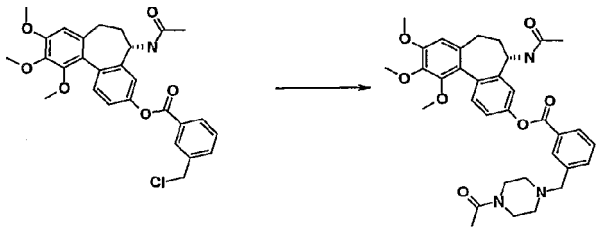
A solution of succinic anhydride (1.72 g ; 17 mmol) and N-acetyl piperazine (2 g ; 15.6 mmol) in dichloromethane (40 ml) was stirred overnight. After evaporation to dryness, the residue was triturated in ether / pentane to give 4-(4-acetylpiperazin-1-yl)-4-oxobutanoic acid as a solid.

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 %

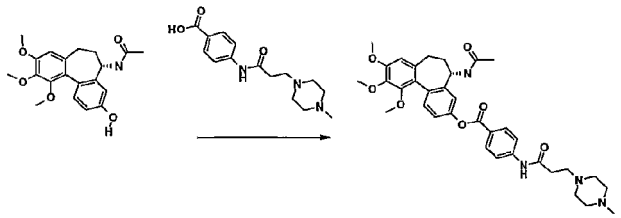
^{1}H NMR (DMSO-d_6) : 1.81-1.97 (m, 1H) ; 1.87 (s, 3H) ; 1.99 (s, 3H) ; 2.04-2.28 (m, 2H) ; 2.28-2.38 (m, 2H) ; 2.39-2.48 (m, 2H) ; 2.48-2.55 (m, 1H, signal partially obscured by DMSO peak) ; 3.42-3.51 (m, 4H) ; 3.56 (s, 3H) ; 3.64 (s, 2H) ; 3.81 (s, 3H) ; 3.87 (s, 3H) ; 4.54-4.67 (m, 1H) ; 6.84 (s, 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



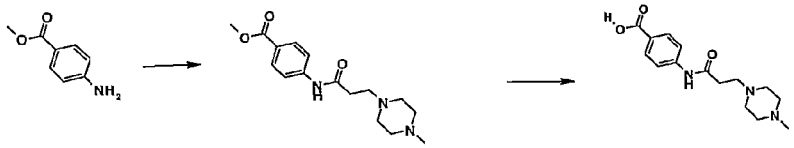
The title compound was prepared using a similar method to that in Example 1 but replacing 5-(4-acetylpiperazin-1-yl)-5-oxopentanoic acid by 4-{3-(4-methylpiperazin-1-yl)propionylamino]benzoic acid.

Yield : 55 %

^1H 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 $[MH]^+$

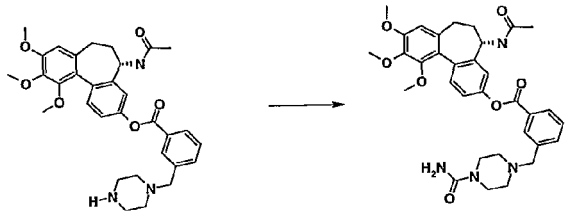
A solution of methyl 4-[3-(4-methylpiperazin-1-yl) propionylamino]benzoate (0.69 g ; 2.26 mmol) in methanol (10 ml) was treated with 2N sodium hydroxide solution (1.25 ml ; 2.48 mmol) at 60°C for 6 hours. After evaporation to dryness, the residue was triturated in acetone. The insoluble material was 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.

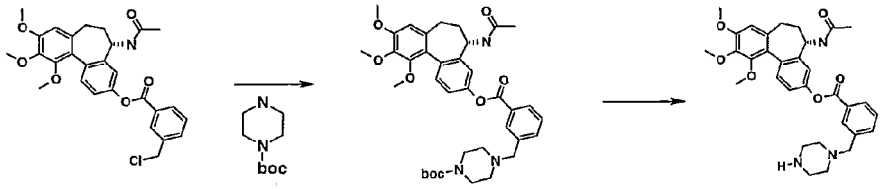
Yield : 87 %

^{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.

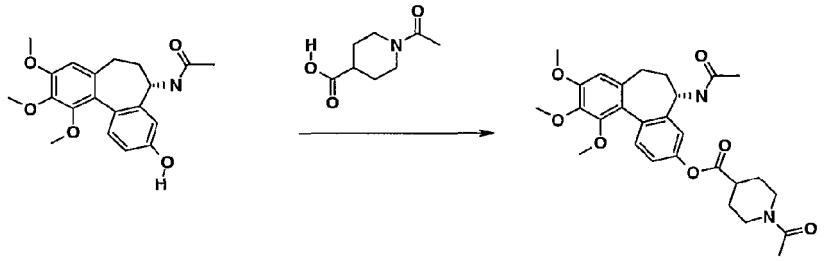
Yield : 55 %

^{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 %

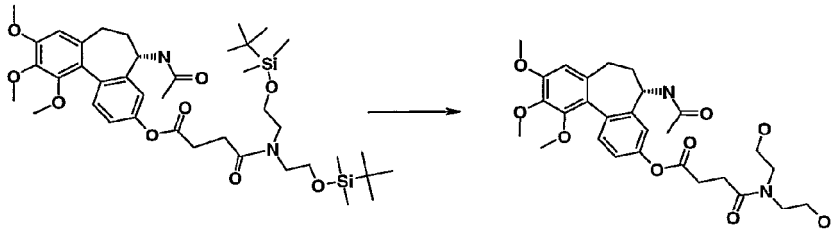
^{1}H NMR (DMSO-d_6) : 1.45-1.60 (m, 1H) ; 1.61-1.75 (m, 1H) ; 1.88-1.93 (m, 2H) ; 1.87 (s, 3H) ; 1.94-2.09 (m, 2H) ; 2.02 (s, 3H) ; 2.10-2.24 (m, 1H) ; 2.53-2.60 (m , 1H, signal partially obscured by DMSO peak) ; 2.75-2.85 (m, 1H) ; 2.88-2.98 (m, 1H) ; 3.14-3.25 (m, 1H) ; 3.26 (bs, 1H) ; 3.50 (s, 3H) ; 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.

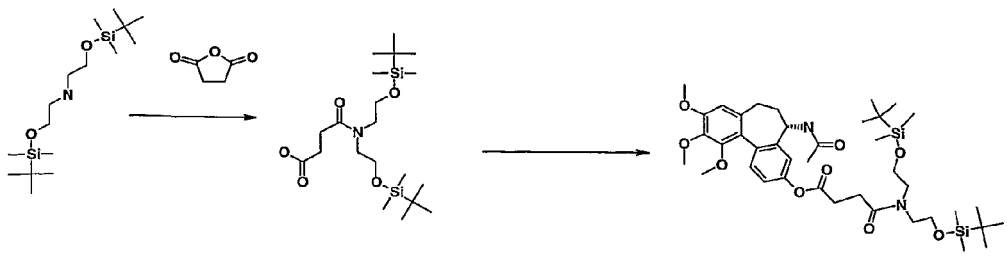
Yield : 66 %

^{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).

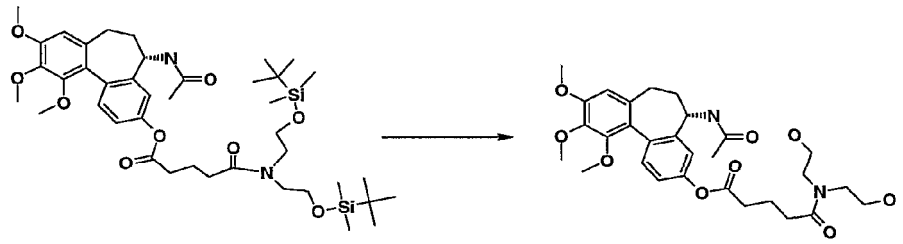
A solution of 3-{N,N-di[2-(tert-butyldimethylsilyloxy)ethyl]carbamoyl}propanoic acid (0.552 g ; 1.27 mmol), EDCI (0.244 g ; 1.27 mmol), DMAP (0.036 g ; 0.294 mmol) in dichloromethane (30 ml) was stirred under argon atmosphere for 30 mins. N-acetyl colchinol (0.35 g ; 0.98 mmol) was added and the mixture 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.

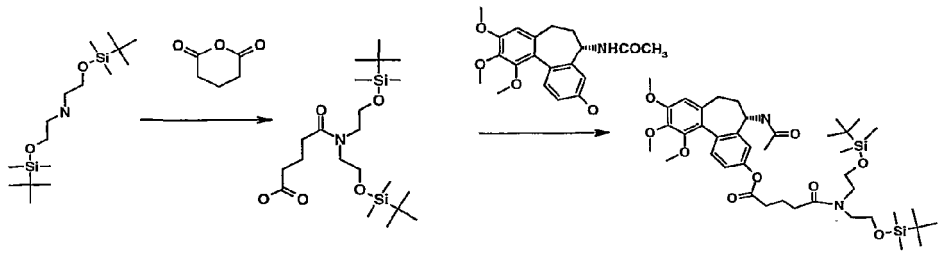
Yield : 37 %

^{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).

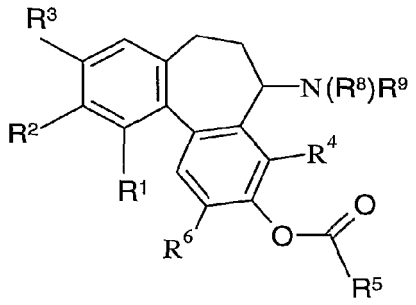
4-{N,N-di[2-(tert-Butyldimethylsilyloxy)ethyl]carbamoyl}butanoic acid was reacted with N-acetyl colchinol using the similar conditions to that in Example 7, but using 4-{N,N-di[2-(tert-butyldimethylsilyloxy)ethyl]carbamoyl}butanoic acid in place of 3-{N,N-di[2-(tert-butyldimethylsilyloxy)ethyl]carbamoyl}propanoic 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:

$\mathbf{R^1}$, $\mathbf{R^2}$ 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 two of R^1, R^2 and R^3 are C_{1-4}alkoxy;

$\mathbf{R^4}$ and $\mathbf{R^6}$ 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;

$\mathbf{R^5}$ 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);

$\mathbf{X^1}$ 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 $\mathbf{R^{10}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

$\mathbf{Y^1}$ is C_{1-3}alkylene;

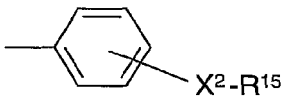
B is carboxy, sulpho, phosphoryloxy, hydroxy, amino, N-(C_{1-4}alkyl)amino, N,N-di (C_{1-3}alkyl)amino, $-R^{12}$ or $-NHC(R^{13})COOH$; (wherein $\mathbf{R^{12}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently 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)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:

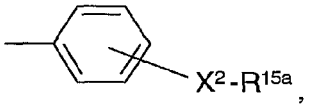
A is ethylene or phenylene;

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:

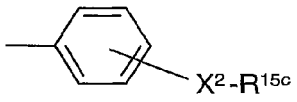
A is ethylene or phenylene;

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);

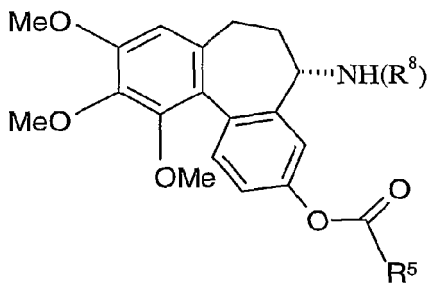
3) R^{22b} -C_{1-4}alkyl- (wherein R^{22b} is as hereinabove defined); or

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):

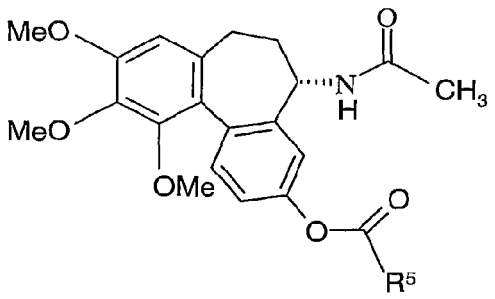


(II)

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:

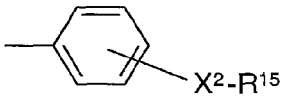
A is ethylene or phenylene;

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:

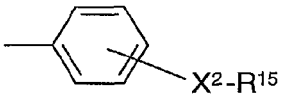
A is ethylene or phenylene;

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:

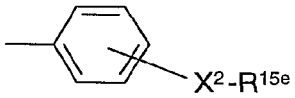
A is phenylene;

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)benzoatc;

(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.

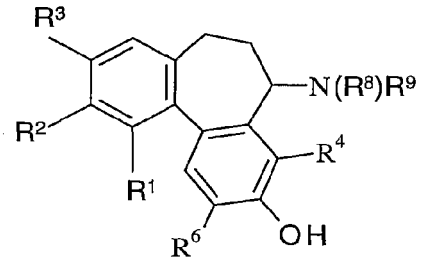
12. The use of a compound according any one of claims 1 to 10, or a pharmaceutically-acceptable salt, solvate or pro-drug thereof, in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm-blooded animal.

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:

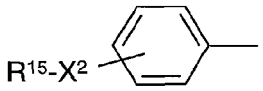
(a) reacting a compound of the formula (X):



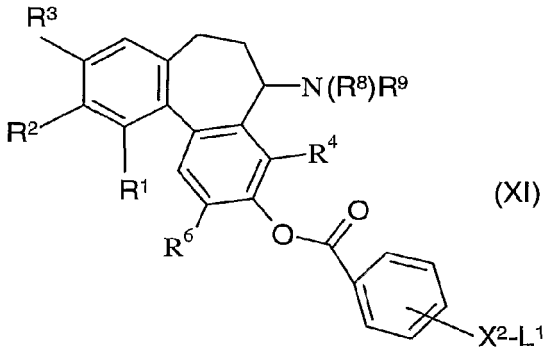
(X)

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);

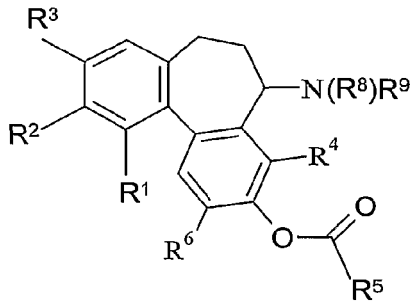
ii) removing any protecting groups;

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:

$\mathbf{R^1}$, $\mathbf{R^2}$ 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 two of R^1, R^2 and R^3 are C_{1-4}alkoxy;

$\mathbf{R^4}$ and $\mathbf{R^6}$ 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;

$\mathbf{R^5}$ 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);

$\mathbf{X^1}$ 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 $\mathbf{R^{10}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

$\mathbf{Y^1}$ is C_{1-3}alkylene;

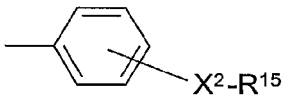
B is carboxy, sulpho, phosphoryloxy, hydroxy, amino, N-(C_{1-4}alkyl)amino, N,N-di (C_{1-3}alkyl)amino, $-R^{12}$ or $-NHC(R^{13})COOH$; (wherein $\mathbf{R^{12}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently 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)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:

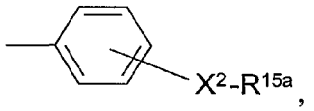
A is ethylene or phenylene;

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-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:

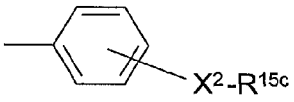
A is ethylene or phenylene;

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);

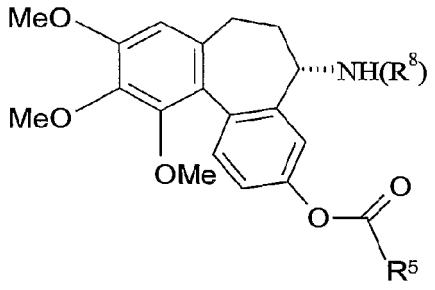
3) R^{22b} -C_{1-4}alkyl- (wherein R^{22b} is as hereinabove defined); or

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):

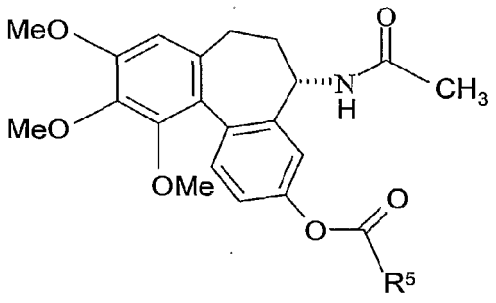


(II)

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:

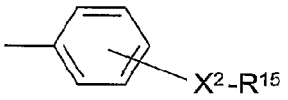
A is ethylene or phenylene;

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:

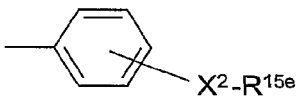
A is phenylene;

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-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.

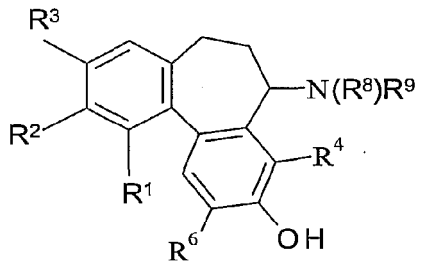
12. The use of a compound according any one of claims 1 to 10, or a pharmaceutically-acceptable salt, solvate or pro-drug thereof, in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm-blooded animal.

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:

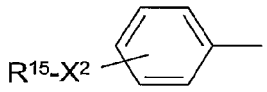
(a) reacting a compound of the formula (X):



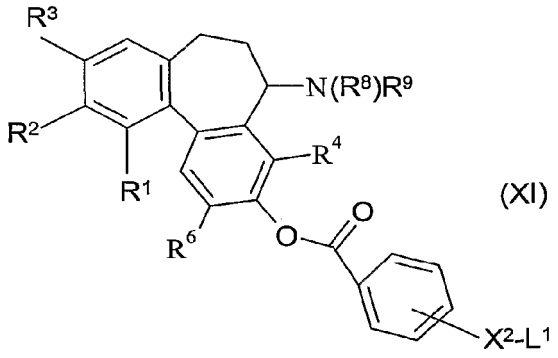
(X)

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.

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)

INTERNATIONAL SEARCH REPORT

Int. ...lication No
PCT/GB 01/02966

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

Form PCT/ISA/210 (patent family annex) (July 1992)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 January 2002 (31.01.2002)

PCT

(10) International Publication Number
WO 02/08213 A1

(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

(22) International Filing Date: 4 July 2001 (04.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
00401977.4 7 July 2000 (07.07.2000) EP
00401976.6 7 July 2000 (07.07.2000) EP

(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 blond (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-$Y^1(CH_2)_eR^{11}$ 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.

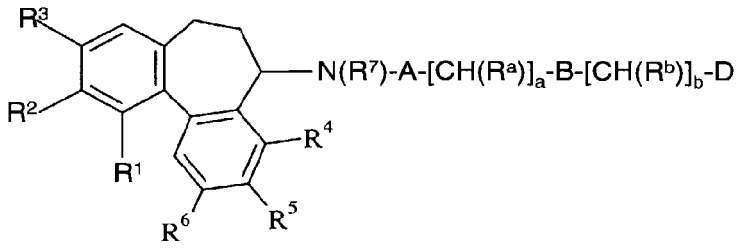
Reversal of neovascularisation by damaging the newly-formed vascular endothelium is expected to have a beneficial therapeutic effect. The present invention is based on the discovery of tricyclic compounds that surprisingly specifically damage newly formed vasculature without affecting the normal, established 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;

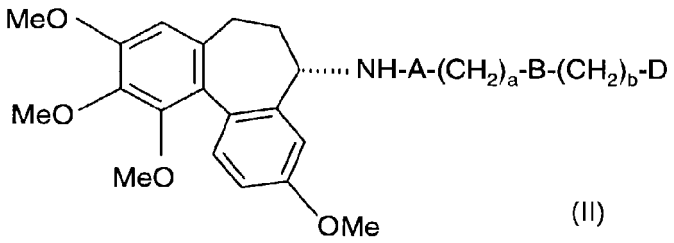
b is 0 or 1;

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;

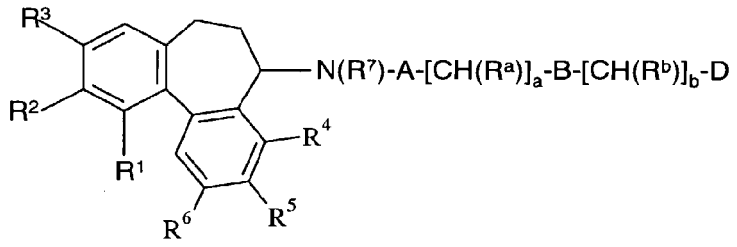
B is -CO-, -NHCO- or a single direct bond;

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:

$\mathbf{R^1}$, $\mathbf{R^2}$ 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_{2-3}alkyl, aminoC_{2-3}alkyl or hydroxyC_{2-3}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_{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 $\mathbf{R^{14}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or

nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O 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;

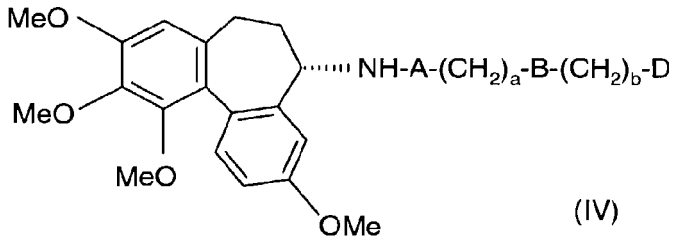
b is 0 or 1;

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-1-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.

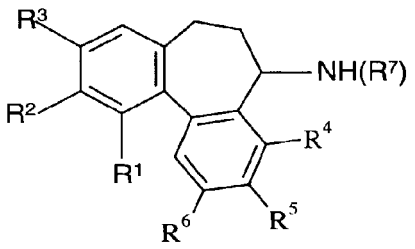
A suitable protecting group for a carboxy group is, for example, an esterifying group, for example a C_{1-4}alkyl group (especially methyl or ethyl) which may be removed, for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide; or for 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 -20^0C 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

$HO\text{-}[CH(R^a)]_a\text{-}B\text{-}[CH(R^b)]_b\text{-}D$ under similar conditions to those described above for the reaction of a compound of the formula (X) with a compound of the formula $L^2\text{-}[CH(R^a)]_a\text{-}B\text{-}[CH(R^b)]_b\text{-}D$ 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 $C{\equiv}N\text{-}[CH(R^a)]_a\text{-}B\text{-}[CH(R^b)]_b\text{-}D$. 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 $R^{17}\text{-}NH_2$ under similar conditions to those described above for the reaction of a compound of the formula (X) with a compound of the formula $L^2\text{-}[CH(R^a)]_a\text{-}B\text{-}[CH(R^b)]_b\text{-}D$ 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:

$HO-[CH(R^7)]_a - B -[CH(R^7)]_b-D$

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 ^{o}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;

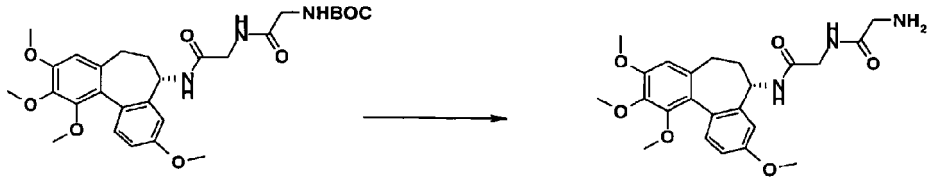
(v) intermediates were not generally fully characterised and purity was assessed by thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), infra-red (IR) or NMR analysis;

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.

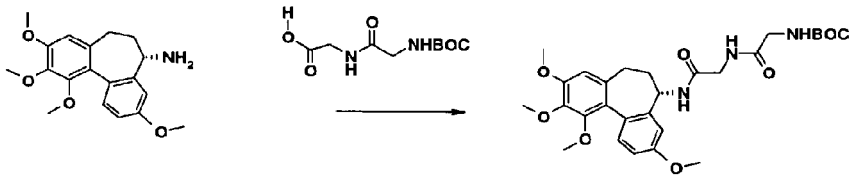
Yield : 65 %

^{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.

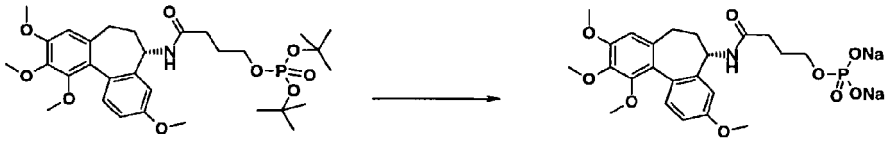
Yield : 65 %.

^{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

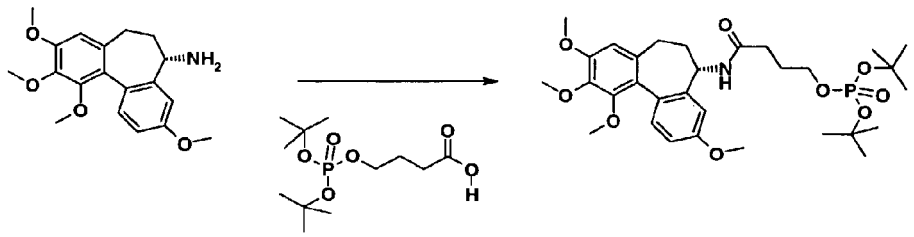


A solution of N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-4-[di(tert-butoxy)phosphoryloxy]butanamide (0.529g; 0.892 mmol) in a mixture of (12N) HCl (5 ml) and dioxan (25 ml) was stirred under argon atmosphere for 4 hours. After evaporation of the dioxan, the pH was adjusted 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:



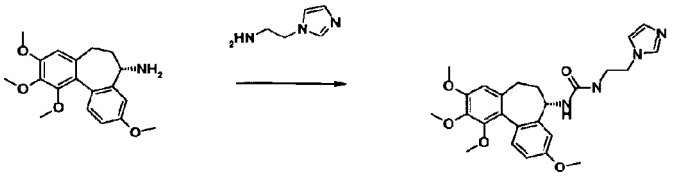
N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-4-[di-(tert-butoxy)phosphoryloxy]butanamide was prepared using a similar method to that of Example 1 by reacting (5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine with 4-[di(tert-butoxy)phosphoryloxy]butanoic 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 %.

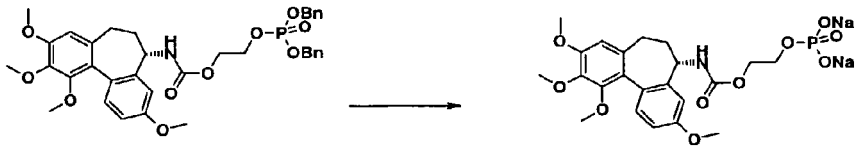
^{1}H NMR (DMSO-d_6) : 1.66-1.77 (m, 1H) ; 1.97-2.10 (m, 1H) ; 2.13-2.25 (m, 1H) ; 2.53 partially obscured by DMSO peak (m, 1H) ; 3.12-3.32 (m, 2H) ; 3.47 (s, 3H) ; 3.77 (s, 3H) ; 3.79 (s, 3H) ; 3.83 (s, 1H) ; 3.94 (t, 3H) ; 4.32-4.42 (m, 1H) ; 5.97 (t, 1H) ; 6.63 (d, 1H) ; 6.77 (s, 1H) ; 6.83-6.92 (m, 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.

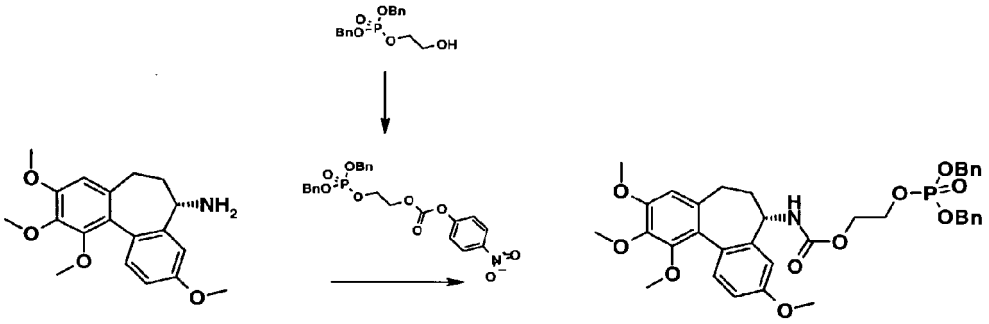
Yield : 83 %

^{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.

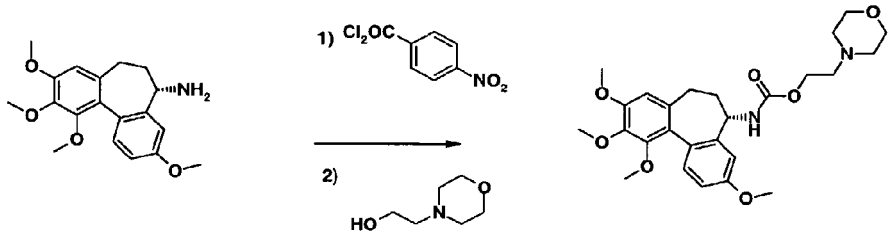
Yield : 85 %

^{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 %

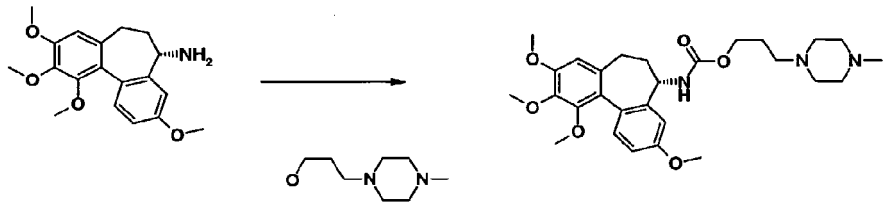
^{1}H NMR Spectrum (DMSO-d_6 + AcO-d_4) : 1.82-2.31 (m, 3H) ; 2.44 (m, 4H) ; 2.49 (m, 2H) ; 2.57 partially obscured by DMSO peak (m, 1H) ; 3.47 (s, 3H) ; 3.56 (m, 4H) ; 3.78 (s, 3H) ; 3.79 (s, 3H) ; 3.83 (s, 3H) ; 4.03 (m, 2H) ; 4.17-4.33 (m, 1H) ; 6.76 (s, 1H) ; 6.88 (dd, 1H) ; 6.93 (d, 1H) ; 7.26 (d, 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 %.

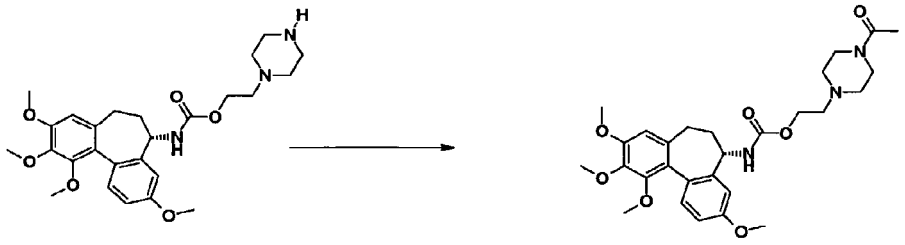
^{1}H NMR (DMSO-d_6) : 1.62-2.44 (m, 20H) ; 2.54 partially obscured by DMSO peak (m, 1H) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.78 (s, 3H) ; 3.82 (s, 3H) ; 6.78 (s, 1H) ; 6.89 (dd, 1H) ; 6.93 (d, 1H) ; 7.27 (d, 1H) ; 7.80 (d, 1H).

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.

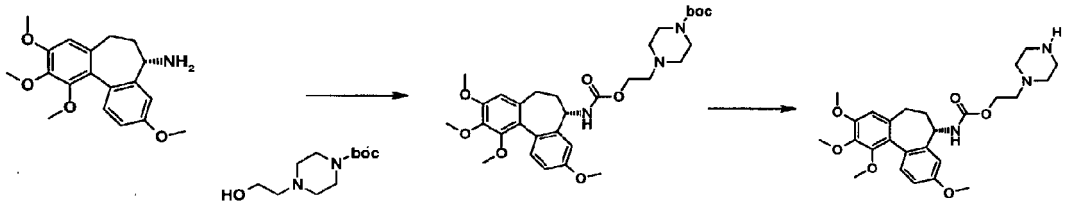
Yield : 90 %

^{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

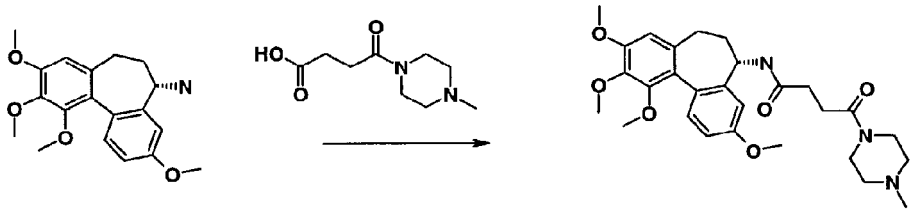
buffer (2 g/l, pH 7) to give 2-(piperazin-4-yl) ethyl- N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl] carbamate.

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 %

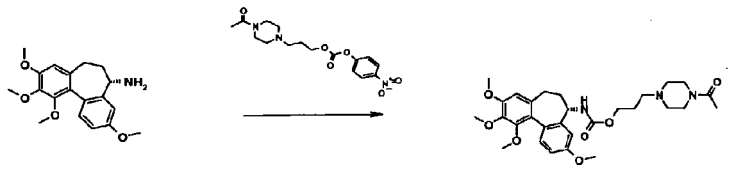
^{1}H NMR (DMSO-d_6) : 1.85-1.96 (m, 1H) ; 2.01-2.15 (m, 1H) ; 2.16 (s, 3H) ; 2.22 (t, 2H) ; 2.26 (t, 2H) ; 2.33-2.42 (m, 1H) ; 2.47-2.53 (m, 1H) ; 3.35-3.46 (m, 4H) ; 3.46 (s, 3H) ; 3.79 (s, 3H) ; 3.82 (s, 3H) ; 3.84 (s, 3H) ; 4.44-4.56 (m, 1H) . 6.79 (s, 1H) ; 6.86 (dd, 1H) ; 6.98 (d, 1H) ; 7.25 (d, 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.

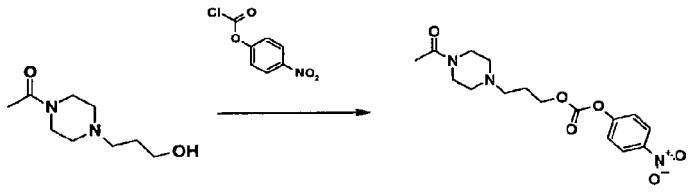
Yield : 80 %

^{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 :

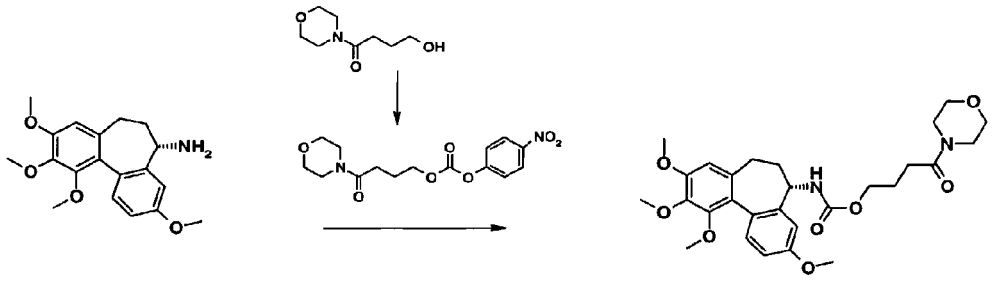


4-Nitrophenyl chloroformate (0.733 g ; 3.63 mmol) was added to a solution of 3-(4-acetylpiperazin-1-yl) propanol [Synthesis (1997), 6, 643-648] (0.645 g ; 3.46 mmol) and triethylamine (0.51 ml ; 3.36 mmol) in dichloromethane (7 ml) under argon atmosphere at 0°C. The mixture was stirred at ambient temperature 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

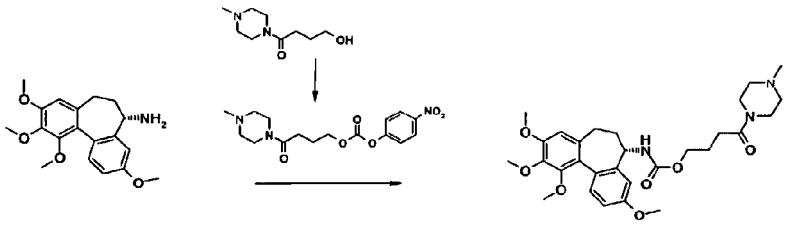
The starting material was prepared using a similar method to that of example 9, starting from 4-morpholino-4-oxobutyl 4-nitrophenyl carbonate.

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

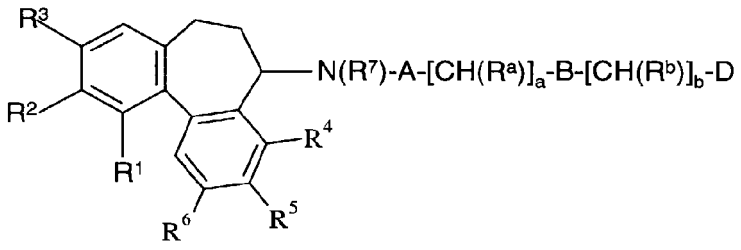
The starting material was prepared using a similar method to that of Example 9, from 4-(4-methylpiperazin-l-yl)-4-oxobutanol.

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;

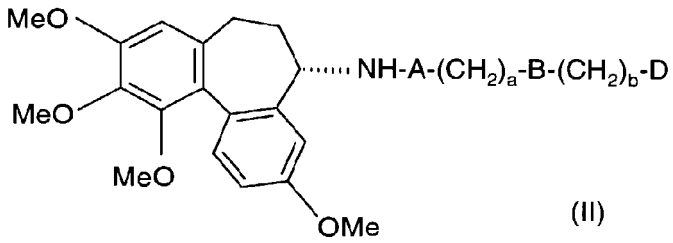
D is carboxy, phosphoryloxy, hydroxy, amino, N-C_{1-4} alkylamino, N,N-di(C_{1-4} alkyl)amino or of the formula $-Y^1(CH_2)_cR^{11}$ (wherein $\mathbf{Y^1}$ is -NHC(O)- or -C(O)NH-; **c** is 1 or 2; $\mathbf{R^{11}}$ is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring nitrogen 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;

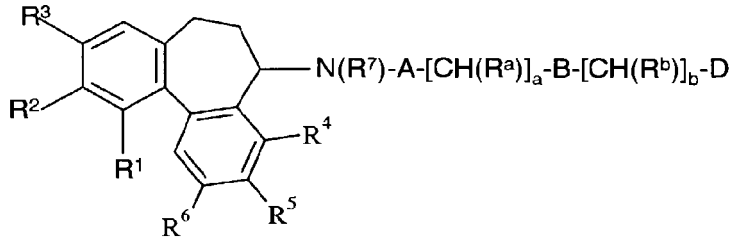
b is 0 or 1;

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:

$\mathbf{R^1}$, $\mathbf{R^2}$ 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_{2-3}alkyl, aminoC_{2-3}alkyl or hydroxyC_{2-3}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_{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 $\mathbf{R^{14}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O 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.

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;

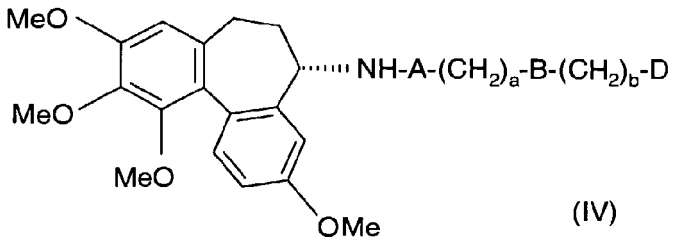
b is 0 or 1;

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]acetamidc;

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.

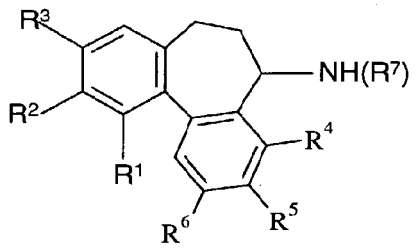
14. A pharmaceutical composition comprising a compound according to any one of claims 1 to 13 or a pharmaceutically acceptable salt, solvate or pro-drug thereof, in association with a pharmaceutically acceptable carrier.

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
IPC 7 C07D295/185 A61K31/495 A61P35/00 C07K5/062 C07F9/09
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.

° 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
18 September 2001	26/09/2001

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	Sánchez García, J.M.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 01/02964

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

Patents JP2017503030

CRF1 receptor antagonist for the treatment of congenital adrenocortical hyperplasia

Abstract

translated from Japanese

CRF1 receptor antagonists directly inhibit ACTH release in patients with CAH, thereby allowing normalization of androgen production while using lower, more physiological doses of hydrocortisone, thus reducing treatment-related side effects. Has the ability to reduce. In one embodiment, a method for treating CAH is provided by administering to a subject in need an effective amount of a CRF1 antagonist, including but not limited to bedtime administration. The

Images (6)



Classifications

- A61K31/519 Pyrimidines; Hydrogenated pyrimidines, e.g. trimethoprim ortho- or peri-condensed with heterocyclic rings

View 4 more classifications

JP2017503030A

Japan

Download PDF Find Prior Art Similar

Other languages: Japanese

Inventor: ディミトリオー、グリゴリアディス、,ディミトリオー、グリゴリアディス、

Worldwide applications

2015 CA MX JP WO AU EP NZ CA KR RU HK CN CN RU HK 2016 IL 2018 US 2019 JP 2020 JP AU US 2021 US IL JP

Application JP2016565135A events

2014-01-21	Priority to US201461929941P
2015-01-21	Application filed by ニューロクライン バイオサイエンシーズ、インコーポレイテッド、ニューロクライン バイオサイエンシーズ、インコーポレイテッド
2017-01-26	Publication of JP2017503030A
2018-03-01	Publication of JP2017503030A5
2019-10-02	Application granted
2019-10-02	Publication of JP6588562B2
Status	Active
2035-01-21	Anticipated expiration

Show all events

Info: Patent citations (57), Cited by (9), Legal events, Similar documents, Priority and Related Applications

External links: Espacenet, Global Dossier, Discuss

Claims (21)

Hide Dependent

translated from Japanese

A method for treating congenital adrenocortical hyperplasia (CAH) by administering to a subject in need a CRF $_1$ receptor antagonist having a dissociation half-life of greater than 30 minutes. The method of claim 1, wherein the CRF $_1$ receptor antagonist has a dissociation half-life of greater than 40 minutes. The method of claim 1, wherein the CRF $_1$ receptor antagonist has a dissociation half-life of greater than 50 minutes. Said 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). The CRF $_1$ receptor antagonists include NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalalmine (N-butyl-N-ethyl-2,5,6-trimethyl-7- (2,4,6- The method according to claim 1, which is 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. 6. The method of any one of claims 1-5, wherein the CRF $_1$ receptor antagonist is administered during or prior to ACTH release with a predicted circadian rhythm. 8. The method of claim 7, wherein the CRF $_1$ receptor antagonist is administered 3-4 hours prior to ACTH release with a predicted circadian rhythm. A method for reducing the levels of 17-OHP and ACTH in a subject having congenital adrenocortical hyperplasia (CAH) comprising administering to the subject a CRF $_1$ receptor antagonist at bedtime. Including the method. 10. The method of claim 9, wherein the CRF $_1$ receptor antagonist is administered at or prior to ACTH release with a predicted circadian rhythm. 10. The method of claim 9, wherein the CRF $_1$ receptor antagonist is administered 3-4 hours prior to ACTH release with a predicted circadian rhythm. Said CRF $_1$ receptor antagonist is compound I (NBI-77860; 2,5-dimethyl-3- [2-methyl-4- (methyloxy) phenyl] -N-[(1S) -1- (3-methyl- The method according to any one of claims 9 to 11, which is 1,2,4-oxadiazol-5-yl) propyl] pyrazolo [1,5-a] pyrimidin-7-amine). The CRF $_1$ receptor antagonists include NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalalmine (N-butyl-N-ethyl-2,5,6-trimethyl-7- (2,4,6- The method according to any one of claims 9 to 11, which is (trimethylphenyl) pyrrolo [3,2-e] pyrimidin-4-amine) or DMP904. A CRF $_1$ receptor antagonist for use in treating congenital adrenal hyperplasia (CAH), wherein the CRF $_1$ receptor antagonist has a dissociation half-life greater than 30 minutes, CRF $_1$ receptor antagonist. The CRF $_1$ receptor antagonist has a dissociation half-life greater than 40 minutes, CRF $_1$ receptor antagonist of claim 14. The CRF $_1$ receptor antagonist has a dissociation half-life greater than 50 minutes, CRF $_1$ receptor antagonist of claim 14. Said CRF $_1$ receptor antagonist is compound I (NBI-77860; 2,5-dimethyl-3- [2-methyl-4- (methyloxy) phenyl] -N-[(1S) -1- (3-methyl- The CRF $_1$ receptor antagonist according to claim 14, which is 1,2,4 oxadiazol-5-yl) propyl] pyrazolo [1,5-a] pyrimidin-7-amine). The CRF $_1$ receptor antagonists include NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalalmine (N-butyl-N-ethyl-2,5,6-trimethyl-7- (2,4,6- The CRF $_1$ receptor antagonist according to claim 14, which is (trimethylphenyl) pyrrolo [3,2-e] pyrimidin-4-amine) or DMP904. The CRF $_1$ receptor antagonist is suitable for administration at bedtime, CRF $_1$ receptor antagonist

according to any one of claims 14 to 18. The CRF_1 receptor antagonist is suitable for administration at the time of ACTH release by circadian rhythms predicted or before that, CRF_1 receptor antagonist according to any one of claims 14 to 18. The CRF_1 receptor antagonist is suitable for administration to 3-4 hours prior to ACTH release by circadian rhythm expected, CRF_1 receptor antagonist according to any one of claims 14 to 18.

Description

translated from Japanese

(background)
(Technical field)
Compositions and methods for treating congenital adrenocortical hyperplasia are described herein.

(Description of related technology)
Congenital adrenocortical hyperplasia (CAH) is a group of autosomal recessive genetic disorders that produce little or no cortisol biosynthesis. The most frequent form of the disease is a 21-hydroxylase deficiency caused by mutations in the CYP21A2 gene located on chromosome 6p21, accounting for about 95% of CAH cases (for review see, for example, Speiser et al. , Int. J. Pediatr. Endocrinol. 2010; 494173 (2010)). These mutations can range from a complete loss of enzyme activity required for cortisol synthesis in the adrenal cortex to a range of partial losses resulting in disease severity that is a direct consequence of certain mutations. This continuous form of 21-hydroxylase deficiency is more commonly known as salt-loss and simple maleized forms (grouped as classical CAH) and non-classical CAH (NCCAH) or "late-onset" CAH. It has been broadly classified into a mild form, which is usually diagnosed in late childhood or early adulthood. Nonclassical CAH patients have either homozygous or complex heterozygotes, often with a classical CAH allele. These patients have sufficient enzymatic activity (> 20-50% normal), so that they have no salt loss or cortisol deficiency, have normal external genitals at birth, and many it remains asymptomatic throughout life (Trap et al., Steroids 77 (4), 342-46 (2012)). In the less frequent form of the disease (which accounts for 5% of cases), mutations in the 11β-hydroxylase gene CYP11B1 result in CAH (11β-OH CAH).
Both genetic variations result in congenital adrenocortical hyperplasia, cortisol deficiency and excessive adrenocorticotropic hormone (ACTH) production, along with androgen overproduction. These patients require lifelong management with glucocorticoids and the problems associated with such treatment. Thus, treatment regimens are very necessary to improve health, well-being, quality of life, and to manage associated disorders in patients with CAH.

Speiser et al. , Int. J. et al. Pediatr. Endocrinol. 2010. 494173 (2010) Trapp et al. , Steroids 77 (4). 342-46 (2012)

(Summary)
Corticotropin releasing factor (CRF) activates CRF_1 receptor, a class B G protein-coupled receptor (GPCR). CRF_1 antagonists directly inhibit ACTH release in patients with CAH, thereby allowing normalization of androgen production while using lower, more physiological doses of hydrocortisone and reducing treatment-related side effects Have

In one embodiment, a method for treating CAH is provided by administering to a subject in need an effective amount of a CRF_1 antagonist, including but not limited to bedtime administration, is done.

In a more specific embodiment, the CRF_1 antagonist has a dissociation half-life ($t_{1/2}$) greater than 30 minutes, and in another embodiment greater than 40 minutes, and in another embodiment greater than 50 minutes. Have.

Embodiment 1 A method for treating congenital adrenocortical hyperplasia (CAH) by administering to a subject in need a CRF_1 receptor antagonist having a dissociation half-life of greater than 30 minutes.

Embodiment 2 The method of embodiment 1, wherein the CRF_1 receptor antagonist has a dissociation half-life of greater than 40 minutes.

Embodiment 3 The method of embodiment 1, wherein the CRF_1 receptor antagonist has a dissociation half-life of greater than 50 minutes.

Embodiment 4 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) according to any one of embodiments 1-3. .

Embodiment 5 The CRF_1 receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmine (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 to 5, wherein the CRF_1 receptor antagonist is administered at bedtime.

Embodiment 7 The method of any one of Embodiments 1 to 6, wherein the CRF_1 receptor antagonist is administered during or prior to ACTH release by a predicted circadian rhythm.

Embodiment 8 The method of embodiment 7, wherein the CRF_1 receptor antagonist is administered 3-4 hours before ACTH release with a predicted circadian rhythm.

Embodiment 9 A method for reducing the levels of 17-OHP and ACTH in a subject having congenital adrenocortical hyperplasia (CAH) comprising: subjecting the subject to a CRF_1 receptor antagonist at bedtime Administering a step of administering

Embodiment 10 The method of embodiment 9, wherein the CRF_1 receptor antagonist is administered during or prior to ACTH release by a predicted circadian rhythm.

Embodiment 11 The method of embodiment 9 or embodiment 10, wherein the CRF_1 receptor antagonist is administered 3-4 hours prior to ACTH release with a predicted circadian rhythm.

Embodiment 12 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) according to any one of embodiments 9-11. .

Embodiment 13 The CRF_1 receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmine (N-butyl-N-ethyl-2,5,6-trimethyl-7- (2,4 , 6-trimethylphenyl) pyrrolo [3,2-e] pyrimidin-4-amine), or DMP904.

A CRF_1 receptor antagonist for use in treating an embodiment 14 Congenital adrenal hyperplasia (CAH), wherein the CRF_1 receptor antagonist has a dissociation half-life greater than 30 minutes, CRF_1 receptors Antagonists.

Embodiment 15 wherein CRF_1 receptor antagonist has a dissociation half-life greater than 40 minutes, CRF_1 receptor antagonists described 14.

Embodiment 16 wherein CRF $_1$ receptor antagonist has a dissociation half-life greater than 50 minutes, CRF $_1$ receptor antagonist of embodiment 14.

Embodiment 17 The CRF $_1$ receptor antagonist is compound I (NBI-77860; 2,5-dimethyl-3- [2-methyl-4- (methyloxy) phenyl] -N-[(1S) -1- (3 CRF according to any one of embodiments 14 to 16, which is -methyl-1,2,4-oxadiazol-5-yl) propyl] pyrazolo [1,5-a] pyrimidin-7-amine). $_1$ receptor antagonist.

Embodiment 18 The CRF $_1$ receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmine (N-butyl-N-ethyl-2,5,6-trimethyl-7- (2,4 , 6-trimethylphenyl) pyrrolo [3,2-e] pyrimidin-4-amine), or DMP904 CRF $_1$ receptor antagonist according to any one of embodiments 14-16.

CRF $_1$ receptor antagonist of embodiment 19 wherein the CRF $_1$ receptor antagonists, which have any one of embodiments 14 to 18 suitable for administration at bedtime.

Embodiment 20 wherein CRF $_1$ receptor antagonists, CRF $_1$ receptor antagonist according to which has any one of embodiments 14-19 suitable for administration at the time of ACTH release by circadian rhythms predicted or before that .

Embodiment 21 wherein CRF $_1$ receptor antagonists, CRF $_1$ receptor according to which has any one of embodiments 14 to 20 suitable for administration to 3-4 hours prior to ACTH release by circadian rhythms predicted Antagonists.

In other embodiments, the methods and uses described above and herein provide the amount of glucocorticoid or mineralocorticoid to a full grown subject (eg, a human subject) with CAH. At least 10%, 15%, 20%, 30%, 40%, 50% from the recommended daily dose of GC to be administered (eg, hydrocortisone (HC), prednisone, prednisolone, dexamethasone, or fludrocortisone) And a step of reducing by 60%. In other embodiments, the methods and uses described above and herein provide that an amount of glucocorticoid or mineralocorticoid is administered to a growing subject (eg, a human subject) having CAH. Reducing at least 10%, 15%, 20%, 30%, 40%, 50%, 60% from the recommended daily dose of GC (eg, hydrocortisone) or mineral corticoid (eg, fludrocortisone) is further included.

These and other embodiments will be apparent upon reference to the following detailed description. To this end, various references describing in more detail certain background information, procedures, compounds and compositions are set forth herein, each of which is incorporated herein by reference in its entirety. Is done.

Terms not specifically defined herein are intended to be given the meanings given to them by those skilled in the art in view of the present disclosure and context. However, as used herein, unless specified to the contrary, the above terms have the meaning indicated. Throughout this specification, references to "one embodiment" or "an (one) embodiment" include at least one particular feature, structure, or characteristic described in connection with the above embodiment. Means. Thus, the appearances of the phrases "in one embodiment" or "in one (one) 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 herein and in the appended claims, the singular forms "a" (one), "an" (one), and "the" (above, this, that) "includes a reference to the plural unless the context clearly indicates otherwise. Thus, for example, reference to "a non-human animal" can refer to one or more non-human animals, or a plurality of such animals, and "a cell" Alternatively, reference to "the cell" includes reference to one or more cells and equivalents thereof (eg, a plurality of cells) known to those of skill in the art, and the like. If a method step is described or claimed and said steps are described to occur in a particular order, the second step "before" (ie, before) the second step occurs (or takes place) The description of the first step has the same meaning when the second step is rewritten to a state that occurs (or is performed) "following" the first step. The term "about" refers to a number or numerical range where the referenced number or numerical range is within experimental variability (or within statistical experimental error). It is an approximation, meaning that its number or numerical range can vary between 1% and 15% of the stated number or numerical range. Note also that the term "or (or or)" is generally used in its sense including "and / or" (and / or) unless the context clearly indicates otherwise. Shall. The term "at least one" has the same meaning and understanding as the term "one or more", for example when referring to at least one compound or at least one composition.

FIG. 1 shows a schematic diagram of a steroid synthesis pathway in the adrenal gland. The most common form of CAH is caused by a 21-hydroxylase (also called 21-α hydroxylase) deficiency, resulting in a decrease in cortisol and an increase in androgens (eg, testosterone and estrogens). A rarer type of classical CAH is 11β-hydroxylase deficiency.

FIG. 2 presents a graph illustrating the effect of Compound I (NBI-77860) on ACTH levels in adrenalectomized rats. Rats were given orally 30 mg / kg Compound I (NBI-77860). Data are expressed as mean plasma concentration of ACTH (± SEM).

FIGS. 3A-C represent graphs illustrating the effect of CRF $_1$ receptor antagonists on ACTH concentrations (distinguished by their dissociation half-life) in adrenalectomized rats.

FIG. 4 is a study design schematic of the clinical trial described in Example 6, designed to evaluate the safety, tolerability and plasma exposure of NBI-77860, and HPA (hypothalamic-pituitary-adrenal).) Represents the effect of this compound on endogenous levels of system hormones.

FIG. 5 represents mean 17-OHP (upper panel) and ACTH levels (lower panel) data during the 24 hour period after administration during the clinical study described in Example 6.

FIG. 6 depicts specific individual subject responses for 17-OHP (upper panel) and ACTH levels (lower panel) over time after administration of 300 mg and 600 mg of NBI-77860 and placebo.

(Detailed explanation)
As described herein, CRF $_1$ receptor antagonists were found to directly inhibit ACTH release in patients with CAH, thereby allowing normalization of androgen production. Administration of a CRF $_1$ receptor antagonist allows the use of lower and more physiological doses of hydrocortisone in subjects with CAH, thus reducing treatment-related side effects.

Newborn screening for CAH is performed by an immunoassay that measures 17-OHP levels in heelstick capillary blood specimens obtained within 72 hours of birth. The blood samples are analyzed for 17-OHP by a commercial dissociation enhanced lanthanide fluorescence immunoassay (DELFIA; PerkinElmer, Waltham Massachets) (White et al., J. Pediatr. 163: 10-12 (2013)).

Nine states in the United States are using the second number of screening tests between 8 and 14 days after birth using biochemical and molecular genetic testing methods, with five more strongly Recommended. Such biochemical methods include immunoassay or liquid chromatography using organic solvent extracts followed by tandem mass spectrometry to determine the steroid ratio of 17-OHP, androstenedione, and 21-deoxycortisol to cortisol. (See, for example, Speiser et al., Int. J. Pediatr. Endocrinol. 2010: 494173, 2010). Genetic screening examines CYP21A2 mutations associated with CAH. Although not widely used in the United States, the addition of a second screen could potentially improve the sensitivity of the entire screening process (here, about 72% for the first screen alone).

Without newborn screening results, young girls with classic CAH are typically identified due to the presence of external genitals with ambiguous gender. Because men have normal external genitals when born, they are not diagnosed unless newborn screening is performed or other medical complications are noticed. Infants who are not initially diagnosed with CAH and experience the salt-losing form of the disease are later diagnosed in the context of insufficient weight gain, vomiting, hyperkalemia and hyponatremia within the first few weeks of life. The

Treatment of CAH is based on normalization of hormone and steroid levels using various drug therapies from diagnosis in childhood to adulthood. Glucocorticoids are currently the standard treatment in CAH and are used to both correct endogenous cortisol deficiency and reduce elevated pituitary ACTH levels (which drive increased androgen production) is done. Unlike the treatment of Addison's disease (decrease in adrenal function), where cortisol replacement is sufficient, treatment of CAH must also reduce ACTH production and control subsequent androgen excess. Thus, the goal of glucocorticoid treatment is to prevent cortisol supplementation and ACTH suppression in women to prevent masculinization and menstrual disturbances and to inhibit testicular adrenal rest tumor in men. Including. Mineralocorticoid supplementation is required to achieve normal plasma renin activity for maintenance of normal blood pressure, electrolyte balance, and volume state in those patients with a salt-loss form of CAH.

Glucocorticoid treatment regimens support normal physiology and also ensure that sufficient cortisol is available during events that can elicit strong stress responses (eg, comorbidities, exercise, hypotension) Must be secured. Careful monitoring is also necessary to avoid the occurrence of iatrogenic Cushing syndrome due to glucocorticoid overtreatment in an attempt to sufficiently suppress androgen production or Addison syndrome due to inadequate treatment. While overtreatment with mineralocorticoids can cause hypertension, inadequate treatment can lead to hypotension, salt loss, fatigue and increased demand for glucocorticoids Typical laboratory tests to monitor the efficacy of treatment include measurement of 17-OHP, androstenedione, testosterone, renin activity, and electrolyte plasma concentrations.

Adult patients with CAH have an increased prevalence of cardiovascular risk factors including obesity, hypertension, and insulin resistance (eg, Kim et al., Semin Reprod. Med. 27 (4): 316-21 (2009)). From a large cohort study of pediatric and adult CAH patients (n = 244), patients frequently experience inadequate hormonal control and the aforementioned adverse outcomes despite being prescribed various glucocorticoid treatment regimens. (See, for example, Finkelstain et al., J. Clin. Endocrinol Metab. 97 (12): 4429-38 (2012)).

Treatment of CAH involves glucocorticoids (usually hydrocortisone in children but often more potent drugs with a narrow therapeutic index, such as dexamethasone in adults), and mineralocorticoids (if needed for salt loss) This includes attempts to normalize cortisol deficiency (usually fludrocortisone). However, the glucocorticoid dose required to achieve sufficient suppression of excess androgens is usually sufficient for normal physiological doses used only for cortisol replacement, as in patients with Addison's disease. It has exceeded. This increased exposure to glucocorticoids can result in iatrogenic Cushing syndrome, increased cardiovascular risk factors, impaired glucose tolerance, and decreased bone mineral density in CAH patients (eg, Elnecave et al M., J. Pediatr Endocrinol.Metab.21: 1155-62 (2008); King et al., J.Clin.Endocrinol.Metab.91 (3): 8566-69 (2006); Migeon et al. Metab. Clin. North Am. 30: 193-206 (2001)).

Adrenocorticotropic hormone releasing factor (CRF) was isolated from sheep hypothalamus and identified as a 41 amino acid peptide. CRF has been found to produce significant changes in the functions of the endocrine system, nervous system, and immune system. CRF is the main physiological of basal and stress-induced release of adrenocorticotropic hormone ("ACTH"), β-endorphin, and other proopiomelanocortins ("POMC") (anterior pituitary derived peptides) It is thought to be a regulator (see, eg, Vale et al., Science 213: 1394-1397, 1981). Secretion of CRF causes ACTH release from corticotrophs in the anterior pituitary gland through binding to the CRF $_1$ receptor, a member of the class B family of G protein coupled receptors.

Due to the physiological importance of CRF, the development of biologically active small molecules that have significant CRF $_1$ receptor binding activity and can antagonize the CRF $_1$ receptor is still a desired goal, anxiety, it continues to be the subject of ongoing research and development for the treatment of depression, irritable bowel syndrome, post-traumatic stress disorder, and substance abuse.

The pituitary hormone ACTH is under the control of the hypothalamic corticotropin releasing factor (CRF) and stimulates cholesterol uptake and drives the synthesis of pregnenolone that initiates steroidogenesis in the adrenal gland (see FIG. 1). about). The adrenal cortex is composed of three zones, which produce distinct classes of hormones, many of which are driven by ACTH, which mobilizes cholesterol through this pathway. Loss of these enzymes as a result of mutation or deletion causes an increase in substrate concentration. The most common form of CAH resulting from mutations or deletions in the 21-hydroxylase gene (CYP21A2) is a potent androgen due to the accumulation of the steroid precursors progesterone and 17-hydroxyprogesterone (17-OHP), is produced by the adrenal glands. Plasma levels of 17-OHP can reach 10 to 1000 times the normal concentration in these cases. These increases result in overproduction of androgens, specifically androstenedione, testosterone, and dihydroxytestosterone, causing masculinization in women. Furthermore, 21-hydroxylase deficiency in CAH causes inadequate biosynthesis of glucocorticoids and mineralocorticoids, specifically cortisol and aldosterone. Cortisol is an important negative feedback regulator of hypothalamic CRF secretion and pituitary ACTH release. The lack of glucocorticoid synthesis and release eliminates inhibition on the hypothalamus and pituitary gland, which increases ACTH levels. Excessive ACTH stimulation causes cord and reticulum hypertrophy, resulting in adrenal hyperplasia.

In one embodiment, the 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 herein as "Compound I") This has the following structure:

Compound I is a potent CRF $_1$ antagonist with binding pKi = 8.2 (eg Tellew et al., Bioorg. Med. Chem. Lett. 20: 7259, 2010 and International Patent Application Publication No. WO 2006/044958). (Both of which are incorporated herein by reference in their entirety)). As described herein, Compound I has a potent ACTH-lowering effect as shown in adrenalectomized rats.

In another embodiment, CRF $_1$ receptor antagonists useful for treating CAH are US Pat. Nos. 6,586,456, 6,806,282, 6,531,475, 664,261, 6,610,678, WO 98/08846, WO 98/11075, WO 99/10350, WO 2006/059888, WO 2006/044821, WO 2006/102194, WO 2006/107784, WO 2006/116412, WO 2006/126718, WO 2007/066955, WO 2007/069671, WO 2008/036541, WO 2008/036579, WO 2008/051533, WO 2008/082003, WO 2008/083070, WO 200 / 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 2011/092295, and WO 2011/043381.

In yet 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-[(1S) -2-cyclopropyl-1- (3-fluoro-4-methylphenyl) ethyl] -5-methyl-N- (2-propyn-1-yl) -2-thiazolamine] , SSR-126374, ONO-2333, NBI-34041, JNJ-1955567470, GSK586529, PF-00572778, CP-376395, antalarmine (N-butyl-N-ethyl-2,5,6-trimethyl-7- (2, 4,6-trimethylphenyl) pyrrolo [3,2- e] pyrimidine-4-amine), and DMP904.

In yet another embodiment, the CRF $_1$ receptor antagonist has a dissociation half-life ($t_{1/2}$) greater than 30 minutes, and in another embodiment greater than 40 minutes, and in another embodiment greater than 50 minutes. Have. 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, antalarmine (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 is also to be understood that when a particular position is designated as hydrogen, such hydrogen (H) can be replaced with deuterium (D). . Incorporation of deuterium instead of hydrogen is known to produce significant effects on the physiological and pharmacological activity of the substituted compound. To this end, it should be understood that deuterium substitution of hydrogen means that the deuterium abundance at that position is substantially greater than the natural deuterium abundance. Thus, in one embodiment, representative compounds include the following.

In another embodiment, any of the aforementioned compounds may incorporate a stable or radioactive isotope. Thus, the use of isotopically-labelled compounds identical to those described herein is also contemplated, wherein one or more atoms are atoms that differ from some atomic mass or mass number normally found in nature. Substituted by an atom having a mass or mass number. Examples of isotopes that can be incorporated into these compounds include hydrogen, carbon, nitrogen, oxygen, phosphorus, fluorine and chlorine isotopes, eg, deuterium (^{2} H) as discussed above, and ^{3} H, ^{13} C, ^{14} C, ^{15} N, ^{18} O, ^{17} O, ^{31} P, ^{32} P, ^{35} S, ^{18} F, and ^{36} Cl are included, but are not limited thereto. Certain isotopically-labelled compounds, for example those into which radioactive isotopes such as ^{3} H and ^{14} C are incorporated, are also useful in drug or substrate tissue distribution assays. Tritium hydrogen (^{3} H) and carbon 14 (^{14} C) isotopes are particularly preferred for their ease of preparation and detectability. Substitution with heavier isotopes (eg, deuterium (^{2} H)) may provide certain metabolic benefits resulting from greater metabolic stability, eg, increased in vivo half-life or reduced dose requirements, Therefore, it may be preferable in some environments. 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 main glucocorticoid) in rats and eliminates negative feedback control of the HPA system at both the hypothalamic and pituitary (adrenocortical hormone producing cells) levels, and thus plasma ACTH (See, for example, Mims et al., J. Natl. Med. Assoc. 69: 145-47 (1977)) Intravenous injection of peptide CRF $_{1}$ receptor antagonists has been shown to reduce high plasma ACTH levels in adrenalectomized (ADX) rats (see, eg, Rivier et al., J. Med. Chem. 12: 42: 3175). 82 (1999)). These findings were reproduced with the low molecular weight NBI-77860 (Compound I). In adrenalectomized rats, Compound I has a potent capacity for ACTH reduction. The greatest reduction in ACTH correlated with the peak plasma concentration of NBI-77860, however, the duration of the ACTH-lowering effect was beyond drug plasma exposure. Thus, there is a predictable relationship between integrated plasma exposure of NBI-77860 and in vivo efficacy after oral administration in adrenalectomized rats.

The effectiveness of a compound as a CRF receptor antagonist can be determined by various assays The CRF antagonists described herein can inhibit the specific binding of CRF to its receptor and, as a result, can antagonize the activity associated with CRF. A compound can be evaluated for activity as a CRF antagonist by one or more commonly accepted assays, including the assays described in the Examples. CRF antagonists useful in the methods described herein include compounds that exhibit affinity for the CRF receptor.

While not wishing to be bound by theory, in the treatment of CAH, CRF receptor antagonists potentially block ACTH release from pituitary corticotrophic cells, thereby reducing androgen production. Allows for a more sophisticated treatment methodology for cortisol supplementation. Animal and human studies have shown the pharmacological effect of Compound I (NBI-77860) on ACTH release. Standard biomarker assessments used by endocrinologists when monitoring treatment efficacy can be used to monitor the effects of this CRF $_{1}$ receptor antagonist. Plasma levels of 17-OHP, androstenedione, testosterone, cortisol and ACTH, and urinary metabolites of these steroids are easily measured in both children and adults, giving quick and meaningful data regarding the effects of treatment.
(Pharmaceutical compositions and methods for treatment)

The present disclosure relates to a pharmaceutical composition for use in a method for treating CAH comprising any one of the CRF antagonist compounds described herein and a pharmaceutically acceptable excipient. Provide further. Pharmaceutically acceptable excipients are physiologically and pharmaceutically suitable, non-toxic and inert substances or components that do not interfere with the activity of the active ingredient; It can be said. The CRF antagonist compound is used in a pharmaceutical composition for use in a treatment or preventive (or prophylactic) treatment (eg. reducing exacerbation of CAH disease or the occurrence or recurrence of one or more symptoms of the disease). It can be formulated. 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 are described, for example, by Rowe et al. , Handbook of Pharmaceutical Excipients: A Comprehensive Guide to Uses, Properties, and Safety, 5 th Ed. , 2006 and Remington: The Science and Practice of Pharmacy (Gennaro, 21 st 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, buffering agents and the like may be provided in the pharmaceutical compositions. In addition, antioxidants and suspending agents can also be used.

Therapeutic and / or prophylactic benefits include, for example, improved clinical outcome (both therapeutic treatment and prophylactic or preventative measures), where the objective is to reduce undesirable physiological changes or disorders. Preventing, delaying or delaying (reducing), or preventing or delaying or delaying (reducing) the spread or severity of such disorders. As discussed herein, beneficial or desirable clinical results from treating a subject include, but are not limited to: Does it result from a disease, condition or disorder to be treated? Or a reduction, reduction, or alleviation of symptoms associated with them; a decrease in the occurrence of symptoms; an improvement in quality of life; a condition that is free of disease (ie, subject has symptoms (based on which the disease is diagnosed) Decrease the likelihood or tendency to show); decrease the degree of disease; stable (ie, not aggravating) disease; delay or delay in disease progression; improvement or reduction of the disease state As well as detectable or undetectable, regression (whether partial or whole); and / or overall survival. "Treatment" can also mean prolonging survival as compared to expected survival if the subject does not receive treatment. Subjects in need of treatment include those who already have the condition or disorder, as well as subjects who are prone to or at risk of developing the disease, condition or disorder, and the disease or condition. Or people whose disability should be prevented (ie, reducing the likelihood of the disease, disorder or condition occurring). The subject can be a human or non-human mammal (eg, rat, mouse, dog, cat, farm animal, zoo animal).

Optimal dosages can generally be determined using experimental models and / or clinical trials. The optimal dose can depend on the subject's body mass, weight, or blood volume. In general, the amount of a compound described herein present in a dose ranges from about 0.1 mg to about 30 mg / kg subject body weight. In certain embodiments, the single dose is about 50-1000 mg. The use of the minimum dose that is sufficient to provide effective treatment is usually preferred. Subjects can generally be monitored for therapeutic efficacy by clinical assessment and using assays suitable for the condition being treated or prevented. This assay is familiar to those skilled in the art and is described herein. The level of the compound administered to the subject is other biologicals in the biological fluid, eg, blood, blood fraction (eg, serum) and / or urine, and / or derived from the subject. Can be monitored by determining the level of the compound in a laboratory sample. Any method practiced in the art to detect the compound can be used to measure the level of the compound during the course of a treatment regimen.

The dose of the composition for treating CAH or related diseases or disorders comprising at least one of the compounds described herein depends on the condition of the subject, ie the stage of the disease, the disease. The severity of symptoms caused, general health, and age, gender and weight, and other factors apparent to those skilled in the medical arts. Similarly, the dose of the compound can be determined according to parameters understood by those skilled in the medical arts.

The pharmaceutical compositions described herein comprising at least one of the CRF $_{1}$ antagonist compounds described herein are among several routes that efficiently deliver an effective amount of the compound. Any one can be administered to a subject in need. Such administration routes include, for example, oral, parenteral, intestinal, rectal, nasal, buccal, sublingual, intramuscular, and transdermal. Compositions administered by these routes of administration and the like are described in more detail herein.

Administration of the disclosed compounds or compositions includes night administration or administration prior to going to bed (ie, bedtime administration). As used herein, bedtime administration is clinically relevant to the CRF $_1$ antagonist at or before (eg, 2 to 5 hours before) the release of ACTH according to the expected circadian rhythm. Administration that is intended to deliver a concentration. This ACTH release is typically 1-2 am, and most orally administered drugs have a Tmax of several hours, so, for example, 3 to 3 of ACTH release due to the expected circadian rhythm. Administration at 10pm, 4 hours before, is desirable. This same prepulse bedtime administration may be adapted for shift workers (eg, people who work at night and sleep during the day), in which case the administration may not necessarily occur at night . Thus, administration depends on the expected circadian rhythm of ACTH release and can vary depending on the individual's (ie, subject, patient's) specific work and sleep patterns. In certain embodiments, the CRF $_1$ receptor antagonists described herein (eg, NBI-77860, or NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalalmine (N-butyl-N— Ethyl-2,5,6-trimethyl-7- (2,4,6-trimethylphenyl) pyrrolo [3,2-e] pyrimidin-4-amine), or any one of DMP904) Administered about 2 to 5 hours before (ie, first) the ACTH release due to circadian rhythm. In other embodiments, the CRF $_1$ receptor antagonist is administered to the subject during about 2-4 hours or 3-5 hours prior to ACTH release with the expected circadian rhythm. In a more specific embodiment, the CRF $_1$ receptor antagonist is administered to the subject during about 3-4 hours prior to the release of ACTH according to the expected circadian rhythm

Also provided herein are methods for reducing 17-OHP and / or ACTH levels in a subject with CAH (ie, patient, individual) by administering a CRF $_1$ receptor antagonist. In certain embodiments, the antagonist is administered at or prior to ACTH release with an expected circadian rhythm. In certain other embodiments, the CRF $_1$ receptor antagonist is administered during about 2-4 hours or about 3-5 hours prior to the expected circadian rhythm ACTH release. In a more specific embodiment, the CRF $_1$ receptor antagonist is administered to the subject during about 3-4 hours prior to ACTH release with the expected circadian rhythm.

Described herein including administering to a female subject in need a CRF $_1$ receptor antagonist in the manner described herein that causes a decrease in the levels of ACTH and 17-OHP in said subject. Methods can result in reduced release of androgens (eg, testosterone and androstenedione. The dose of glucocorticoid can be concomitantly reduced by a clinically significant amount, which in turn reduces side effects. Produce.

The amount of glucocorticoid (GC) and mineralocorticoid (MC) for maintenance therapy in patients with CAH and young growing patients and for maintenance therapy in mature patients is known to those skilled in the art . For example, guidelines can be found in Speiser et al. (J. Clin. Endocrinol. Metab. 95: 4133-60 (2010) (incorporated by reference in its entirety) and shown in Tables 1 and 2 therein, in particular, young growth with CAH. With respect to patients in the art, those skilled in the art will recognize that administration too high with GC can interfere with stature growth in the patient, and thus a book for treating patients with CRF $_1$ receptor antagonists. The methods described herein can include reducing the dose of GC in a clinically significant manner.

In certain embodiments, the method for treating CAH by administering a CRF $_1$ receptor antagonist administers GC at a dose lower than the currently recommended GC dose for treating a subject with CAH. A step can be further included. If the subject is a mature patient, the GC dose (eg, hydrocortisone (HC), prednisone, prednisolone, dexamethasone, or fludrocortisone recommended for maintenance therapy in mature subjects) is: 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 Fludrocortisone The dose can be reduced to the extent of about 10%, 15%, 20%, 30%, 40%, 50%, 60% or more. In growing subjects with CAH, the recommended dose of GC (eg, HC) can be reduced from a total recommended dose of 10-15 mg / m^2 / day and / or 0.05-0.2 mg. The total dose of fludrocortisone / day is about 10%, 15%, 20%, 30%, 40%, 50%, respectively, in subjects receiving a CRF $_1$ receptor antagonist as described herein. It can be reduced by as much as 60% or more. In one embodiment, a method described herein comprising administering an effective amount of a CRF $_1$ antagonist to a subject in need comprises a clinically significant reduction in ACTH levels compared to placebo. Produce. In a specific embodiment, administering an effective amount of a CRF $_1$ antagonist to a subject in need results in a clinically significant reduction in ACTH levels compared to placebo, wherein the reduction is at least 25%. In another embodiment, administering an effective amount of a CRF $_1$ antagonist to a subject in need results in a clinically significant decrease in ACTH levels compared to placebo, wherein the decrease is at least 50%. For guidelines on the administration of glucocorticoids and mineralocorticoids, see Speiser et al. See (above).

In another embodiment, a method described herein comprising administering an effective amount of a CRF $_1$ antagonist to a subject in need comprises a 17-OHP level clinically compared to a placebo. A significant decrease occurs. In another specific embodiment, administering an effective amount of a CRF $_1$ antagonist to a subject in need results in a clinically significant decrease in 17-OHP levels compared to placebo, wherein The decrease is at least 25%. In yet another embodiment, administering an effective amount of a CRF $_1$ antagonist to a subject in need results in a clinically significant reduction in 17-OHP levels compared to placebo, wherein the reduction is at least 50%.

In one embodiment, a method described herein comprising administering an effective amount of a CRF $_1$ antagonist to a subject in need comprises both ACTH and 17-OHP levels compared to placebo. Produces a clinically significant decrease in In certain embodiments, administering an effective amount of a CRF $_1$ antagonist to a subject in need results in a clinically significant decrease in both ACTH and 17-OHP levels compared to placebo. Where the reduction is at least 25%. In another specific embodiment, administering an effective amount of a CRF $_1$ antagonist to a subject in need results in a clinically significant decrease in both ACTH and 17-OHP levels compared to placebo. Occurs, where the reduction is at least 50%.

The pharmaceutical composition can be in the form of a solution. Alternatively, they can be in the form of a solid (eg, powder, tablet, etc.). A composition comprising any one of the compounds described herein can be formulated for sustained or slow release. Such compositions can generally be prepared using well-known techniques and can be administered, for example, by oral, rectal or subcutaneous implantation, or by implantation at the desired target site. The sustained release formulation may comprise a compound dispersed in a carrier matrix and / or contained within a reservoir surrounded by a rate controlling membrane. Excipients for use within such formulations may be biocompatible and biodegradable; preferably, the formulation provides a relatively constant level of active ingredient release. The amount of active compound contained within the sustained release formulation will depend on the site of implantation, the rate of release and the expected duration, 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, eg, conventional additives (eg, lactose, mannitol, Corn starch or potato starch); with binders; with disintegrants; with lubricants; and, if desired, with diluents, buffering agents, wetting agents, preservatives, colorants and flavoring agents, tablets, Powders, granules or capsules can be made. The compounds can be formulated with buffering agents and / or enteric coatings to provide protection of the compounds from the low pH of the stomach environment. The compounds included in the composition can be formulated for oral delivery, for example, with a flavoring agent in a liquid, solid or semi-solid formulation and / or with an enteric coating. Oral formulations can be provided as gelatin capsules that can contain the active compound together with a powdered carrier such as lactose, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similar carriers and diluents can be used to make compressed tablets.

Example 1: CRF receptor binding activity CRF antagonists as used in the methods described herein are described in Grigoriadis et al. (See, eg, Mol. Pharmacol vol 50, pp 679-686, 1996) and Hoare et al. (See, eg, Mol. Pharmacol 63: 751-765, 2003) and can be assessed for binding activity to the CRF receptor by a standard radioligand binding assay as generally described. By utilizing a radiolabeled CRF ligand, the assay can be used to assess the binding activity of the compounds described herein with any CRF receptor subtype.

Briefly, the binding assay involves replacing a radiolabeled CRF ligand with the CRF receptor. More specifically, the binding assay is performed in 96-well assay plates using 1-10 μg cell membranes derived from cells stably transfected with human CRF receptor. Each well contains a compound of interest or a reference ligand (eg. sorbazine, urocortin I, or CRF), 0.05 mL of [^{125}I] tyrosine-sorbazine (final concentration about 150 pM or as determined by Scatchard analysis). K_D) and about 0.05 mL assay buffer (eg Dulbecco's phosphate buffered saline, 10 mM magnesium hydrochloride, 2 mM EGTA) containing 0.1 mL cell membrane suspension containing the CRF receptor. The mixture is incubated for 2 hours at 22 ° C., followed by separation of bound and free radioligand by rapid filtration through glass fiber filters. After three washes, the filter is dried and the radioactivity (^{125}I Auger electrons) is counted using a scintillation counter. All radioligand binding data can be analyzed using the nonlinear least square curve fitting program Prism (GraphPad Software inc) or XLfit (ID Business Solutions Ltd).

Example 2: CRF $_1$ receptor agonist activity

Fleck et al. (As previously reported in J. Pharmacology and Experimental Therapeutics, 341 (2): 516-531, 2012) (hereinafter referred to as "Feck et al", incorporated herein by reference in its entirety). Activity of the produced CRF $_1$ receptor antagonists. Such activity is reported as a kinetically derived affinity (K_i) calculated from the association rate constant (k_1) and dissociation rate constant (k_{-1}) by the following equation:

$K_i = k_{-1} / k_1$

See also Fleck et al. in As reported, kinetic K_i of the CRF $_1$ receptor antagonist listed in Table 1 below were reported:

By this same technique, the kinetic Ki of compound I (NBI-77860) was found to be as follows.

Example 3: Dissociation half-life of the CRF $_1$ receptor antagonist ($t_{1/2}$)

The dissociation half-life ($t_{1/2}$) of a CRF $_1$ receptor antagonist as used in the methods described herein is determined by the method of Flek et al. It is evaluated by the technique described in. As described therein, the dissociation rate constant for labeled and unlabeled ligands is expressed as k_{-1} while the drug dissociation half-life of the receptor ($t_{1/2}$) (this is equal to the median residence time) is calculated from the dissociation rate constant (k_{-1}) by the following equation:

$t_{1/2} = 0.693 / k_{-1}$

Fleck et al. As reported in, the dissociation half-life ($t_{1/2}$) of the CRF $_1$ receptor antagonists listed in Table 2 below was reported.

By this same technique, the dissociation half-life of Compound I (NBI-77860) was found to be as follows:

Thus, CRF $_1$ receptor antagonists having a dissociation half-life ($t_{1/2}$) of greater than 30 minutes include antalarmine, NBI-34041, DMP904, NBI-30775, SSR125543A, and NBI-77860 (Compound I) (But is not limited to these). These same compounds also are representative of the CRF $_1$ receptor antagonist having a dissociation half-life greater than 40 minutes $_{(t\ 1/2)}$ and dissociation half-life greater than 50 minutes to $_{(t\ 1/2)}$.

Example 4: Reduction of ACTH in adrenalectomized rats

Compound I (NBI-77860) (see, for example, Tellew et al., Bioorg. Med. Chem. Lett. 2010, 20: 7259; see WO2006044958) binds pKI 8.2, kinetic KI 49nM (above, Table 1) and dissociated $t_{1/2}$ 58 min (Table 2 above) are potent CRF $_1$ antagonists.

Intravenous injection of peptide CRF $_1$ receptor antagonists has been shown to reduce high plasma ACTH levels in adrenalectomized (ADX) rats (eg, Rivier et al., J. Med. Chem. 12: 42: 3175). 82 (1999)). These findings were reproduced with the low molecular weight NBI-77860. When administered orally to ADX rats (n = 6 / group), a 30 mg / kg single dose of NBI-77860 significantly reduced plasma ACTH levels for up to 5 hours (see FIG. 2) The duration of efficacy correlated with the peak plasma concentration over time over drug plasma exposure. There is a predictable relationship between NBI-77860 integrated plasma exposure and in vivo efficacy after oral administration in adrenalectomized rats

Fleck et al. Also report the effects of CRF $_1$ receptor antagonists, distinguished by their dissociation half-life on plasma ACTH levels in adrenalectomized rats; ie, NBI 30775, NBI 34041 and NBI 35965. At the highest dose (10 mg / kg), all three ligands drastically reduced ACTH (1 hour after injection, FIGS. 3A-C). After a longer duration, a clear difference emerged between NBI 35965 and the other two ligands. The ACTH level returned to vehicle level by 2 hours for NBI 35965 (FIG. 3C), whereas the response persisted for 4-6 hours for NBI 30775 and NBI 34041 (FIG. 3A). And FIG. 3B).

Example 5: Pharmacodynamic effect of Compound I in humans

In this study, the pharmacodynamic effects of NBI-77860 (Compound I) on human subjects were compared with placebo and a single dose of alprazolam (0.75 mg) by the HPA system produced by metyrapone (0.04 g / kg). Evaluated by observing the effect of a single oral dose on response. Methyrapone blocks cortisol synthesis in the adrenal gland (thus mimicking the cortisol deficiency of CAH) and is associated with a sharp increase in ACTH levels.

Primary analysis was performed by determining ACTH values (AUC (30 minutes to 4 hours)) between 30 minutes and 4 hours after administration. In this analysis, the following observations were made compared to placebo. A significant reduction in ACTH AUC (30 minutes to 4 hours) was observed with alprazolam and NBI-77860, 400 mg treatment. A non-significant decrease in ACTH AUC (30 minutes to 4 hours) was observed with NBI-77860, 50 mg treatment. An increase in AUC (30 minutes to 4 hours) was observed with NBI-77860, 10 mg treatment.

The analysis performed on ACTH values (AUC (2-4 hours)) between 2 and 4 hours post-dose (period when exposure reached C_{max} values) compared to the placebo treatment period , NBI-77860, 400 mg and 50 mg, the ACTH level was significantly reduced during the treatment period; however, the reduction was not observed at the 10 mg dose of NBI-77860.

Example 6: Clinical study

In this study, NBI-77860 (Compound I) was administered with the title "Phase 1, single-blind, placebo-controlled fixation to evaluate the safety and tolerability of NBI-77860 in adult women with congenital adrenocortical hyperplasia. Evaluated in a clinical study called "Phase 1, Single-Blind, Placebo-Controlled, Fixed-Sequence, Single-Dose Study" (IND 117,388). The study was a single-blind, placebo-controlled, single-center, fixed-order, single-dose clinical trial in adult female classic CAH patients. This was designed to evaluate the safety, tolerance, and plasma exposure of NBI-77860, and the effect of this compound on endogenous levels of HPA hormones.

A total of 8 female subjects (aged 19-58 years) medically diagnosed with classic 21-hydroxylase deficient CAH received NBI-77860 300 mg, 600 mg, and 3 different treatment periods. A single bedtime dose (hs) of placebo was administered (see study design schematic in FIG. 4). The subject's usual morning dose of concurrent steroid treatment was delayed until a 16 hour blood sample was collected (ie, up to about 1400 hours).

The PD endpoint for this study is the HPA biomarker 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 were included. The initial analysis of PD variables was a survey of

grouped subject data for each biomarker (mean% from pre-dose levels for the two active dosing conditions compared to placebo conditions) Expressed as a change). Data for average 17-OHP and ACTH levels over the 24 hour period after administration is provided in FIG.

A consistent and clinically significant decrease from pre-dose levels of both 17-OHP and ACTH was observed in these CAH patients throughout the post-dose period after administration of NBI-77860 compared to placebo. In addition to group mean data, individual responses were assessed and treatment "responders" were at least 50 in 17-OHP and ACTH under active NBI-77860 compared to placebo during the morning peak period. Conservatively defined as those subjects with% reduction. This responder analysis resulted in a fairly large responder rate of 50% in the study (none of these patients were responders during the first placebo treatment period). Furthermore, the 300 mg dose produced the same effects on 17-OHP and ACTH as the 600 mg dose. Examples of individual subject responses for these biomarkers are provided in FIG.

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 mentioned in this specification and / or listed in the application data sheet are U.S. provisional patent applications. 61,929,941 (filed January 21, 2014); 61,981,033 (filed April 17, 2014); and 62 / 069,155 (October 27, 2014) All of which are hereby incorporated by reference herein. Aspects of the above embodiments can be modified to provide still further embodiments if necessary to use the various patents, applications, and published concepts.

These and other changes can be made to the above embodiments in light of the above detailed description. In general, in the following claims, the terminology used shall be construed to limit the scope of the claims to the specific embodiments disclosed in the specification and the claims. Rather, it is intended to 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 above disclosure

Patent Citations (57)

Publication number	Priority date	Publication date	Assignee	Title
JP2010504344A *	2006-09-20	2010-02-12	イーライ リリー アンド カンパニー	Thiazolepyrazolopyrimidines as CRF1 receptor antagonists
Family To Family Citations				
JPS4949582B1	1969-01-20	1974-12-27		
FR2692893B1	1992-06-24	1994-09-02	Sanofi Elf	Branched alkylamino thiazole derivatives, processes for their preparation and pharmaceutical compositions containing them
US6664261B2	1996-02-07	2003-12-16	Neurocrine Biosciences, Inc.	Pyrazolopyrimidines as CRF receptor antagonists
TW477678	1996-08-27	2002-03-01	Pfizer	Pyrido six-membered nitrogen-containing cyclic ring derivatives having corticotropin releasing factor antagonist activity and pharmaceutical composition containing same
EP0927171B1	1996-09-16	2002-02-20	Du Pont Pharmaceuticals Company	Pyrazinones and triazinones and their derivatives thereof
AU7644698A	1997-04-22	1998-11-13	Janssen Pharmaceutica N.V.	Crf antagonistic quino- and quinazolines
EP0937081A1	1997-08-22	1999-08-25	Du Pont Pharmaceuticals Company	NITROGEN SUBSTITUTED IMIDAZO 4,5-c]PYRAZOLES AS CORTICOTROPIN RELEASING HORMONE ANTAGONISTS
US6313147B1	1998-11-12	2001-03-11	Neurocrine Biosciences, Inc	CRF receptor antagonists and methods relating thereto
AU3931400A	1999-04-02	2000-10-23	Neurogen Corporation	N-benzimidazolylmethyl- and n-indolylmethyl-benzamides and their use as crf modulators
FR2796380B3	1999-07-15	2001-08-17	Sanofi Synthelabo	NOVEL AMINOTHIAZOL DERIVATIVES, THEIR PREPARATION AND THE PHARMACEUTICAL COMPOSITIONS CONTAINING THEM
FR2802530B1	1999-12-17	2002-02-22	Sanofi Synthelabo	NOVEL BRANCHED SUBSTITUTED AMINO DERIVATIVES OF 3-AMINO-1-PHENYL-1H [1,2,4] TRIAZOLE, METHODS FOR THEIR PREPARATION AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM
US7276592B2	2001-07-13	2007-10-02	Bristol-Myers Squibb Pharma Company	Substituted thiazoles and oxazoles as corticotropin releasing hormone ligands
CA2556160A1	2004-02-13	2005-09-01	Pfizer Products Inc.	Therapeutic combinations of atypical antipsychotics with corticotropin releasing factor antagonists
JP2008509933A	2004-08-13	2008-04-03	ニキスフェリー・テクノロジーズ・インク	Pharmaceutical formulation comprising microparticles or nanoparticles of a delivery agent
AT430746T	2004-10-19	2009-05-15	Smithkline Beecham Cork Ltd	CRF receptor antagonists and associated methods
EA012393B1	2005-03-21	2009-10-30	Эли Лилли Энд Компани	Imidazopyridazine compounds
EP1879896A1	2005-04-05	2008-01-23	Eli Lilly And Company	Imidazopyridazine compounds
TWI370208B	2005-04-27	2012-08-21	Takeda Pharmaceutical	Fused heterocyclic compounds
US20090234117A1	2005-05-27	2009-09-17	Toshihiko Kashiwagi	Pyrazolopyrimidine Derivative

GB0519957D0	2005-09-30	2005-11-09	Sb Pharmco Inc	Chemical compound
WO2007054719A2	2005-11-10	2007-05-18	Topotarget Uk Limited	Histone deacetylase (hdac) inhibitors (pxdlol) for the treatment of cancer alone or in combination with chemotherapeutic agent
EP1961745A1	2005-12-12	2008-08-27	Ono Pharmaceutical Co., Ltd.	Bicyclic heterocyclic compound
JPWO2007069671A1	2005-12-15	2009-05-21	小野薬品工業株式会社	Bicyclic heterocyclic compounds
PT1982178E	2006-02-07	2013-10-24	Max Planck Gesellschaft	Methods for the treatment of affective disorders
EP1834641A1	2006-03-16	2007-09-19	Sanofi-Aventis	Use of CRF1 receptor antagonists for preparing a drug for treating metabolic syndrome and/or obesity and/or dyslipoproteinemia
CA2652416A1	2006-05-22	2007-11-29	Vanda Pharmaceuticals, Inc.	Treatment for depressive disorders
ES2428543T3	2006-09-20	2013-11-08	Eli Lilly And Company	Thiophenepyrazolopyrimidine Compounds
US20100056515A1	2006-10-25	2010-03-04	Kazuyoshi Aso	Benzimidazole compounds
EP2125753A1	2006-12-29	2009-12-02	Takeda Pharmaceutical Company Limited	Fused heterocyclic compounds having crf antagonistic activity
WO2008083070A1	2006-12-29	2008-07-10	Neurogen Corporation	Crf1 receptor ligands comprising fused bicyclic heteroaryl moieties
TW200902019A	2007-04-26	2009-01-16	Ono Pharmaceutical Co	Bicyclic heterocyclic compound
WO2009008552A1	2007-07-11	2009-01-15	Taisho Pharmaceutical Co., Ltd.	8-aryl-4-alkylpyrrolo[2,3,4-de]quinolin-5(4h)-one and 8-aryl-4-alkyl-4,5-dihydropyrrolo[2,3,4-de]quinolin-5-ol derivatives
WO2009144532A1	2008-05-30	2009-12-03	Pfizer Limited	Novel compounds
EP2307024B1	2008-07-31	2013-01-09	Bristol-Myers Squibb Company	Substituted carbamate derivatives as modulators of corticotropin-releasing factor receptor activity
US7932256B2	2008-07-31	2011-04-26	Bristol-Myers Squibb Company	(S)-4-(1-cyclopropyl-2-methoxyethyl)-6-(6-(difluoromethoxy)-2,5-dimethylpyridin-3-ylamino)-5-oxo-4,5-dihydropyrazine-2-carbonitrile: a pyrazinone modulator of corticotropin-releasing factor receptor activity
US7994203B2	2008-08-06	2011-08-09	Novartis Ag	Organic compounds
US8273900B2	2008-08-07	2012-09-25	Novartis Ag	Organic compounds
CN102245117B	2008-11-03	2014-04-16	衛恩藥新有限公司	Adjustable rod assembly
US8598196B2	2009-02-20	2013-10-08	Emory University	Compounds, compositions, methods of synthesis, and methods of treatment
HU0900267A2	2009-04-30	2011-03-28	Sanofi Aventis	Process for preparing of thiazole amines and intermediates thereof
AR078521A1	2009-10-08	2011-11-16	Eisai R&D Man Co Ltd	PIRAZOLOTIAZOL COMPOUND
WO2011043387A1	2009-10-08	2011-04-14	エーザイ・アール・アンド・ディー・マネジメント株式会社	Pyrazoloxazole compound
JP2013518085A	2010-02-01	2013-05-20	ノバルティス アーゲー	Pyrazolo [5,1b] oxazole derivatives as CRF-1 receptor antagonists
AR080056A1	2010-02-01	2012-03-07	Novartis Ag	CICLOHEXIL-AMIDA DERIVATIVES AS ANTAGONISTS OF CRF RECEIVERS
ES2527849T3	2010-02-02	2015-01-30	Novartis Ag	Cyclohexylamide derivatives as CRF receptor antagonists
CN109745316A	2012-04-13	2019-05-14	Epizyme股份有限公司	Combination therapy for treating cancer
JP2015516979A	2012-04-23	2015-06-18	ホルスボールマッシュマイヤー・ニューロケミー・ゲーエムベーハー・Ｈｏｌｓｂｏｅｒｍａｓｃｈｍｅｙｅｒ Ｎｅｕｒｏｃｈｅｍｉｅ Ｇｍｂｈ	CRHR1 antagonists for use in treating patients with CRH overactivity
EP2841595A2 *	2012-04-23	2015-03-04	Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V	Genetic predictors of response to treatment with crhr1 antagonists
CA2935974A1	2014-01-21	2015-07-30	Neurocrine Biosciences, Inc.	Crf1 receptor antagonists for the treatment of congenital adrenal hyperplasia
WO2016069177A1	2014-10-22	2016-04-28	The Trustees Of The University Of Pennsylvania	Method of treating depression and other stress related disorders

EP3277683B1	2015-04-02	2019-02-27	HMNC Value GmbH	Method of treatment using genetic predictors of a response to treatment with ssr-125543
WO2018012066A1 *	2016-07-14	2018-01-18	株式会社コナミデジタルエンタテインメント	Game system, terminal device, and program
EP3548035A4	2016-11-30	2020-07-22	Case Western Reserve University	Combinations of 15-pgdh inhibitors with corticosteroids and/or tnf inhibitors and uses thereof
WO2018219884A1	2017-06-02	2018-12-06	Bayer Pharma Aktiengesellschaft	Self-microemulsifying drug delivery systems
US20210361664A1	2017-08-14	2021-11-25	Spruce Biosciences, Inc.	Corticotropin releasing factor receptor antagonists
JP2022508317A	2018-12-07	2022-01-19	ニューロクライン バイオサイエンシーズ，インコーポレイテッド	CRF1 receptor antagonist for treating congenital adrenal hyperplasia, its pharmaceutical formulation and solid form

* Cited by examiner, † Cited by third party

Cited By (9)

Publication number	Priority date	Publication date	Assignee	Title
JP2019081814A *	2014-01-21	2019-05-30	ニューロクライン バイオサイエンシーズ，インコーポレイテッド	Crf1 receptor antagonists for treatment of congenital adrenal hyperplasia
JP2020530831A *	2017-08-14	2020-10-29	スプルース バイオサイエンシズ インコーポレイテッド	Corticotropin-releasing factor receptor antagonist
JP2022508317A *	2018-12-07	2022-01-19	ニューロクライン バイオサイエンシーズ，インコーポレイテッド	CRF1 receptor antagonist for treating congenital adrenal hyperplasia, its pharmaceutical formulation and solid form
Family To Family Citations				
CA2940242A1	2014-02-20	2015-08-27	Alder Biopharmaceuticals, Inc.	Anti-acth antibodies and use thereof
CA3039026A1	2016-09-07	2018-03-15	The Regents Of The University Of California	Allosteric corticotropin-releasing factor receptor 1 (crfr1) antagonists that decrease p-tau and improve cognition
JP2021522340A *	2018-04-27	2021-08-30	スプルース バイオサイエンシーズ，インク．	Methods for Treating Testicular Adrenal Residual Tumors and Ovarian Adrenal Residual Tumors
KR20200052927A *	2019-07-19	2022-04-28	스프룩스 바이오사이언시스 인코포레이티드	How to treat congenital adrenal hyperplasia
WO2021062246A1 *	2019-09-27	2021-04-01	Neurocrine Biosciences, Inc.	Crf receptor antagonists and methods of use
TW202214235A *	2020-06-10	2022-04-16	美商細胞基因生物科學有限公司	Crf1 receptor antagonist for the treatment of congenital adrenal hyperplasia

* Cited by examiner, † Cited by third party, ‡ Family to family citation

Similar Documents

Publication	Publication Date	Title
JP6858525B2	2019-10-02	CRF1 receptor antagonist for the treatment of congenital adrenocortical hyperplasia
Gold et al	2002	Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states
Falhammar et al.	2011	Cardiovascular risk, metabolic profile, and body composition in adult males with congenital adrenal hyperplasia due to 21-hydroxylase deficiency
Kirkgoz et al.	2018	Primary adrenal insufficiency in children: Diagnosis and management
EP1836695A1	2006-02-13	Pharmaceutical combination of an aldosterone synthase inhibitor and a glucocorticoid receptor antagonist or a cortisol synthesis inhibitor or a corticotropin releasing factor antagonist
Basta-Kaim et al.	2011	Hyperactivity of the hypothalamus–pituitary–adrenal axis in lipopolysaccharide-induced neurodevelopmental model of schizophrenia in rats: effects of antipsychotic drugs
JP2006508951A	2006-03-16	Methods for treating migraine
JP2004525135A	2004-08-19	Methods for treating stress disorders using glucocorticoid receptor-specific antagonists
Nordenström et al.	2022	Current and novel treatment strategies in children with congenital adrenal hyperplasia

AU2020319970A1	2022-03-03	Methods of treating congenital adrenal hyperplasia
EP1342543A1	2007-10-10	Pharmaceutical composition coprising an aldosterone synthase inhibitor and a mineralocorticoid receptor antagonist
Charmandari et al	2002	Treatment with flutamide decreases cortisol clearance: implications for therapy in congenital adrenal hyperplasia
CA2446508C	2012-04-10	Methods for treating delirium using glucocorticoid receptor-specific antagonists
US20220288091A1	2022-09-15	Method for Improving Insulin Sensitivity
Anderson	2017	Cellular regulation of cortisol in vivo by 11-beta hydroxysteroid dehydrogenase type 1
Fitzgerald et al	2009	The neuroendocrine system
Markopoulou	9	This electronic theses or dissertation has been downloaded from the King's Research Portal at https://kclpure.kcl.ac.uk/portal

Priority And Related Applications

Child Applications (1)

Application	Priority date	Filing date	Relation	Title
JP2019043465A	2014-01-21	2019-03-11	Division	CRF1 receptor antagonist for the treatment of congenital adrenal hyperplasia

Priority Applications (7)

Application	Priority date	Filing date	Title
US201461929941P	2014-01-21	2014-01-21	*US Provisional Application*
US61/929,941		2014-01-21	
US201461981033P	2014-04-17	2014-04-17	*US Provisional Application*
US61/981,033		2014-04-17	
US201462069155P	2014-10-27	2014-10-27	*US Provisional Application*
US62/069,155		2014-10-27	
PCT/US2015/012315	2014-01-21	2015-01-21	Crf1 receptor antagonists for the treatment of congenital adrenal hyperplasia

Applications Claiming Priority (1)

Application	Filing date	Title
JP2019043465A	2019-03-11	CRF1 receptor antagonist for the treatment of congenital adrenal hyperplasia

Legal Events

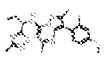
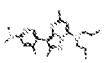
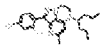
Date	Code	Title	Description
2018-01-19	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20180119
2018-01-19	A621	Written request for application examination	**Free format text**: JAPANESE INTERMEDIATE CODE: A621 **Effective date**: 20180119
2018-12-11	A131	Notification of reasons for refusal	**Free format text**: JAPANESE INTERMEDIATE CODE: A131 **Effective date**: 20181211
2019-03-11	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20190311
2019-08-02	TRDD	Decision of grant or rejection written	
2019-08-13	A01	Written decision to grant a patent or to grant a registration (utility model)	**Free format text**: JAPANESE INTERMEDIATE CODE: A01 **Effective date**: 20190813

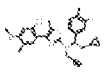
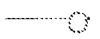
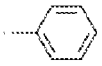
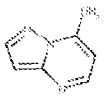
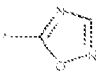
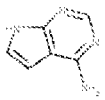
2019-09-12	A61	First payment of annual fees (during grant procedure)	**Free format text:** JAPANESE INTERMEDIATE CODE: A61 **Effective date:** 20190905
2019-09-13	R150	Certificate of patent or registration of utility model	**Ref document number:** 6585625 **Country of ref document:** JP **Free format text:** JAPANESE INTERMEDIATE CODE: R150
2022-09-24	R250	Receipt of annual fees	**Free format text:** JAPANESE INTERMEDIATE CODE: R250

Concepts

machine-extracted

Download Filter table

Name	Image	Sections	Count	Query match
receptor antagonist		title,claims,abstract,description	91	0.000
Hyperplasia		title,claims,description	10	0.000
Congenital anomaly		title,claims,description	9	0.000
adrenocortical		title,claims,description	9	0.000
CRHR1		title,abstract	3	0.000
CRHR1		title,abstract	3	0.000
Corticotropin-Releasing Hormone		claims,abstract,description	137	0.000
corticotropin		claims,abstract,description	80	0.000
ACTH and synthetic analog preparations		claims,abstract	8	0.000
compounds		claims,description	60	0.000
3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine		claims,description	39	0.000
17α-hydroxyprogesterone		claims,description	26	0.000
Dissociation Half-Life		claims,description	25	0.000
circadian rhythms		claims,description	21	0.000
circadian rhythm		claims,description	18	0.000
3-[6-(dimethylamino)-4-methylpyridin-3-yl]-2,5-dimethyl-N,N-dipropylpyrazolo[1,5-a]pyrimidin-7-amine		claims,description	12	0.000
SSR-126374		claims,description	12	0.000
268545-87-5		claims,description	10	0.000
crinecerfont		claims,description	8	0.000

				
methyloxidanyl		claims,description	3	0.000
phenyl group		claims,description	3	0.000
propyl group		claims,description	3	0.000
pyrazolo[1,5-a]pyrimidin-7-amine		claims,description	3	0.000
Adrenogenital Syndrome		claims,description	2	0.000
Congenital Adrenal Hyperplasia		claims,description	2	0.000
chronic kidney disease		claims	30	0.000
1,2,4-oxadiazol-5-yl group		claims	2	0.000
7H-pyrrolo[2,3-d]pyrimidin-4-amine		claims	1	0.000
Cortisol		abstract,description	30	0.000
hydrocortisone		abstract,description	30	0.000
antagnostic		abstract,description	23	0.000
antagonist		abstract,description	23	0.000
androgen		abstract,description	13	0.000
manufacturing process		abstract,description	8	0.000
normalization		abstract,description	4	0.000
side effect		abstract,description	4	0.000
VNR048W		abstract	1	0.000
crf1		abstract	1	0.000

Show all concepts from the description section

Data provided by IFI CLAIMS Patent Services

About Send Feedback Public Datasets Terms Privacy Policy

(19)日本国特許庁(JP)　(12)公表特許公報(A)　(11)特許出願公表番号
特表2017-503030
(P2017-503030A)
(43)公表日　平成29年1月26日(2017.1.26)

(51)Int.Cl.		F I		テーマコード（参考）
A61K 45/00	(2006.01)	A61K	45/00	4C084
A61K 31/519	(2006.01)	A61K	31/519	4C086
A61P 5/38	(2006.01)	A61P	5/38	

審査請求 未請求　予備審査請求 未請求　（全 38 頁）

(21)出願番号　特願2016-565135 (P2016-565135)
(86)(22)出願日　平成27年1月21日 (2015.1.21)
(85)翻訳文提出日　平成28年8月24日 (2016.8.24)
(86)国際出願番号　PCT/US2015/012315
(87)国際公開番号　WO2015/112842
(87)国際公開日　平成27年7月30日 (2015.7.30)
(31)優先権主張番号　61/929,941
(32)優先日　平成26年1月21日 (2014.1.21)
(33)優先権主張国　米国(US)
(31)優先権主張番号　61/981,033
(32)優先日　平成26年4月17日 (2014.4.17)
(33)優先権主張国　米国(US)
(31)優先権主張番号　62/069,155
(32)優先日　平成26年10月27日 (2014.10.27)
(33)優先権主張国　米国(US)

(71)出願人　398005054
ニューロクライン　バイオサイエンシーズ，インコーポレイテッド
アメリカ合衆国　カリフォルニア　９２１３０，サン　ディエゴ，エル　カミノ　レアル　１２７９０
(74)代理人　100078282
弁理士　山本　秀策
(74)代理人　100113413
弁理士　森下　夏樹
(72)発明者　グリゴリアディス，　ディミトリ　イー．
アメリカ合衆国　カリフォルニア　９２１３０，　サンディエゴ，　ジンジャーウッド　コーブ　１０６２６

Ｆターム(参考)　4C084 AA17　NA14　ZC08　ZC42

最終頁に続く

(54)【発明の名称】先天性副腎皮質過形成の処置のためのＣＲＦＩレセプターアンタゴニスト

(57)【要約】
ＣＲＦ$_1$レセプターアンタゴニストは、ＣＡＨを有する患者においてＡＣＴＨ放出を直接阻害し、それによって、ヒドロコルチゾンのより低い、より生理学的な用量を使用しながらアンドロゲン生成の正常化を可能にし、従って、処置関連の副作用を低減する能力を有する。一実施形態において、必要性のある被験体に、有効量のＣＲＦ$_1$アンタゴニストを投与する工程（就寝時投与が挙げられる（が、これらに限定されない））によって、ＣＡＨを処置するための方法が提供される。

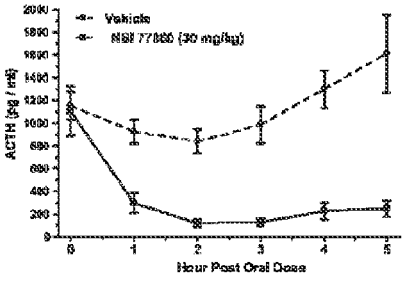


FIG. 2

【特許請求の範囲】
【請求項１】
３０分間を超える解離半減期を有するＣＲＦ$_1$レセプターアンタゴニストを必要性のある被験体に投与する工程によって、先天性副腎皮質過形成（ＣＡＨ）を処置するための方法。
【請求項２】
前記ＣＲＦ$_1$レセプターアンタゴニストは、４０分間を超える解離半減期を有する、請求項１に記載の方法。
【請求項３】
前記ＣＲＦ$_1$レセプターアンタゴニストは、５０分間を超える解離半減期を有する、請求項１に記載の方法。
【請求項４】
前記ＣＲＦ$_1$レセプターアンタゴニストは、化合物Ｉ（ＮＢＩ－７７８６０；　２，５－ジメチル－３－［２－メチル－４－（メチルオキシ）フェニル］－Ｎ－［（１Ｓ）－１－（３－メチル－１，２，４－オキサジアゾール－５－イル）プロピル］ピラゾロ［１，５－ａ］ピリミジン－７－アミン）である、請求項１に記載の方法。
【請求項５】
前記ＣＲＦ$_1$レセプターアンタゴニストは、ＮＢＩ－３０７７５、ＮＢＩ－３４０４１、ＳＳＲ－１２６３７４、ＳＳＲ－１２５５４３、アンタラルミン（Ｎ－ブチル－Ｎ－エチル　２，５，６　トリメチル　７　（２，４，６　トリメチルフェニル）ピロロ［３，２－ｅ］ピリミジン－４－アミン）、もしくはＤＭＰ９０４である、請求項１に記載の方法。
【請求項６】
前記ＣＲＦ$_1$レセプターアンタゴニストは、就寝時に投与される、請求項１～５のいずれか１項に記載の方法。
【請求項７】
前記ＣＲＦ$_1$レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出の時にもしくはその前に投与される、請求項１～５のいずれか１項に記載の方法。
【請求項８】
前記ＣＲＦ$_1$レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出の３～４時間前に投与される、請求項７に記載の方法。
【請求項９】
先天性副腎皮質過形成（ＣＡＨ）を有する被験体における１７－ＯＨＰおよびＡＣＴＨのレベルを低下させるための方法であって、該方法は、該被験体に、ＣＲＦ$_1$レセプターアンタゴニストを就寝時に投与する工程を包含する、方法。
【請求項１０】
前記ＣＲＦ$_1$レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出の時にもしくはその前に投与される、請求項９に記載の方法。
【請求項１１】
前記ＣＲＦ$_1$レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出の３～４時間前に投与される、請求項９に記載の方法。
【請求項１２】
前記ＣＲＦ$_1$レセプターアンタゴニストは、化合物Ｉ（ＮＢＩ－７７８６０；　２，５－ジメチル－３－［２－メチル－４－（メチルオキシ）フェニル］－Ｎ－［（１Ｓ）－１－（３－メチル－１，２，４－オキサジアゾール－５－イル）プロピル］ピラゾロ［１，５－ａ］ピリミジン－７－アミン）である、請求項９～１１のいずれか１項に記載の方法。
【請求項１３】
前記ＣＲＦ$_1$レセプターアンタゴニストは、ＮＢＩ－３０７７５、ＮＢＩ－３４０４１、ＳＳＲ－１２６３７４、ＳＳＲ－１２５５４３、アンタラルミン（Ｎ－ブチル－Ｎ－エ

チル－２，５，６－トリメチル－７－（２，４，６－トリメチルフェニル）ピロロ［３，２－ｅ］ピリミジン－４－アミン）、もしくはＤＭＰ９０４である、請求項９～１１のいずれか１項に記載の方法。

【請求項１４】

先天性副腎皮質過形成（ＣＡＨ）を処置するにあたって使用するためのCRF_1レセプターアンタゴニストであって、ここで該CRF_1レセプターアンタゴニストは、３０分間を超える解離半減期を有する、CRF_1レセプターアンタゴニスト。

【請求項１５】

前記CRF_1レセプターアンタゴニストは、４０分間を超える解離半減期を有する、請求項１４に記載のCRF_1レセプターアンタゴニスト。

【請求項１６】

前記CRF_1レセプターアンタゴニストは、５０分間を超える解離半減期を有する、請求項１４に記載のCRF_1レセプターアンタゴニスト。

【請求項１７】

前記CRF_1レセプターアンタゴニストは、化合物Ｉ（ＮＢＩ－７７８６０；　２，５－ジメチル－３－［２－メチル－４－（メチルオキシ）フェニル］－Ｎ－［（１Ｓ）－１－（３－メチル－１，２，４－オキサジアゾール－５－イル）プロピル］ピラゾロ［１，５－ａ］ピリミジン－７－アミン）である、請求項１４に記載のCRF_1レセプターアンタゴニスト。

【請求項１８】

前記CRF_1レセプターアンタゴニストは、ＮＢＩ－３０７７５、ＮＢＩ－３４０４１、ＳＳＲ－１２６３７４、ＳＳＲ－１２５５４３、アンタラルミン（Ｎ－ブチル－Ｎ－エチル－２，５，６－トリメチル－７－（２，４，６－トリメチルフェニル）ピロロ［３，２－ｃ］ピリミジン－４－アミン）、もしくはＤＭＰ９０４である、請求項１４に記載のCRF_1レセプターアンタゴニスト。

【請求項１９】

前記CRF_1レセプターアンタゴニストは、就寝時に投与するために適している、請求項１４～１８のいずれか１項に記載のCRF_1レセプターアンタゴニスト。

【請求項２０】

前記CRF_1レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出の時にもしくはその前に投与するために適している、請求項１４～１８のいずれか１項に記載のCRF_1レセプターアンタゴニスト。

【請求項２１】

前記CRF_1レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出の３～４時間前に投与するために適している、請求項１４～１８のいずれか１項に記載のCRF_1レセプターアンタゴニスト。

【発明の詳細な説明】

【技術分野】

【０００１】

（背景）

（技術分野）

先天性副腎皮質過形成を処置するための組成物および方法が、本明細書で記載される。

【背景技術】

【０００２】

（関連技術の説明）

先天性副腎皮質過形成（ＣＡＨ）は、コルチゾール生合成をほとんどもしくは全く生じない常染色体劣性遺伝障害の一群である。上記疾患の最も頻度の高い形態は、ＣＡＨ症例のうちの約９５％を占める、染色体６ｐ２１に位置するＣＹＰ２１Ａ２遺伝子における変異によって引き起こされる２１－ヒドロキシラーゼ欠損である（総説に関しては、例えば、Speiser et al., Int. J. Pediatr. Endocr

ｉｎｏｌ．　２０１０：４９４１７３　（２０１０）を参照のこと）。これら変異は、特定の変異の直接的結果である疾患重症度を生じる、副腎皮質におけるコルチゾール合成に必要とされる酵素活性の完全な喪失からある範囲の部分的喪失までの範囲に及び得る。２１－ヒドロキシラーゼ欠損のこの連続型は、塩類喪失形態および単純男性化形態（古典的ＣＡＨとしてグループ分けされる）、ならびに非古典的ＣＡＨ（ＮＣＣＡＨ）もしくは「遅発性」ＣＡＨとして公知の、より軽度の形態（これは通常、後期小児期もしくは早期成人期に診断される）へと広く分類されてきた。非古典的ＣＡＨ患者は、ホモ接合性もしくはしばしば、古典的ＣＡＨ対立遺伝子を伴って、複合ヘテロ接合体のいずれかを有する。これら患者は、十分な酵素活性（正常の＞２０～５０％）を有し、その結果、彼らは塩類喪失もしくはコルチゾール欠損を有さず、誕生時に正常な外性器を有し、そして多くは、生涯を通じて無症候性のままである（Ｔｒａｐｐ　ｅｔ　ａｌ．，　Ｓｔｅｒｏｉｄｓ　７７（４）：３４２－４６　（２０１２））。上記疾患の頻度の少ない形態（これは症例のうちの５％を占める）では、１１β－ヒドロキシラーゼ遺伝子ＣＹＰ１１Ｂ１の変異が、ＣＡＨ（１１β－ＯＨ　ＣＡＨ）を生じる。

両方の遺伝的変異は、アンドロゲンの過剰生成とともに、先天性副腎皮質過形成、コルチゾール欠損および過剰な副腎皮質刺激ホルモン（ＡＣＴＨ）生成を生じる。これら患者には、グルココルチコイドでの終生の管理およびこのような処置と関連して付随する問題が要求される。よって、健康状態、幸福、クオリティ・オブ・ライフを改善するために、およびＣＡＨを有する患者における関連障害を管理するために、処置レジメンが非常に必要である。

【先行技術文献】

【非特許文献】

【０００３】

【非特許文献１】Ｓｐｅｉｓｅｒ　ｅｔ　ａｌ．，　Ｉｎｔ．　Ｊ．　Ｐｅｄｉａｔｒ．　Ｅｎｄｏｃｒｉｎｏｌ．　２０１０：４９４１７３　（２０１０）

【非特許文献２】Ｔｒａｐｐ　ｅｔ　ａｌ．，　Ｓｔｅｒｏｉｄｓ　７７（４）：３４２－４６　（２０１２）

【発明の概要】

【課題を解決するための手段】

【０００４】

（要旨）

副腎皮質刺激ホルモン放出因子（ＣＲＦ）は、クラスＢ　Ｇプロテイン共役レセプター（ＧＰＣＲ）であるＣＲＦ$_1$レセプターを活性化する。ＣＲＦ$_1$アンタゴニストは、ＣＡＨを有する患者におけるＡＣＴＨ放出を直接阻害し、それによって、ヒドロコルチゾンのより低い、より生理学的な用量を使用しながらアンドロゲン生成の正常化を可能にし、処置関連副作用を低減する能力を有する。

【０００５】

一実施形態において、必要性のある被験体に、有効量のＣＲＦ$_1$アンタゴニストを投与する工程（就寝時投与が挙げられる（が、これらに限定されない））によって、ＣＡＨを処置するための方法が提供される。

【０００６】

より具体的な実施形態において、上記ＣＲＦ$_1$アンタゴニストは、３０分間を超える、および別の実施形態では４０分間を超える、および別の実施形態では５０分間を超える解離半減期（$t_{1/2}$）を有する。

【０００７】

実施形態１　３０分間を超える解離半減期を有するＣＲＦ$_1$レセプターアンタゴニストを必要性のある被験体に投与する工程によって、先天性副腎皮質過形成（ＣＡＨ）を処置するための方法。

【０００８】

実施形態２　前記ＣＲＦ$_1$レセプターアンタゴニストは、４０分間を超える解離半減期

を有する、実施形態１に記載の方法。
【０００９】
実施形態３　前記ＣＲＦ$_1$レセプターアンタゴニストは、５０分間を超える解離半減期を有する、実施形態１に記載の方法。
【００１０】
実施形態４　前記ＣＲＦ$_1$レセプターアンタゴニストは、化合物Ｉ（ＮＢＩ－７７８６０；　２，５－ジメチル－３－［２－メチル－４－（メチルオキシ）フェニル］－Ｎ－［（１Ｓ）－１－（３－メチル－１，２，４－オキサジアゾール－５－イル）プロピル］ピラゾロ［１，５－ａ］ピリミジン－７－アミン）である、実施形態１～３のいずれか１つに記載の方法。
【００１１】
実施形態５　前記ＣＲＦ$_1$レセプターアンタゴニストは、ＮＢＩ－３０７７５、ＮＢＩ－３４０４１、ＳＳＲ－１２６３７４、ＳＳＲ－１２５５４３、アンタラルミン（Ｎ－ブチル－Ｎ－エチル－２，５，６－トリメチル－７－（２，４，６－トリメチルフェニル）ピロロ［３，２－ｅ］ピリミジン－４－アミン），もしくはＤＭＰ９０４である、実施形態１～３のいずれか１つに記載の方法。
【００１２】
実施形態６　前記ＣＲＦ$_1$レセプターアンタゴニストは、就寝時に投与される、実施形態１～５のいずれか１つに記載の方法。
【００１３】
実施形態７　前記ＣＲＦ$_1$レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出の時にもしくはその前に投与される、実施形態１～６のいずれか１つに記載の方法。
【００１４】
実施形態８　前記ＣＲＦ$_1$レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出の３～４時間前に投与される、実施形態７に記載の方法。
【００１５】
実施形態９　先天性副腎皮質過形成（ＣＡＨ）を有する被験体における１７－ＯＨＰおよびＡＣＴＨのレベルを低下させるための方法であって、該方法は、該被験体に、ＣＲＦ$_1$レセプターアンタゴニストを就寝時に投与する工程を包含する、方法。
【００１６】
実施形態１０　前記ＣＲＦ$_1$レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出の時にもしくはその前に投与される、実施形態９に記載の方法。
【００１７】
実施形態１１　前記ＣＲＦ$_1$レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出の３～４時間前に投与される、実施形態９または実施形態１０に記載の方法。
【００１８】
実施形態１２　前記ＣＲＦ$_1$レセプターアンタゴニストは、化合物Ｉ（ＮＢＩ－７７８６０；　２，５－ジメチル－３－［２－メチル－４－（メチルオキシ）フェニル］－Ｎ－［（１Ｓ）－１－（３－メチル－１，２，４－オキサジアゾール－５－イル）プロピル］ピラゾロ［１，５－ａ］ピリミジン－７－アミン）である、実施形態９～１１のいずれか１つに記載の方法。
【００１９】
実施形態１３　前記ＣＲＦ$_1$レセプターアンタゴニストは、ＮＢＩ－３０７７５、ＮＢＩ－３４０４１、ＳＳＲ－１２６３７４、ＳＳＲ－１２５５４３、アンタラルミン（Ｎ－ブチル－Ｎ－エチル－２，５，６－トリメチル－７－（２，４，６－トリメチルフェニル）ピロロ［３，２－ｅ］ピリミジン－４－アミン），もしくはＤＭＰ９０４である、実施形態９～１１のいずれか１つに記載の方法。
【００２０】

実施形態１４　先天性副腎皮質過形成（ＣＡＨ）を処置するにあたって使用するためのＣＲＦ$_1$レセプターアンタゴニストであって、ここで該ＣＲＦ$_1$レセプターアンタゴニストは、３０分間を超える解離半減期を有する、ＣＲＦ$_1$レセプターアンタゴニスト。

【００２１】

実施形態１５　前記ＣＲＦ$_1$レセプターアンタゴニストは、４０分間を超える解離半減期を有する、１４に記載のＣＲＦ$_1$レセプターアンタゴニスト。

【００２２】

実施形態１６　前記ＣＲＦ$_1$レセプターアンタゴニストは、５０分間を超える解離半減期を有する、実施形態１４に記載のＣＲＦ$_1$レセプターアンタゴニスト。

【００２３】

実施形態１７　前記ＣＲＦ$_1$レセプターアンタゴニストは、化合物Ｉ（ＮＢＩ－７７８６０；　２，５－ジメチル－３－［２－メチル－４－（メチルオキシ）フェニル］－Ｎ－［（１Ｓ）－１－（３－メチル－１，２，４－オキサジアゾール－５－イル）プロピル］ピラゾロ［１，５－ａ］ピリミジン－７－アミン）である、実施形態１４～１６のいずれか１つに記載のＣＲＦ$_1$レセプターアンタゴニスト。

【００２４】

実施形態１８　前記ＣＲＦ$_1$レセプターアンタゴニストは、ＮＢＩ－３０７７５、ＮＢＩ－３４０４１、ＳＳＲ－１２６３７４、ＳＳＲ－１２５５４３、アンタラルミン（Ｎ－ブチル－Ｎ－エチル－２，５，６－トリメチル－７－（２，４，６－トリメチルフェニル）ピロロ［３，２　ｅ］ピリミジン　４　アミン）、もしくはＤＭＰ９０４である、実施形態１４～１６のいずれか１つに記載のＣＲＦ$_1$レセプターアンタゴニスト。

【００２５】

実施形態１９　前記ＣＲＦ$_1$レセプターアンタゴニストは、就寝時に投与するために適している、実施形態１４～１８のいずれか１つに記載のＣＲＦ$_1$レセプターアンタゴニスト。

【００２６】

実施形態２０　前記ＣＲＦ$_1$レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出の時にもしくはその前に投与するために適している、実施形態１４～１９のいずれか１つに記載のＣＲＦ$_1$レセプターアンタゴニスト。

【００２７】

実施形態２１　前記ＣＲＦ$_1$レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出の３～４時間前に投与するために適している、実施形態１４～２０のいずれか１つに記載のＣＲＦ$_1$レセプターアンタゴニスト。

【００２８】

他の実施形態において、上記でおよび本明細書で記載される方法および使用は、グルココルチコイドもしくは鉱質コルチコイドの量を、ＣＡＨを有する成熟した（ｆｕｌｌ　ｇｒｏｗｎ）被験体（例えば、ヒト被験体）に投与されるＧＣ（例えば、ヒドロコルチゾン（ＨＣ）、プレドニゾン、プレドニゾロン、デキサメタゾン、もしくはフルドロコルチゾン）の推奨される１日用量から、少なくとも１０％、１５％、２０％、３０％、４０％、５０％、６０％低減する工程をさらに包含する。他の実施形態において、上記でおよび本明細書で記載される方法および使用は、グルココルチコイドもしくは鉱質コルチコイドの量を、ＣＡＨを有する成長中の被験体（例えば、ヒト被験体）に投与されるＧＣ（例えば、ヒドロコルチゾン）もしくは鉱質コルチコイド（例えば、フルドロコルチゾン）の推奨される１日用量から、少なくとも１０％、１５％、２０％、３０％、４０％、５０％、６０％低減するＴ程をさらに包含する。

【００２９】

これらおよび他の実施形態は、以下の詳細な説明を参照すれば明らかである。この目的に向けて、ある種の背景情報、手順、化合物および組成物をより詳細に記載する種々の参考文献は、本明細書で示され、それらの全体において各々が本明細書に参考として援用される。

【００３０】
本明細書で具体的に定義されない用語は、本開示および状況に鑑みて、当業者によって上記用語に与えられる意味が与えられるものとする。しかし、本明細書で使用される場合、それとは逆に特定されなければ、上記用語は、示される意味を有する。本明細書全体を通じて「一実施形態」もしくは「ある（一）実施形態」への言及は、上記実施形態に関連して記載される特定の特徴、構造もしくは特性が少なくとも一実施形態に含まれることを意味する。従って、本明細書全体を通じて種々の場所における語句「一実施形態において」もしくは「ある（一）実施形態において」との出現は、全てが必ずしも同じ実施形態をいうわけではない。さらに、上記特定の特徴、構造、もしくは特性は、１以上の実施形態において任意の適切な様式で組み合わされ得る。

【００３１】
また、本明細書および添付の特許請求の範囲で使用される場合、単数形「ａ（１つの、ある）」、「ａｎ（１つの、ある）」および「ｔｈｅ（上記、この、その、）」とは、状況が明らかに別のことを指し示さなければ、複数形への言及をも含む。従って、例えば、「ａ　ｎｏｎ－ｈｕｍａｎ　ａｎｉｍａｌ（ある非ヒト動物）」への言及は、１以上の非ヒト動物、もしくは複数のこのような動物に言及し得、「ａ　ｃｅｌｌ（１つの細胞）」もしくは「ｔｈｅ　ｃｅｌｌ（上記細胞）」への言及は、１以上の細胞および当業者に公知のその均等物（例えば、複数の細胞）への言及を含むなど。方法の工程が記載されるかもしくは特許請求され、上記工程が特定の順序で起こると記載される場合、第２の工程「より前に」（すなわち、その前に）起こる（もしくは行われる）第１の工程の記載は、上記第２の工程が上記第１の工程に「引き続いて」起こる（もしくは行われる）という状態に書き換えられる場合には同じ意味を有する。用語「ａｂｏｕｔ（約、およそ）」とは、数もしくは数値範囲に言及する場合、この言及される数もしくは数値範囲が、実験上の変動性の内にある（もしくは統計上の実験誤差の内にある）近似値であり、従って、その数字もしくは数値範囲が、述べられた数もしくは数値範囲の１％～１５％の間で変動し得ることを意味する。用語「ｏｒ（あるいは、または、もしくは）」は一般に、状況が明らかに別のことを指し示さなければ、「ａｎｄ／ｏｒ（および／または）」を含むその意味で使用されることにも注意するものとする。用語「ａｔ　ｌｅａｓｔ　ｏｎｅ（少なくとも１）」とは、例えば、少なくとも１種の化合物もしくは少なくとも１種の組成物に言及する場合、用語「ｏｎｅ　ｏｒ　ｍｏｒｅ（１以上）」と同じ意味および理解を有する。

【図面の簡単な説明】

【００３２】
【図１】図１は、副腎におけるステロイド合成経路の模式図を示す。ＣＡＨの最も一般的な形態は、２１－ヒドロキシラーゼ（２１－αヒドロキシラーゼともいわれる）欠損によって引き起こされ、コルチゾールの減少およびアンドロゲン（例えば、テストステロンおよびエストロゲン）の増大を生じる。古典的ＣＡＨのより希なタイプは、１１β－ヒドロキシラーゼ欠損である。

【００３３】
【図２】図２は、副腎摘出ラットにおけるＡＣＴＨ濃度に対する化合物Ｉ（ＮＢＩ－７７８６０）の効果を図示するグラフを表す。ラットに、３０ｍｇ／ｋｇの化合物Ｉ（ＮＢＩ－７７８６０）を経口的に与えた。データを、ＡＣＴＨの平均血漿濃度（±ＳＥＭ）として表す。

【００３４】
【図３】図３Ａ～Ｃは、ＣＲＦ１レセプターアンタゴニストの、副腎摘出ラットにおけるＡＣＴＨ濃度に対する効果（それらの解離半減期によって区別される）を図示するグラフを表す。

【００３５】
【図４】図４は、ＮＢＩ－７７８６０の安全性、寛容性および血漿曝露を評価するために設計された、実施例６に記載される臨床試験の研究デザイン模式図、ならびにＨＰＡ（視床下部－下垂体－副腎）系ホルモンの内因性レベルに対するこの化合物の効果を表す。

【００３６】
【図５】図５は、実施例６に記載される臨床試験の間の、投与後２４時間期間の間中の平均１７－ＯＨＰ（上のパネル）およびＡＣＴＨレベル（下のパネル）のデータを表す。
【００３７】
【図６】図６は、３００ｍｇおよび６００ｍｇのＮＢＩ－７７８６０ならびにプラセボの投与後の経時的な１７－ＯＨＰ（上パネル）およびＡＣＴＨレベル（下パネル）に関する具体的な個々の被験体の応答を表す。
【発明を実施するための形態】
【００３８】
（詳細な説明）
本明細書で記載されるように、CRF_1レセプターアンタゴニストは、ＣＡＨを有する患者におけるＡＣＴＨ放出を直接阻害し、それによって、アンドロゲン生成の正常化を可能にすることが見出された。CRF_1レセプターアンタゴニストの投与は、ＣＡＨを有する被験体においてヒドロコルチゾンのより低い、より生理学的な用量の使用を可能にし、従って、処置関連副作用を低減する。
【００３９】
ＣＡＨの新生児スクリーニングは、生後７２時間以内に得られるヒールスティック毛細管血液標本において１７－ＯＨＰレベルを測定するイムノアッセイによって行われる。上記血液サンプルを、市販の解離増強ランタニド蛍光イムノアッセイ（ＤＥＬＦＩＡ；ＰｅｒｋｉｎＥｌｍｅｒ，Ｗａｌｔｈａｍ Ｍａｓｓａｃｈｕｓｅｔｔｓ）（Ｗｈｉｔｅ ｅｔ ａｌ．，Ｊ．Ｐｅｄｉａｔｒ．１６３：１０－１２（２０１３））によって、１７－ＯＨＰについて分析する。
【００４０】
生化学的試験法および分子遺伝学的試験法を利用して生後８～１４日間の間に行われる二番手のスクリーニング試験は、米国の９つの州が使用しており、さらに５つの州が強く推奨している。上記生化学的方法としては、有機溶媒抽出物を用いたイムノアッセイもしくは液体クロマトグラフィー、続いて、コルチゾールに対する１７－ＯＨＰ、アンドロステンジオン、および２１－デオキシコルチゾールのステロイド比を測定するためのタンデム質量分析法が挙げられる（例えば、Ｓｐｅｉｓｅｒ ｅｔ ａｌ．，Ｉｎｔ．Ｊ．Ｐｅｄｉａｔｒ．Ｅｎｄｏｃｒｉｎｏｌ．２０１０：４９４１７３，２０１０を参照のこと）。遺伝子スクリーニングは、ＣＡＨと関連するＣＹＰ２１Ａ２変異を調べる。米国で広く使用されているわけではないが、第２のスクリーニングを追加することで、スクリーニングプロセス全体の感度（ここで第１のスクリーニングのみでは約７２％である）を潜在的に改善できた。
【００４１】
新生児スクリーニングの結果がなければ、古典的ＣＡＨを有する幼い女児は、代表的には、性別が曖昧な外性器の存在に起因して同定される。男性は、生まれたときに正常な外性器を有するので、新生児スクリーニングが行われなければ、または他の医学的合併症に気づかなければ、診断されない。ＣＡＨと最初に診断されず、この疾患の塩類喪失形態を経験する幼児は、生後数週間以内に体重増大が不十分、嘔吐、高カリウム血症および低ナトリウム血症という状況の中で後に診断される。
【００４２】
ＣＡＨの処置は、幼年期から成人期における診断から種々の薬物療法を使用するホルモンおよびステロイドレベルの正常化に基づく。グルココルチコイドは、ＣＡＨでは現在標準的な処置であり、内因性コルチゾール欠損を矯正する、および下垂体からのＡＣＴＨレベル上昇（これは、アンドロゲン生成増大を駆動する）を低減する、両方のために使用される。コルチゾール補充（ｃｏｒｔｉｓｏｌ ｒｅｐｌａｃｅｍｅｎｔ）が十分であるアジソン病（副腎機能低下）の処置とは異なり、ＣＡＨの処置は、ＡＣＴＨ生成も低減して、その後のアンドロゲン過剰をも制御しなければならない。従って、グルココルチコイド処置の目標は、女性においては男性化および月経障害を防止するために、ならびに男性に

おいては精巣副腎残存腫瘍（ｔｅｓｔｉｃｕｌａｒ ａｄｒｅｎａｌ ｒｅｓｔ ｔｕｍｏｒ）を阻害するために、コルチゾール補充およびＡＣＴＨの抑制を含む。鉱質コルチコイド補充は、ＣＡＨの塩類喪失形態を有するそれら患者において通常血圧、電解質バランス、および容積状態の維持のための正常の血漿レニン活性を達成するために必要とされる。

【００４３】

グルココルチコイド処置のレジメンは、正常な生理機能を支援し、そしてまた、強いストレス応答（例えば、併発疾患、運動、低血圧）を誘発し得る事象の間に十分なコルチゾールが利用可能であることを担保しなければならない。アンドロゲン生成を十分に抑制しようとしてグルココルチコイド過剰処置になることに起因する医原性クッシング症候群、または処置不十分に起因するアジソン症候群の発生を回避するためには、注意深いモニタリングもまた必要である。鉱質コルチコイドでの過剰処置は、高血圧症を引き起こし得る一方で、処置不十分は、低血圧、塩類喪失、疲労およびグルココルチコイドの要求の増大をもたらし得る。処置の効力をモニターするための代表的な実験室での検査としては、１７－ＯＨＰ、アンドロステンジオン、テストステロン、レニン活性、および電解質の血漿濃度の測定が挙げられる。

【００４４】

ＣＡＨを有する成人患者は、肥満、高血圧症、およびインスリン抵抗性を含む心血管疾患のリスク因子の増大した保有率を有する（例えば、Ｋｉｍ ｅｔ ａｌ．，Ｓｅｍｉｎ．Ｒｅｐｒｏｄ．Ｍｅｄ．２７（４）：３１６－２１（２００９）を参照のこと）。小児および成人のＣＡＨ患者（ｎ＝２４４）の大コホート研究から、患者は、種々のグルココルチコイド処置レジメンを処方されるにも関わらず、不十分なホルモン制御および前述の有害転帰を頻繁に経験していることが示された（例えば、Ｆｉｎｋｉｅｌｓｔａｉｎ ｅｔ ａｌ．，Ｊ．Ｃｌｉｎ．Ｅｎｄｏｃｒｉｎｏｌ Ｍｅｔａｂ．９７（１２）：４４２９－３８（２０１２）を参照のこと）。

【００４５】

ＣＡＨの処置は、グルココルチコイド（通常は、小児ではヒドロコルチゾンであるが、成人では、デキサメタゾンのような、しばしば治療指数の狭いより強力な薬剤）、および塩類喪失に必要であれば、鉱質コルチコイド（通常はフルドロコルチゾン）でのコルチゾール欠損を正常化する試みを含む。しかし、過剰なアンドロゲンの十分な抑制を達成するために必要とされるグルココルチコイド用量は、通常は、アジソン病を有する患者におけるように、コルチゾール補充のみに使用される通常の生理学的用量を十分に上回っている。グルココルチコイドへのこの増大した曝露は、ＣＡＨ患者において、医原性クッシング症候群、心血管リスク因子の増大、耐糖能障害、および骨密度（ｂｏｎｅ ｍｉｎｅｒａｌ ｄｅｎｓｉｔｙ）の低下をもたらし得る（例えば、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）を参照のこと）。

【００４６】

副腎皮質刺激ホルモン放出因子（ＣＲＦ）を、ヒツジ視床下部から単離し、４１アミノ酸のペプチドとして同定した。ＣＲＦは、内分泌系、神経系、および免疫系の機能において顕著な変化を生じることが見出された。ＣＲＦは、副腎皮質刺激ホルモン（「ＡＣＴＨ」）、β－エンドルフィン、および他のプロオピオメラノコルチン（「ＰＯＭＣ」）（下垂体前葉由来ペプチド）の基底の放出およびストレス誘発性の放出の主要な生理学的調節因子であると考えられる（例えば、Ｖａｌｅ ｅｔ ａｌ．，Ｓｃｉｅｎｃｅ ２１３：１３９４－１３９７，１９８１を参照のこと）。ＣＲＦの分泌は、Ｇプロテイン共役レセプターのクラスＢファミリーのメンバーであるＣＲＦ$_1$レセプターへの結合を介して、下垂体前葉における副腎皮質刺激ホルモン生成細胞（ｃｏｒｔｉｃｏｔｒｏｐｈ）から

のＡＣＴＨ放出を引き起こす。

【００４７】

ＣＲＦの生理学的重要性に起因して、顕著なＣＲＦ$_1$レセプター結合活性を有しかつ上記ＣＲＦ$_1$レセプターに拮抗できる生物学的に活性な低分子の開発は、未だに望まれる目標であり、不安、鬱病、過敏性腸症候群、心的外傷後ストレス障害、および物質乱用の処置のために進行中の研究および開発の主題であり続けている。

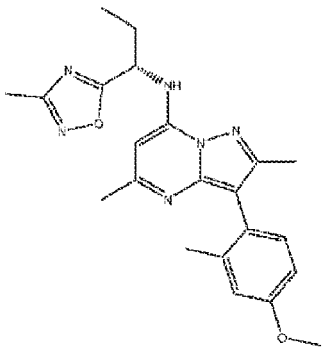
【００４８】

下垂体ホルモンＡＣＴＨは、視床下部の副腎皮質刺激ホルモン放出因子（ＣＲＦ）の制御下にあり、コレステロールの取り込みを刺激し、副腎におけるステロイド生成を開始するプレグネノロンの合成を駆動する（図１を参照のこと）。副腎皮質は、３つのゾーンから構成され、これらは、ホルモンの別個のクラスを生成し、そのうちの多くは、この経路を介してコレステロールを動員させるＡＣＴＨによって駆動される。変異もしくは欠失の結果としてのこれら酵素の欠損は、基質濃度の増大を引き起こす。２１－ヒドロキシラーゼ遺伝子（ＣＹＰ２１Ａ２）における変異もしくは欠失から生じるＣＡＨの最も一般的な形態では、ステロイド前駆体である、プロゲステロンおよび１７－ヒドロキシプロゲステロン（１７－ＯＨＰ）の蓄積が原因で、強力なアンドロゲンが副腎によって生成される。１７－ＯＨＰの血漿レベルは、これら症例において正常濃度の１０～１０００倍にも達し得る。これら増大は、アンドロゲン、具体的には、アンドロステンジオン、テストステロン、およびジヒドロキシテストステロンの過剰生成を生じ、女性において男性化を引き起こす。さらに、ＣＡＨにおける２１　ヒドロキシラーゼ欠損は、グルココルチコイドおよび鉱質コルチコイド、具体的にはコルチゾールおよびアルドステロンの不十分な生合成を引き起こす。コルチゾールは、視床下部のＣＲＦ分泌および下垂体のＡＣＴＨ放出の重要なネガティブフィードバック調節因子である。グルココルチコイド合成および放出の欠如は、視床下部および下垂体に対する抑制を排除し、このことは、ＡＣＴＨレベルを増大させる。過剰なＡＣＴＨ刺激は、束状帯および網状帯の肥大を引き起こし、副腎過形成を生じる。

【００４９】

一実施形態において、ＣＡＨの処置に有用なＣＲＦ$_1$レセプターアンタゴニストは、ＮＢＩ－７７８６０、２，５－ジメチル－３－［２－メチル－４－（メチルオキシ）フェニル］－Ｎ－［（１Ｓ）－１－（３－メチル－１，２，４－オキサジアゾール－５－イル）プロピル］ピラゾロ［１，５－ａ］ピリミジン－７－アミン（本明細書で「化合物１」ともいわれる）であり、これは、以下の構造を有する。

【化１】



化合物I

【００５０】

化合物Ｉは、結合ｐＫｉ＝８．２を有する強力なＣＲＦ$_1$アンタゴニストである（例えば、Ｔｅｌｌｅｗ　ｅｔ　ａｌ．，　Ｂｉｏｏｒｇ．　Ｍｅｄ．　Ｃｈｅｍ．　Ｌｅｔｔ．　２０：７２５９，　２０１０および国際特許出願公開番号ＷＯ　２００６／０４４９

５８（これらはともに、それらの全体において本明細書に参考として援用される）を参照のこと）。本明細書で記載されるとおり、化合物Ｉは、副腎摘出ラットにおいて示されるように強力なＡＣＴＨ低下効果を有する。

【００５１】

別の実施形態において、ＣＡＨの処置に有用な$ＣＲＦ_1$レセプターアンタゴニストは、米国特許第６，５８６，４５６号、同第６，８０６，２８２号、同第６，５３１，４７５号、同第６，６６４，２６１号、同第６，６１０，６７８号、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、およびＷＯ　２０１１／０４３３８１に記載されるとおりの低分子アンタゴニストである。

【００５２】

さらに別の実施形態において、上記$ＣＲＦ_1$レセプターアンタゴニストは、ＮＢＩ－３０７７５、ＣＰ－３１６，３１１、ペキサセルホント、エミセルホント（ｅｍｉｃｅｒｆｏｎｔ）、ＳＳＲ－１２５５４３［４－（２－クロロ－４－メトキシ－５－メチルフェニル）－Ｎ－［（１Ｓ）－２－シクロプロピル－１－（３－フルオロ－４－メチルフェニル）エチル］－５－メチル－Ｎ－（２－プロピン－１－イル）－２－チアゾールアミン］、ＳＳＲ－１２６３７４、ＯＮＯ－２３３３、ＮＢＩ－３４０４１、ＪＮＪ－１９５６７４７０、ＧＳＫ５８６５２９、ＰＦ－００５７２７７８、ＣＰ－３７６３９５、アンタラルミン（Ｎ－ブチル－Ｎ－エチル－２，５，６－トリメチル－７－（２，４，６－トリメチルフェニル）ピロロ［３，２－ｅ］ピリミジン－４－アミン）、およびＤＭＰ９０４である。

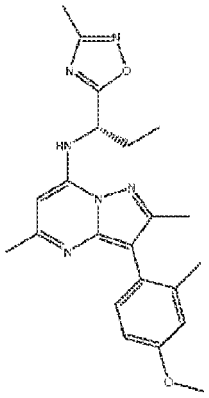
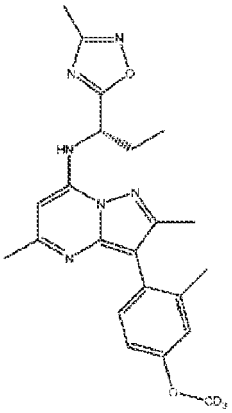
【００５３】

なお別の実施形態において、上記$ＣＲＦ_1$レセプターアンタゴニストは、３０分間を超える、および別の実施形態では４０分間を超える、および別の実施形態では５０分間を超える解離半減期（$t_{1/2}$）を有する。特定の$ＣＲＦ_1$レセプターアンタゴニストの解離半減期は、実施例３で開示される技術によって決定される。これら実施形態の代表的$ＣＲＦ_1$レセプターアンタゴニストとしては、化合物Ｉ、ＮＢＩ－３０７７５、ＮＢＩ－３４０４１、ＳＳＲ－１２５５４３Ａ、アンタラルミン（Ｎ－ブチル－Ｎ－エチル－２，５，６－トリメチル－７－（２，４，６－トリメチルフェニル）ピロロ［３，２－ｅ］ピリミジン－４－アミン）、およびＤＭＰ９０４が挙げられる。

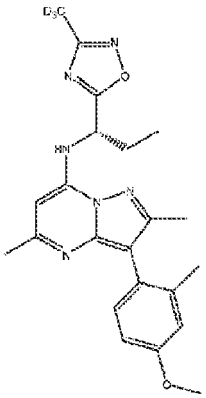
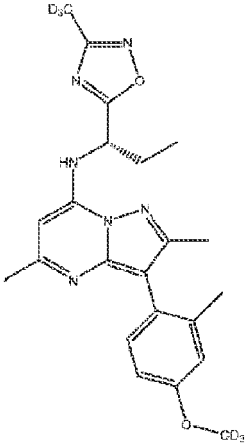
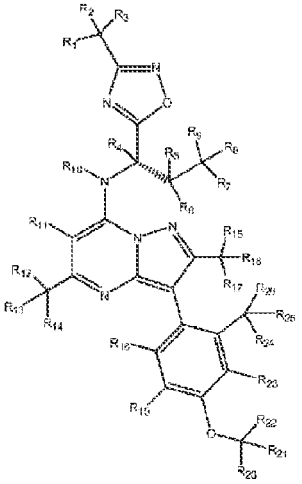
【００５４】

本明細書で記載される化合物に関しては、特定の位置が水素と指定される場合、そのような水素（Ｈ）は、重水素（Ｄ）で置換され得ることもまた、理解されるものとする。水素の代わりに重水素を組み込むことは、その置換された化合物の生理学的活性および薬理学的活性に対して顕著な効果を生じることが公知である。この目的に向けて、水素の重水素置換は、その位置における重水素存在量（ａｂｕｎｄａｎｃｅ）が天然の重水素存在量より実質的に大きいことを意味するということは、理解されるはずである。よって、一実施形態において、代表的化合物としては、以下が挙げられる。

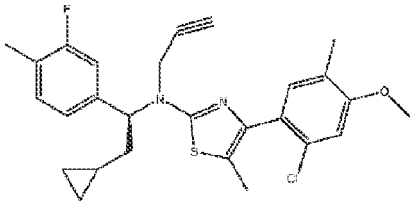
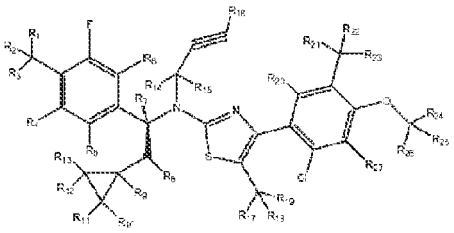
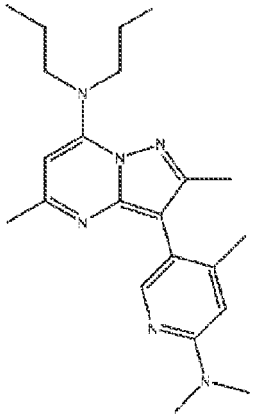
【化２－１】

化合物	構造	化学名
NBI-77860		2,5－ジメチル－3-[2－メチル－4-(メチルオキシ)フェニル]-N-[(1S)-1-(3－メチル－1,2,4－オキサジアゾール－5－イル)プロピル]ピラゾロ[1,5-a]ピリミジン-7-アミン
重水素化 NBI-77860		2,5－ジメチル－3-[2－メチル－4-(d_3-メチルオキシ)フェニル]-N-[(1S)-1-(3－メチル－1,2,4－オキサジアゾール－5－イル)プロピル]ピラゾロ[1,5-a]ピリミジン-7-アミン

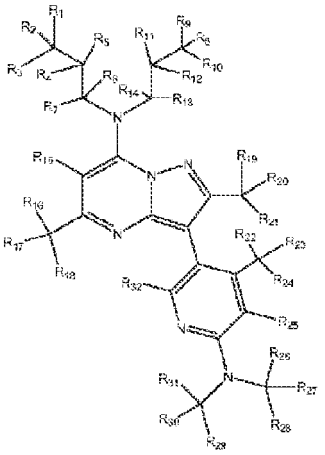
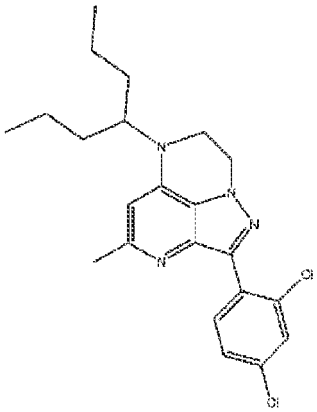
【化２－２】

化合物	構造	化学名
重水素化 NBI-77860		2,5－ジメチル－3-[2－メチル－4-（メチルオキシ）フェニル]-N-[(1S)-1-(3-d_3－メチル－1,2,4－オキサジアゾール－5－イル）プロピル]ピラゾロ[1,5-a]ピリミジン-7-アミン
重水素化 NBI-77860		2,5－ジメチル－3-[2－メチル－4-（d_3-メチルオキシ）フェニル]-N-[(1S)-1-(3-d_3－メチル－1,2,4－オキサジアゾール－5－イル）プロピル]ピラゾロ[1,5-a]ピリミジン-7-アミン
重水素化 NBI-77860		

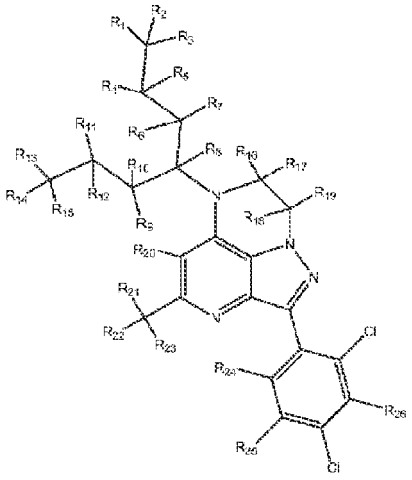
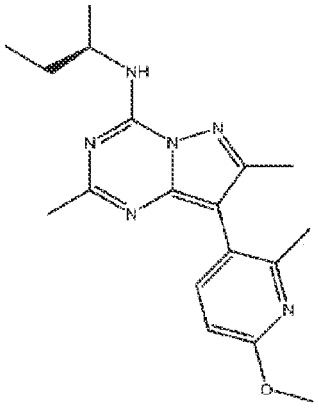
【化２－３】

化合物	構造	化学名
	R_1-R_{28}は、独立してHもしくはD	
SSR-125543		4-(2-クロロ-4-メトキシ-5-メチルフェニル)-*N*-[(1*S*)-2-シクロプロピル-1-(3-フルオロ-4-メチルフェニル)エチル]-5-メチル-*N*-(2-プロピン-1-イル)-2-チアゾールアミン
重水素化 SSR-125543	 R_1-R_{27}は、独立してHもしくはD	
NBI-30775		5-[7-(ジプロピルアミノ)-2,5-ジメチルピラゾロ[1,5-a]ピリミジン-3-イル]-N,N,4-トリメチルピリジン-2-アミン

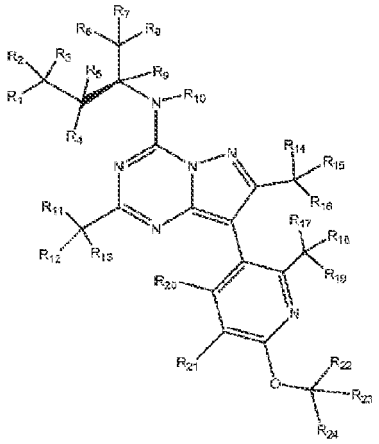
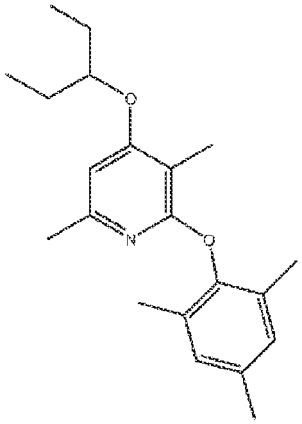
【化２－４】

化合物	構造	化学名
重水素化 NBI-30775	 R_1–R_{32}は、独立してHもしくはD	
NBI-34041		3-(2,4-ジクロロフェニル)-9-(ヘプタン-4-イル)-6-メチル-1,2,5,9-テトラアザトリシクロ[6.3.1.$0^{4,12}$]ドデカ-2,4(12),5,7-テトラエン

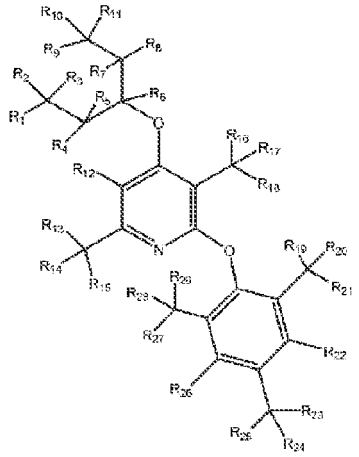
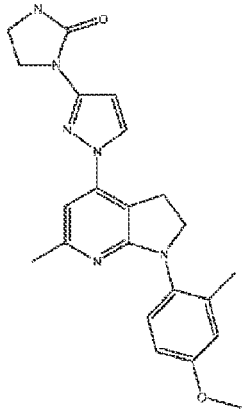
【化２－５】

化合物	構造	化学名
重水素化 NBI-34041	 R_1-R_{26}は、独立してHもしくはD	
ペキサセルホント		N-[(2R)-ブタン-2-イル]-8-(6-メトキシ-2-メチルピリジン-3-イル)-2,7-ジメチルピラゾロ[1,5-a][1,3,5]トリアジン-4-アミン

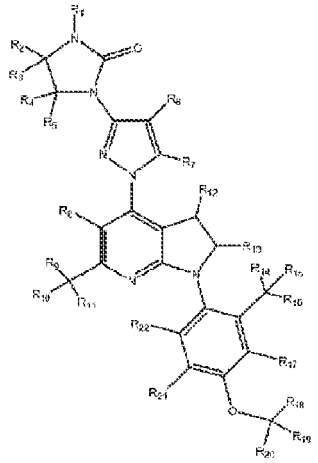
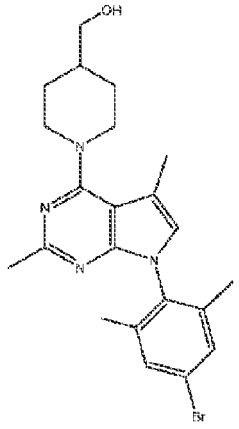
【化２－６】

化合物	構造	化学名
重水素化ペキサセルホント	 R_1–R_{24}は、独立してHもしくはD	
CP-316,311		3,6－ジメチル－4-(ペンタン-3-イルオキシ)-2-(2,4,6-トリメチルフェノキシ)ピリジン

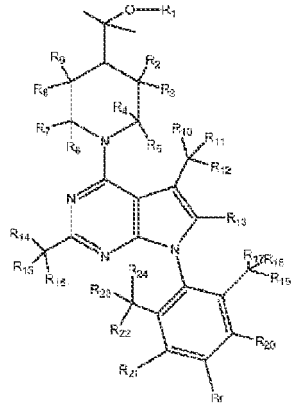
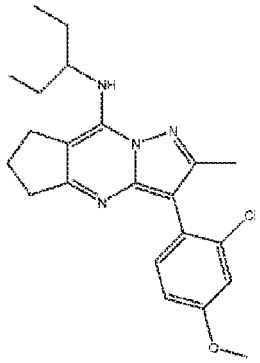
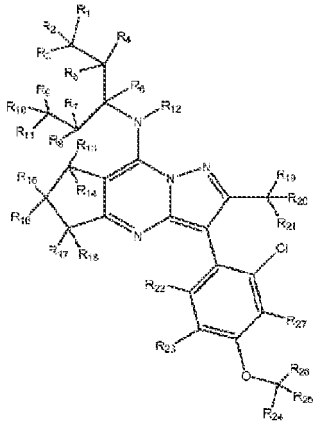
【化２－７】

化合物	構造	化学名
重水素化 CP-316,311	 R_1-R_{29}は、独立してHもしくはD	
GSK876008		

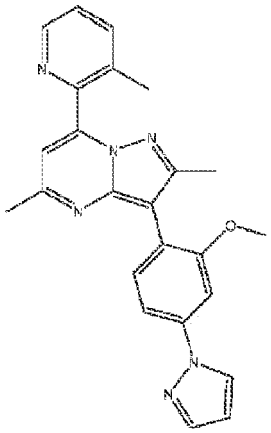
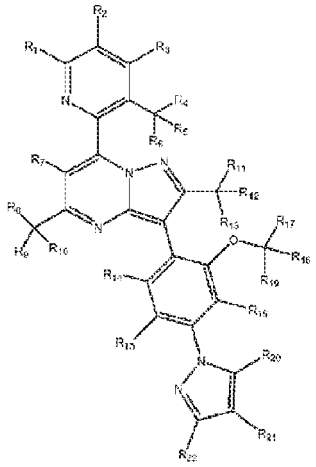
【化２－８】

化合物	構造	化学名
重水素化 GSK876008	 R_1-R_{22}は、独立してHもしくはD	
CRA5626/R3 17573		

【化２－９】

化合物	構造	化学名
重水素化 CRA5626/R3 17573	 R_1-R_{24}は、独立してHもしくはD	
ONO2333Ms		10-(2-クロロ-4-メトキシフェニル)-11-メチル-N-(ペンタン-3-イル)-1,8,12-トリアザトリシクロ[7.3.0.0^{3,7}]ドデカ-2,7,9,11-テトラエン-2-アミン
重水素化 ONO2333	 R_1-R_{27}は、独立してHもしくはD	

【化２－１０】

化合物	構造	化学名
NBI76169		
重水素化 NBI76169	 R_1-R_{22}は、独立してHもしくはD	

【００５５】

別の実施形態において、前述の化合物のうちのいずれも、安定なもしくは放射活性な同位体を組み込み得る。よって、本明細書で記載されるものに同一な同位体標識された化合物の使用もまた企図され、ここで１個以上の原子は、天然において通常見出されるある原子質量もしくは質量数とは異なる原子質量もしくは質量数を有する原子によって置換される。これら化合物へと組み込まれ得る同位体の例としては、水素、炭素、窒素、酸素、リン、フッ素および塩素の同位体、例えば、それぞれ、上記で考察されるとおりの重水素（^{2}Ｈ）、ならびに^{3}Ｈ、^{13}Ｃ、^{14}Ｃ、^{15}Ｎ、^{18}Ｏ、^{17}Ｏ、^{31}Ｐ、^{32}Ｐ、^{35}Ｓ、^{18}Ｆ、および^{36}Ｃｌが挙げられるが、それに限定されない。ある種の同位体標識された化合物、例えば、^{3}Ｈおよび^{14}Ｃのような放射活性同位体が組み込まれているものはまた、薬物もしくは基質組織分布アッセイにおいて有用である。トリチウム水素（^{3}Ｈ）および炭素１４（^{14}Ｃ）同位体は、それらの準備および検出可能性の容易さのために特に好ましい。より重い同位体（例えば、重水素（^{2}Ｈ））での置換は、より大きな代謝安定性、例えば、インビボ半減期の増大もしくは用量要求の低下から生じるある種の治療的利点を提供し得、従って、いくつかの環境では好ましい可能性がある。同位体標識

した化合物は一般に、当該分野で慣用的に実施される手順を行うことによって調製され得る。

（副腎摘出ラットにおける血漿ＡＣＴＨに対する効果）

【００５６】

副腎摘出は、ラットにおいて循環コルチコステロン（主なグルココルチコイド）を排除し、視床下部および下垂体（副腎皮質ホルモン生成細胞）両方のレベルにおいてＨＰＡ系のネガティブフィードバック制御を除去し、従って、血漿ＡＣＴＨを慢性的に上昇させる（例えば、Ｍｉｍｓ ｅｔ ａｌ．，Ｊ．Ｎａｔｌ．Ｍｅｄ．Ａｓｓｏｃ．６９：１４５－４７ （１９７７）を参照のこと）。ペプチドＣＲＦ$_1$レセプターアンタゴニストの静脈内注射は、副腎摘出（ＡＤＸ）ラットにおける高い血漿ＡＣＴＨレベルを低下させることが示された（例えば、Ｒｉｖｉｅｒ ｅｔ ａｌ．，Ｊ．Ｍｅｄ．Ｃｈｅｍ．１２：４２：３１７５－８２ （１９９９）を参照のこと）。これら知見は、上記低分子ＮＢＩ－７７８６０（化合物Ｉ）で再現された。副腎摘出ラットにおいて、化合物Ｉは、ＡＣＴＨ低下に関する強力な能力を有する。ＡＣＴＨにおける最大の低減は、ＮＢＩ－７７８６０のピーク血漿濃度と相関した；しかし、ＡＣＴＨ低下効果の持続時間は、薬物血漿曝露を超えていた。従って、副腎摘出ラットにおいて、ＮＢＩ－７７８６０の統合された血漿曝露と経口投与後のインビボ効力との間に推定可能な関係が存在する。

【００５７】

ＣＲＦレセプターアンタゴニストとしての化合物の有効性は、種々のアッセイ法によって決定され得る。本明細書で記載されるＣＲＦアンタゴニストは、ＣＲＦの、そのレセプターへの特異的結合を阻害し得、結果として、ＣＲＦと関連する活性を拮抗し得る。ある化合物は、１種以上の一般に受け入れられているアッセイ（実施例中に記載されるアッセイが挙げられる）によって、ＣＲＦアンタゴニストとしての活性について評価され得る。本明細書で記載される方法に有用なＣＲＦアンタゴニストは、ＣＲＦレセプターに対する親和性を示す化合物を含む。

【００５８】

理論に拘束されることは望まないが、ＣＡＨの処置において、ＣＲＦレセプターアンタゴニストは、下垂体副腎皮質刺激ホルモン生成細胞からのＡＣＴＨ放出を潜在的にブロックし（それによって、アンドロゲンの生成を低下させる）、コルチゾールの補充のためのより洗練された処置方法論を可能にする。動物およびヒトでの研究は、ＡＣＴＨ放出に対する化合物Ｉ（ＮＢＩ－７７８６０）の薬理学的効果を示した。処置効力をモニターする場合に内分泌学者が使用する標準的な生体マーカー評価は、このＣＲＦ$_1$レセプターアンタゴニストの効果をモニターするために使用され得る。１７－ＯＨＰ、アンドロステンジオン、テストステロン、コルチゾールおよびＡＣＴＨの血漿レベル、ならびにこれらステロイドの尿代謝産物は、小児および成人の両方において容易に測定され、処置の影響に関する迅速かつ有意義なデータを与える。

（処置についての薬学的組成物および方法）

【００５９】

本開示は、本明細書で記載されるＣＲＦアンタゴニスト化合物のうちのいずれか１種および薬学的に受容可能な賦形剤を含む、ＣＡＨを処置するための方法において使用するための薬学的組成物をさらに提供する。薬学的に受容可能な賦形剤は、活性成分の活性に干渉しない、生理学的におよび薬学的に適した、非毒性でかつ不活性な物質もしくは成分である；賦形剤はまた、キャリアともいわれ得る。上記ＣＲＦアンタゴニスト化合物は、処置もしくは防止的（もしくは予防的）処置（例えば、ＣＡＨ疾患の増悪、または上記疾患の１以上の症状の発生もしくは再発を低減する）において使用するための薬学的組成物に製剤化され得る。本明細書で記載される方法および賦形剤は例示であり、決して限定ではない。薬学的に受容可能な賦形剤は、薬学分野において周知であり、例えば、Ｒｏｗｅ ｅｔ ａｌ．，Ｈａｎｄｂｏｏｋ ｏｆ Ｐｈａｒｍａｃｅｕｔｉｃａｌ Ｅｘｃｉｐｉｅｎｔｓ：Ａ Ｃｏｍｐｒｅｈｅｎｓｉｖｅ Ｇｕｉｄｅ ｔｏ Ｕｓｅｓ，Ｐｒｏｐｅｒｔｉｅｓ，ａｎｄ Ｓａｆｅｔｙ，５^{ｔｈ} Ｅｄ．，２００６，および

Ｒｅｍｉｎｇｔｏｎ：Ｔｈｅ Ｓｃｉｅｎｃｅ ａｎｄ Ｐｒａｃｔｉｃｅ ｏｆ Ｐｈａｒｍａｃｙ（Ｇｅｎｎａｒｏ，２１ｓｔ Ｅｄ．Ｍａｃｋ Ｐｕｂ．Ｃｏ．，Ｅａｓｔｏｎ，ＰＡ（２００５））に記載される。薬学的に受容可能な賦形剤の例としては、滅菌生理食塩水および生理学的ｐＨのリン酸緩衝化生理食塩水が挙げられる。保存剤、安定化剤、色素、緩衝化剤などは、上記薬学的組成物の中に提供され得る。さらに、抗酸化剤および懸濁剤もまた、使用され得る。

【００６０】

治療的および／もしくは予防的利益としては、例えば、改善された臨床転帰（治療的処置および予防的もしくは防止的手段の両方）が挙げられ、ここでその目的は、望ましくない生理学的変化もしくは障害を防止するもしくは遅らせるもしくは遅延させる（減らす）こと、またはこのような障害の拡大もしくは重篤度を防止するもしくは遅らせるもしくは遅延させる（減らす）ことである。本明細書で考察される場合、被験体を処置することからの有益なもしくは望ましい臨床結果としては、以下が挙げられるが、これらに限定されない：処置される予定の疾患、状態もしくは障害から生じるかもしくはこれらと関連する症状の減退、減少、もしくは緩和；症状の発生の減少；クオリティ・オブ・ライフの改善；疾患がない状態（すなわち、被験体が症状（これに基づいて、疾患の診断が行われる）を示す見込みもしくは傾向を減少させること）が長くなる；疾患の程度の縮小；疾患の安定した（すなわち、悪化していない）状態；疾患進行の遅滞もしくは遅れ；上記疾患状態の改善もしくは軽減；ならびに検出可能であろうと検出不能であろうと、退縮（部分的であろうが全体であろうが）；ならびに／または全生存。「Ｔｒｅａｔｍｅｎｔ（処置）」はまた、被験体が処置を受けなかった場合に予測される生存と比較したときに、生存を長期化することを意味し得る。処置の必要性のある被験体としては、上記状態もしくは障害を既に有している人々、ならびに上記疾患、状態もしくは障害を有する傾向にあるかまたは発生させるリスクのある被験体、ならびに上記疾患、状態もしくは障害が防止される（すなわち、上記疾患、障害もしくは状態の発生の見込みを減少させる）べきである人々が挙げられる。被験体は、ヒトもしくは非ヒト哺乳動物（例えば、ラット、マウス、イヌ、ネコ、家畜、動物園動物）であり得る。

【００６１】

至適用量は、一般に、実験モデルおよび／もしくは臨床試験を使用して決定され得る。上記至適用量は、上記被験体のボディーマス、体重、もしくは血液容積に依存し得る。一般に、ある用量の中に存在する、本明細書で記載される化合物の量は、約０．１ｍｇ～約３０ｍｇ／ｋｇ被験体体重の範囲に及ぶ。ある種の実施形態において、単一用量は、約５０～１０００ｍｇである。有効な治療を提供するために十分である最少用量の使用は、通常好ましい。被験体は、一般に、臨床評価によって、および処置もしくは防止されている状態に適したアッセイを使用して、治療有効性についてモニターされ得る。このアッセイは、当業者が精通しており、本明細書に記載される。被験体に投与される化合物のレベルは、生物学的流体中の、例えば、血液、血液画分（例えば、血清）および／もしくは尿中の、ならびに／または上記被験体に由来する他の生物学的サンプル中の上記化合物のレベルを決定することによってモニターされ得る。上記化合物を検出するために当該分野で実施される任意の方法は、治療レジメンの経過の中で化合物のレベルを測定するために使用され得る。

【００６２】

本明細書で記載される化合物のうちの少なくとも１種を含む、ＣＡＨまたは関連疾患もしくは障害を処置するための組成物の用量は、上記被験体の状態、すなわち、上記疾患のステージ、上記疾患によって引き起こされる症状の重篤度、全身の健康状態、ならびに年齢、性別および体重、ならびに医療分野の当業者に明らかな他の因子に依存し得る。同様に、上記化合物の用量は、医療分野の当業者によって理解されるパラメーターに従って決定され得る。

【００６３】

本明細書で記載されるＣＲＦ$_1$アンタゴニスト化合物のうちの少なくとも１種を含む本

明細書で記載される薬学的組成物は、上記化合物の有効量を効率的に送達するいくつかの経路のうちのいずれか１つによって、必要性のある被験体に投与され得る。このような投与経路としては、例えば、経口、非経口、腸、直腸、鼻内、口内、舌下、筋肉内、および経皮が挙げられる。これら投与経路などによって投与される組成物は、本明細書でより詳細に記載される。

【００６４】

開示される化合物もしくは組成物の投与は、夜間投与もしくは寝る前の投与（すなわち、就寝時投与）を含む。本明細書で使用される場合、就寝時投与とは、予測される概日リズムによるＡＣＴＨ放出の時にもしくはその前に（例えば、２～５時間前）、上記CRF_1アンタゴニストの臨床的に関連する濃度を送達するように意図された投与をいう。このＡＣＴＨ放出は、代表的には午前１～２時であるので、そして大部分の経口投与された薬物は数時間というＴｍａｘを有するので、例えば、予測される概日リズムによるＡＣＴＨ放出の３～４時間前である午後１０時の投与は、望ましい。この同じプレパルス就寝時投与は、シフト労働者（例えば、夜間に働いて日中に睡眠をとる人々）のために適合させられ得、この場合には、投与は、必ずしも夜間に起こらなくてもよい。従って、投与は、予測される概日リズムによるＡＣＴＨ放出に依存し、個体の（すなわち、被験体、患者の）特定の労働および睡眠パターンに依存して変動し得る。ある種の実施形態において、本明細書で記載されるCRF_1レセプターアンタゴニスト（例えば、ＮＢＩ－７７８６０、またはＮＢＩ－３０７７５、ＮＢＩ－３４０４１、ＳＳＲ－１２６３７４、ＳＳＲ－１２５５４３、アンタラルミン（Ｎ　ブチル　Ｎ　エチル　２，５，６　トリメチル　７　（２，４，６－トリメチルフェニル）ピロロ［３，２－ｅ］ピリミジン－４－アミン）、もしくはＤＭＰ９０４のうちのいずれか１種）は、予測される概日リズムによるＡＣＴＨ放出前（すなわち、先に、先だって）の約２～５時間の間に投与される。他の実施形態において、上記CRF_1レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出前の約２～４時間の間もしくは３～５時間の間に、上記被験体に投与される。より具体的な実施形態において、CRF_1レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出前の約３～４時間の間に上記被験体に投与される。

【００６５】

ＣＡＨを有する被験体（すなわち、患者、個体）における１７－ＯＨＰおよび／もしくはＡＣＴＨレベルを、CRF_1レセプターアンタゴニストを投与する工程によって低下させるための方法もまた、本明細書で提供される。ある種の実施形態において、上記アンタゴニストは、予測される概日リズムによるＡＣＴＨ放出の時にもしくはその前に投与される。他のある種の実施形態において、上記CRF_1レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出の前の約２～４時間の間もしくは約３～５時間の間に投与される。より具体的な実施形態において、CRF_1レセプターアンタゴニストは、予測される概日リズムによるＡＣＴＨ放出の前の約３～４時間の間に上記被験体に投与される。

【００６６】

CRF_1レセプターアンタゴニストを必要性のある女性被験体に、上記被験体においてＡＣＴＨおよび１７－ＯＨＰのレベルの低下を引き起こす本明細書で記載される様式で投与する工程を包含する本明細書で記載される方法は、アンドロゲン（例えば、テストステロンおよびアンドロステンジオンの放出の低下を生じ得る。グルココルチコイドの用量は、臨床的に有意な量によって付随して低下させられ得、これは、翻って副作用の低下を生じる。

【００６７】

ＣＡＨを有する患者および若く成長中である患者における維持療法のための、ならびに成熟した患者における維持療法のためのグルココルチコイド（ＧＣ）および鉱質コルチコイド（ＭＣ）の量は、当業者に公知である。例えば、ガイドラインは、Ｓｐｅｉｓｅｒ　ｅｔ　ａｌ．（Ｊ．Ｃｌｉｎ．Ｅｎｄｏｃｒｉｎｏｌ．Ｍｅｔａｂ．９５：４１３３－６０　（２０１０）（その全体において参考として援用される）に記載され、そ

の中の表１および表２に示される。特に、ＣＡＨを有する若く成長中である患者に関しては、当業者は、ＧＣでの高すぎる投与が上記患者において身長の成長（ｓｔａｔｕｒａｌ ｇｒｏｗｔｈ）を妨げ得ることを認識する。よって、ＣＲＦ$_1$レセプターアンタゴニストで患者を処置するための本明細書で記載される方法は、臨床的に有意な様式でＧＣの用量を低下させる工程を包含し得る。

【００６８】

ある種の実施形態において、ＣＲＦ$_1$レセプターアンタゴニストを投与する工程によってＣＡＨを処置するための方法は、ＣＡＨを有する被験体を処置するために現在推奨されるＧＣ用量より低い用量で、ＧＣを投与する工程をさらに包含し得る。上記被験体が成熟した患者である場合、ＧＣの用量（例えば、成熟した被験体における維持療法のために推奨されるヒドロコルチゾン（ＨＣ）、プレドニゾン、プレドニゾロン、デキサメタゾン、もしくはフルドロコルチゾンの用量）は、１５～２５ｍｇ／日　ＨＣ；５～７．５ｍｇ／日　プレドニゾン、４～６　ｍｇ／日　プレドニゾロン；０．２５～０．５ｍｇ／日　デキサメタゾン、もしくは０．０５～０．２ｍｇ／日　フルドロコルチゾンという推奨用量から、約１０％、１５％、２０％、３０％、４０％、５０％、６０％以上の程度、低下させられ得る。ＣＡＨを有する成長中である被験体において、ＧＣ（例えば、ＨＣ）の推奨用量は、１０～１５ｍｇ／ｍ^2／日という合計推奨用量から低下させられ得、そして／または０．０５～０．２ｍｇ／日のフルドロコルチゾンという合計用量は、各々、本明細書で記載されるとおりのＣＲＦ$_1$レセプターアンタゴニストを受ける被験体において約１０％、１５％、２０％、３０％、４０％、５０％、６０％以上の程度低下させられ得る。一実施形態において、必要性のある被験体に有効量のＣＲＦ$_1$アンタゴニストを投与する工程を包含する本明細書で記載される方法は、プラセボと比較して、ＡＣＴＨレベルの臨床的に有意な低下を生じる。具体的実施形態において、必要性のある被験体に有効量のＣＲＦ$_1$アンタゴニストを投与する工程は、プラセボと比較して、ＡＣＴＨレベルの臨床的に有意な低下を生じ、ここでその低下は、少なくとも２５％である。別の実施形態において、必要性のある被験体に有効量のＣＲＦ$_1$アンタゴニストを投与する工程は、プラセボと比較して、ＡＣＴＨレベルの臨床的に有意な低下を生じ、ここでその低下は、少なくとも５０％である。グルココルチコイドおよび鉱質コルチコイドの投与に関するガイドラインについては、Ｓｐｅｉｓｅｒ　ｅｔ　ａｌ．（前出）を参照のこと。

【００６９】

別の実施形態において、必要性のある被験体に有効量のＣＲＦ$_1$アンタゴニストを投与する工程を包含する本明細書で記載される方法は、プラセボと比較して、１７－ＯＨＰレベルの臨床的に有意な低下を生じる。別の具体的実施形態において、必要性のある被験体に有効量のＣＲＦ$_1$アンタゴニストを投与する工程は、プラセボと比較して、１７－ＯＨＰレベルの臨床的に有意な低下を生じ、ここでその低下は、少なくとも２５％である。さらに別の実施形態において、必要性のある被験体に有効量のＣＲＦ$_1$アンタゴニストを投与する工程は、プラセボと比較して、１７－ＯＨＰレベルの臨床的に有意な低下を生じ、ここでその低下は、少なくとも５０％である。

【００７０】

一実施形態において、必要性のある被験体に有効量のＣＲＦ$_1$アンタゴニストを投与する工程を包含する本明細書で記載される方法は、プラセボと比較して、ＡＣＴＨレベルおよび１７－ＯＨＰレベルの両方の臨床的に有意な低下を生じる。ある種の実施形態において、必要性のある被験体に有効量のＣＲＦ$_1$アンタゴニストを投与する工程は、プラセボと比較して、ＡＣＴＨレベルおよび１７－ＯＨＰレベルの両方の臨床的に有意な低下を生じ、ここで上記低下は、少なくとも２５％である。別の具体的実施形態において、必要性のある被験体に有効量のＣＲＦ$_1$アンタゴニストを投与する工程は、プラセボと比較して、ＡＣＴＨレベルおよび１７－ＯＨＰレベルの両方の臨床的に有意な低下を生じ、ここで上記低下は、少なくとも５０％である。

【００７１】

上記薬学的組成物は、液剤の形態にあり得る。あるいは、それらは、固体（例えば、散

剤、錠剤など）の形態にあり得る。本明細書で記載される化合物のうちのいずれか１種を含む組成物は、徐放性もしくは緩慢な放出のために製剤化され得る。このような組成物は、一般に、周知の技術を使用して調製され得、例えば、経口、直腸もしくは皮下移植によって、または所望の標的部位での移植によって、投与され得る。徐放性製剤は、キャリアマトリクスの中に分散された、および／もしくは速度制御膜によって囲まれたレザバ内に含まれた化合物を含み得る。このような製剤内で使用するための賦形剤は、生体適合性であり、生分解性でもあり得る；好ましくは、上記製剤は、活性成分放出の比較的一定なレベルを提供する。徐放性製剤内に含まれる活性化合物の量は、移植部位、放出の速度および予測される持続時間、ならびに処置もしくは防止される予定の状態の性質に依存する。

【００７２】

経口製剤に関しては、本明細書で記載される化合物のうちの少なくとも１種は、単独で使用されるかまたは適切な添加剤と併用されて、例えば、従来の添加剤（例えば、ラクトース、マンニトール、コーンスターチもしくはジャガイモデンプン）とともに；結合剤とともに；崩壊剤とともに；滑沢剤とともに；および望ましい場合には、希釈剤、緩衝化剤、湿潤剤、保存剤、着色剤、および矯味矯臭剤とともに、錠剤、散剤、粒剤もしくはカプセル剤を作製し得る。上記化合物は、胃の環境の低ｐＨからの上記化合物の保護を提供するための緩衝化剤および／もしくは腸溶性コーティングとともに製剤化され得る。上記組成物に含まれる化合物は、例えば、液体、固体もしくは半固体の製剤の中で矯味矯臭剤とともに、および／または腸溶性コーティングとともに、経口送達のために製剤化され得る。経口製剤は、粉末化キャリア（例えば、ラクトース、デンプン、セルロース誘導体、ステアリン酸マグネシウム、ステアリン酸など）とともに上記活性化合物を含み得るゼラチンカプセルとして提供され得る。類似のキャリアおよび希釈剤は、圧縮された錠剤を作製するために使用され得る。

【実施例】

【００７３】

実施例１：ＣＲＦレセプター結合活性

本明細書で記載される方法において使用されるとおりのＣＲＦアンタゴニストは、Ｇｒｉｇｏｒｉａｄｉｓ ｅｔ ａｌ．（例えば、Ｍｏｌ． Ｐｈａｒｍａｃｏｌ ｖｏｌ ５０， ｐｐ ６７９－６８６， １９９６を参照のこと）およびＨｏａｒｅ ｅｔ ａｌ．（例えば、Ｍｏｌ． Ｐｈａｒｍａｃｏｌ ６３： ７５１－７６５， ２００３を参照のこと）によって一般に記載されるとおりの標準的放射性リガンド結合アッセイによって、上記ＣＲＦレセプターへの結合活性について評価され得る。放射性標識したＣＲＦリガンドを利用することによって、上記アッセイは、本明細書で記載される化合物と任意のＣＲＦレセプターサブタイプとの結合活性を評価するために使用され得る。

【００７４】

簡潔には、上記結合アッセイは、放射性標識したＣＲＦリガンドを上記ＣＲＦレセプターから置き換えることを包含する。より具体的には、上記結合アッセイは、ヒトＣＲＦレセプターで安定してトランスフェクトされた細胞に由来する１～１０μｇ 細胞膜を使用して、９６ウェルアッセイプレートの中で行われる。各ウェルは、目的の化合物もしくは参照リガンド（例えば、ソーバジン、ウロコルチンＩ、もしくはＣＲＦ）、０．０５ｍＬの［^{125}Ｉ］ヨードシンソーバジン（終濃度約１５０ｐＭもしくはスキャッチャード分析によって決定される場合のほぼK_D）および上記ＣＲＦレセプターを含む細胞膜懸濁物０．１ｍＬを含む、約０．０５ｍＬ アッセイ緩衝液（例えば、ダルベッコのリン酸緩衝化生理食塩水、１０ｍＭ 塩酸マグネシウム、２ｍＭ ＥＧＴＡ）を受容する。上記混合物を、２時間、２２℃でインキュベートし、続いて、ガラス繊維フィルタでの迅速濾過によって、結合した放射性リガンドと遊離の放射性リガンドとを分離する。３回洗浄した後、上記フィルタを乾燥させ、放射活性（^{125}Ｉのオージェ電子）を、シンチレーションカウンターを使用して計数する。全ての放射性リガンド結合データを、非線形最小自乗曲線フィッティングプログラムＰｒｉｓｍ（ＧｒａｐｈＰａｄ Ｓｏｆｔｗａｒｅ Ｉｎｃ）もしくはＸＬｆｉｔ （ＩＤ Ｂｕｓｉｎｅｓｓ Ｓｏｌｕｔｉｏｎｓ Ｌｔｄ）を使

用して分析し得る。

実施例２：CRF_1レセプターアゴニスト活性

【００７５】

Ｆｌｅｃｋ ｅｔ ａｌ．（Ｊ． Ｐｈａｒｍａｃｏｌｏｇｙ ａｎｄ Ｅｘｐｅｒｉｍｅｎｔａｌ Ｔｈｅｒａｐｅｕｔｉｃｓ，３４１（２）：５１８－５３１，２０１２）（本明細書中以降「Ｆｌｅｃｋ ｅｔ ａｌ．」、その全体において参考として援用される）に報告されるように、以前同定されたCRF_1レセプターアンタゴニストの活性が示される。このような活性は、以下の式によって、会合速度定数（k_1）および解離速度定数（k_{-1}）から計算される、速度論的に導出される親和性（K_i）として報告される：

$$K_i = k_{-1} / k_1$$

【００７６】

また、Ｆｌｅｃｋ ｅｔ ａｌ．で報告されるように、以下の表１に列挙される上記CRF_1レセプターアンタゴニストの速度論的K_iが報告された：

【表１－１】

表1

代表的CRF_1レセプターアンタゴニスト

リガンド	k_1 ($10^6 M^{-1}分^{-1}$)	速度論的 K_i (nM)
NBI-27914	9.4 ± 3	25

【表１－２】

リガンド	k_1 ($10^6 M^{-1}分^{-1}$)	速度論的 K_i (nM)
CP-316,311	13 ± 2	12
NBI-46200	6.2 ± 2	22
DMP696	7.7 ± 2	9.5
ペキサセルホント	2.6 ± 0.1	19
NBI-35965	20 ± 2	2.3
ONO-2333Ms	4.4 ± 2.2	15
アンタラルミン	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

【００７７】

この同じ技術によって、化合物Ｉ（ＮＢＩ－７７８６０）の速度論的Ｋｉは、以下のとおりであることが見出された：

【表１－３】

表1(続き)

リガンド	k_1 ($10^6M^{-1}分^{-1}$)	速度論的 K_i (nM)
NBI-77860	0.24±0.05	48±9

実施例３：ＣＲＦ$_1$レセプターアンタゴニストの解離半減期（ｔ$_{1/2}$）

【００７８】

本明細書で記載される方法において使用されるとおりのＣＲＦ$_1$レセプターアンタゴニストの解離半減期（ｔ$_{1/2}$）を、Ｆｌｅｃｋ　ｅｔ　ａｌ．に記載される技術によって評価する。その中で記載されるように、標識されたおよび標識されていないリガンドに関する解離速度定数は、ｋ$_{-1}$と表される一方で、上記レセプターの薬物解離の半減期（ｔ$_{1/2}$）（これは、滞留時間中央値（ｍｅｄｉａｎ　ｒｅｓｉｄｅｎｃｅ　ｔｉｍｅ）に等しい）は、以下の式によって、解離速度定数（ｋ$_{-1}$）から計算される：

$$t_{1/2} = 0.693 / k_{-1}$$

【００７９】

Ｆｌｅｃｋ　ｅｔ　ａｌ．において報告されるように、以下の表２に列挙されるＣＲＦ$_1$レセプターアンタゴニストの解離半減期（ｔ$_{1/2}$）が報告された。

【表２－１】

表2

代表的化合物の解離半減期

リガンド	k_{-1} ($分^{-1}$)	解離 $t_{1/2}$ (分)
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
ベキサセルホント	0.049 ± 0.001	14
NBI-35965	0.048 ± 0.005	16
ONO-2333Ms	0.063 ± 0.029	17
アンタラルミン	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

【００８０】
この同じ技術によって、化合物Ｉ（ＮＢＩ－７７８６０）の解離半減期は、以下のとおりであることが見出された：
【表２－２】

表2(続き)

リガンド	k_{-1}, $分^{-1}$	解離 $t_{1/2}$, 分
NBI-77860	0.012 ± 0.0003	58

【００８１】
よって、３０分間を超える解離半減期（$t_{1/2}$）を有するＣＲＦ$_1$レセプターアンタゴニストとしては、アンタラルミン、ＮＢＩ－３４０４１、ＤＭＰ９０４、ＮＢＩ－３０７７５、ＳＳＲ１２５５４３Ａ、およびＮＢＩ－７７８６０（化合物Ｉ）が挙げられる（が、これらに限定されない）。これら同じ化合物はまた、４０分間を超える解離半減期（$t_{1/2}$）および５０分間を超える解離半減期（$t_{1/2}$）を有するＣＲＦ$_1$レセプター

アンタゴニストの代表である。

実施例４：副腎摘出ラットにおけるＡＣＴＨの低下

【００８２】

化合物Ｉ（ＮＢＩ－７７８６０）（例えば、Ｔｅｌｌｅｗ ｅｔ ａｌ．，Ｂｉｏｏｒｇ．Ｍｅｄ．Ｃｈｅｍ．Ｌｅｔｔ．２０１０，２０：７２５９；ＷＯ２００６０４４９５８を参照のこと）は、結合ｐＫｉ ８．２、速度論的Ｋｉ ４９ｎＭ（上記の表１）、および解離$t_{1/2}$ ５８分（上記の表２）を有する強力なＣＲＦ$_1$アンタゴニストである。

【００８３】

ペプチドＣＲＦ$_1$レセプターアンタゴニストの静脈内注射は、副腎摘出（ＡＤＸ）ラットにおいて高い血漿ＡＣＴＨレベルを低下させることが示された（例えば、Ｒｉｖｉｅｒ ｅｔ ａｌ．，Ｊ．Ｍｅｄ．Ｃｈｅｍ．１２：４２：３１７５－８２（１９９９）を参照のこと）。これら知見は、上記低分子ＮＢＩ－７７８６０で再現された。ＡＤＸラット（ｎ＝６／群）に経口投与された場合、ＮＢＩ－７７８６０の３０ｍｇ／ｋｇ単一用量は、最大５時間にわたって血漿ＡＣＴＨレベルを有意に低下させた（図２を参照のこと）。効力の持続時間は、薬物血漿曝露を超える時間経過とともに、ピーク血漿濃度と相関した。副腎摘出ラットにおいて、ＮＢＩ－７７８６０の統合された血漿曝露（ｉｎｔｅｇｒａｔｅｄ ｐｌａｓｍａ ｅｘｐｏｓｕｒｅ）と経口投与後のインビボ効力との間には、推定可能な関係が存在する。

【００８４】

Ｆｌｅｃｋ ｅｔ ａｌ．はまた、副腎摘出ラットにおける血漿ＡＣＴＨレベルに対する、それらの解離半減期によって区別されるＣＲＦ$_1$レセプターアンタゴニスト；すなわち、ＮＢＩ ３０７７５、ＮＢＩ ３４０４１およびＮＢＩ ３５９６５の効果を報告する。最高用量（１０ｍｇ／ｋｇ）では、３種全てのリガンドがＡＣＴＨを急激に低下させた（注射後１時間、図３Ａ～Ｃ）。より長い持続時間の後、明らかな差異が、ＮＢＩ ３５９６５と他の２種のリガンドとの間に出てきた。上記ＡＣＴＨレベルは、ＮＢＩ ３５９６５に関しては２時間までにビヒクルレベルへと戻った（図３Ｃ）のに対して、その応答は、ＮＢＩ ３０７７５およびＮＢＩ ３４０４１に関しては、４～６時間にわたって持続した（図３Ａおよび図３Ｂ）。

実施例５：ヒトにおける化合物Ｉの薬力学的効果

【００８５】

この研究において、ヒト被験体に対するＮＢＩ－７７８６０（化合物Ｉ）の薬力学的効果を、プラセボおよび単一用量のアルプラゾラム（０．７５ｍｇ）に関して、メチラポン（０．０４ｇ／ｋｇ）によって生成されるＨＰＡ系応答に対する単一経口用量の効果を観察することによって評価した。メチラポンは、副腎におけるコルチゾール合成をブロックし（それによって、ＣＡＨのコルチゾール欠損を模倣する）、ＡＣＴＨレベルの急激な反射上昇と関連する。

【００８６】

一次分析を、投与後３０分～４時間の間のＡＣＴＨ値（ＡＵＣ（３０分～４時間））を決定することによって行った。この分析において、プラセボと比較して、以下の観察がなされた。ＡＣＴＨ ＡＵＣ（３０分～４時間）の有意な低下を、アルプラゾラムおよびＮＢＩ－７７８６０、４００ｍｇ処置で観察した。ＡＣＴＨ ＡＵＣ（３０分～４時間）の有意でない低下は、ＮＢＩ－７７８６０、５０ｍｇ処置で認められた。ＡＵＣ（３０分～４時間）の増大は、ＮＢＩ－７７８６０、１０ｍｇ処置で認められた。

【００８７】

投与後２時間～４時間の間のＡＣＴＨ値（ＡＵＣ（２時間～４時間））（曝露がC_{max}値に達した場合の期間）に対して行った分析では、プラセボ処置期間と比較して、ＮＢＩ－７７８６０、４００ｍｇおよび５０ｍｇ処置期間においてＡＣＴＨレベルが有意に低下した；しかし、上記低下は、１０ｍｇ用量のＮＢＩ－７７８６０では認められなかった。

実施例６：臨床研究

【００８８】

この研究において、ＮＢＩ－７７８６０（化合物Ｉ）を、標題「先天性副腎皮質過形成を有する成人女性におけるＮＢＩ－７７８６０の安全性および寛容性を評価するためのフェーズＩ、単盲検のプラセボ対照固定順単一用量研究（Ｐｈａｓｅ　１，　Ｓｉｎｇｌｅ－Ｂｌｉｎｄ，　Ｐｌａｃｅｂｏ－Ｃｏｎｔｒｏｌｌｅｄ，　Ｆｉｘｅｄ－Ｓｅｑｕｅｎｃｅ，　Ｓｉｎｇｌｅ－Ｄｏｓｅ　Ｓｔｕｄｙ）」（ＩＮＤ　１１７，３８８）という臨床研究において評価した。上記研究は、成人女性の古典的ＣＡＨ患者における単盲検のプラセボ対照単一施設固定順単一用量臨床試験であった。これを、ＮＢＩ－７７８６０の安全性、寛容性、および血漿曝露、ならびにＨＰＡ系ホルモンの内因性レベルに対するこの化合物の効果を評価するためにデザインした。

【００８９】

古典的な２１－ヒドロキシラーゼ欠損ＣＡＨと医学的に診断された合計８名の女性被験体（年齢１９～５８歳）に、３つの別個の処置期間の間に、ＮＢＩ－７７８６０　３００ｍｇ、６００ｍｇ、およびプラセボの単一就寝時用量（ｈｓ）を投与した（図４の研究デザイン模式図を参照のこと）。投与後１６時間の血液サンプルが集められるまで（すなわち、約１４００時間まで）、上記被験体の、同時のステロイド処置の通常の朝の用量を遅らせた。

【００９０】

この研究に関するＰＤエンドポイントは、この患者集団における目的のＨＰＡ系生体マーカー；すなわち、１７－ヒドロキシプロゲステロン（１７－ＯＨＰ；主要なＰＤエンドポイントとして）、副腎皮質刺激ホルモン（ＡＣＴＨ）、アンドロステンジオン、テストステロン、および血清コルチゾールのレベルを含んだ。ＰＤ変数の初期分析は、各生体マーカーに関するグループ分けされた被験体データの調査であった（プラセボ条件と比較して、上記２種の活性物質（ａｃｔｉｖｅ）投与条件に関する投与前レベルからの平均％変化として表される）。投与後２４時間の期間全体の平均１７－ＯＨＰおよびＡＣＴＨのレベルのデータを、図５に提供する。

【００９１】

１７－ＯＨＰおよびＡＣＴＨの両方の投与前レベルからの一貫したかつ臨床的に有意義な低下を、これらＣＡＨ患者において、プラセボと比較して、ＮＢＩ－７７８６０の投与後の投与後期間全体で観察した。群平均データに加えて、個々の応答を評価し、処置「応答者」を、朝のピーク期間の間にプラセボと比較して、活性なＮＢＩ－７７８６０の下で１７－ＯＨＰおよびＡＣＴＨにおいて少なくとも５０％低下を有するそれら被験体として控えめに定義した。この応答者分析から、上記研究において５０％というかなり大きな応答者割合が得られた（これら患者のうちのいずれも、最初のプラセボ処置期間の間には応答者でなかった）。さらに、上記３００ｍｇ用量は、上記６００ｍｇ用量とほぼ同一の、１７－ＯＨＰおよびＡＣＴＨに対する効果を生じた。これら生体マーカーに関する個々の被験体の応答の例を、図６に提供する。

【００９２】

上記の種々の実施形態は、さらなる実施形態を提供するために組み合わされ得る。本明細書中で言及されるおよび／または出願データシート中に列挙される全ての米国特許、米国特許出願公開、米国特許出願、外国特許、外国特許出願および非特許刊行物は、米国仮特許出願第６１，９２９，９４１号（２０１４年１月２１日出願）；同第６１，９８１，０３３号（２０１４年４月１７日出願）；および同第６２／０６９，１５５号（２０１４年１０月２７日出願）を含め、それらの全体において本明細書に参考として援用される。上記実施形態の局面は、上記種々の特許、出願および公開の概念を使用するために必要であれば、なおさらなる実施形態を提供するために改変され得る。

【００９３】

これらおよび他の変更は、上記の詳細な説明に鑑みて上記実施形態に対して行われ得る。一般に、以下の特許請求の範囲において、使用される用語は、特許請求の範囲を、本明

細書および特許請求の範囲の中で開示される具体的実施形態に限定すると解釈されるものとするのではなく、均等物の全範囲（上記範囲に対して、このような特許請求項の範囲が権利付与される）とともに、全ての考えられる実施形態を含むと解釈されるものとする。よって、特許請求の範囲は、上記開示によって限定されない。

【図１】

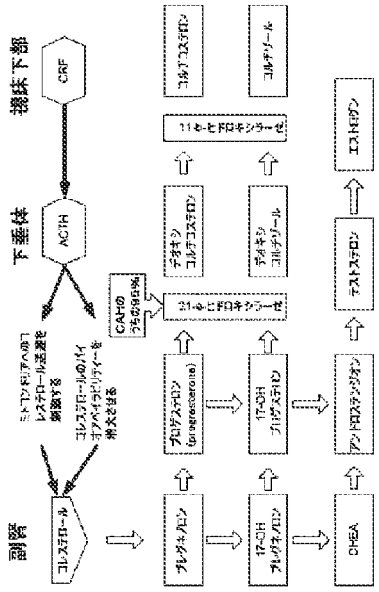


FIG. 1

【図２】

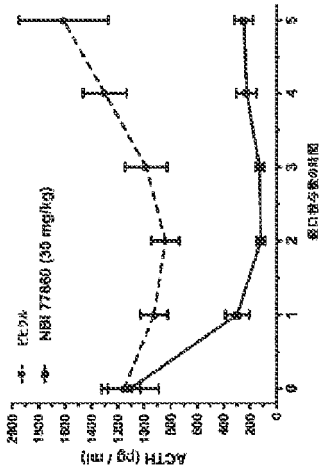


FIG. 2

【図３】

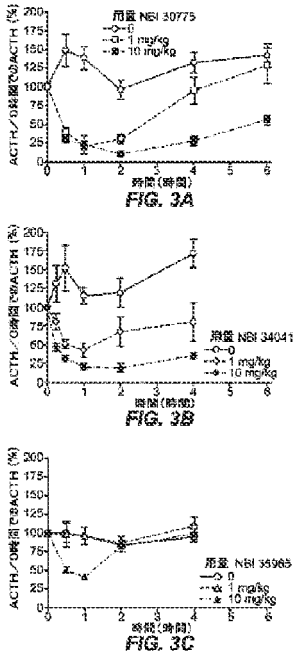


FIG. 3A

FIG. 3B

FIG. 3C

【図４】

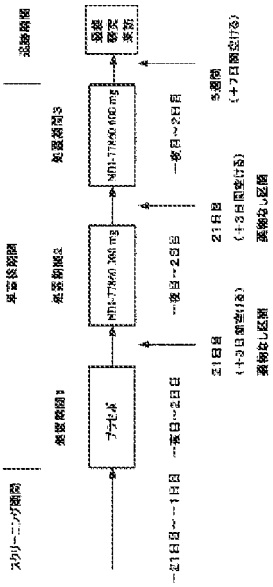


FIG. 4

【図５】

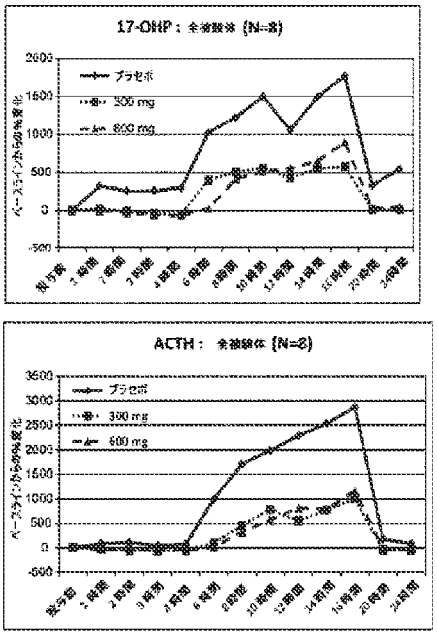


FIG. 5

【図６】

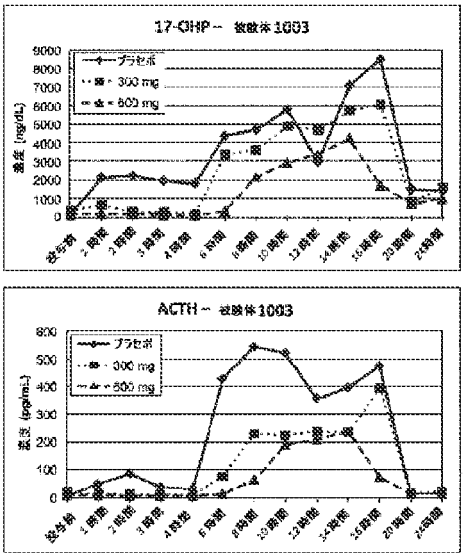


FIG. 6

【国際調査報告】

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 :

"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
6 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)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/012315

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

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/012315

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, XP000851739, ISSN: 0013-7227, DOI: 10.1210/EN.137.12.5747 the whole document -----	1-21

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

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
US 8030304 B2	04-10-2011	AR 062866 A1	10-12-2008
		AT 481405 T	15-10-2010
		AU 2007297421 A1	27-03-2008
		BR PI0717023 A2	11-03-2014
		CA 2663511 A1	27-03-2008
		CL 2007002693 A1	18-04-2008
		CN 101516867 A	26-08-2009
		CR 10582 A	13-08-2009
		DK 2094709 T3	06-12-2010
		EA 200970303 A1	30-10-2009
		EP 2094709 A1	02-09-2009
		ES 2350282 T3	20-01-2011
		HK 1132505 A1	25-02-2011
		HR P20100516 T1	31-10-2010
		IL 197614 A	31-10-2012
		JP 5161226 B2	13-03-2013
		JP 2010504344 A	12-02-2010
		KR 20090052366 A	25-05-2009
		MA 30798 B1	01-10-2009
		MY 146368 A	15-08-2012
		NZ 575572 A	28-10-2011
		PE 13772008 A1	18-09-2008
		PT 2094709 E	04-10-2010
		RS 51545 B	30-06-2011
		SI 2094709 T1	31-01-2011
		TW 200821312 A	16-05-2008
		UA 96458 C2	10-11-2011
		US 2010222339 A1	02-09-2010
		WO 2008036579 A1	27-03-2008

Form PCT/ISA/210 (patent family annex) (April 2005)

フロントページの続き

(81)指定国 AP(BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), EA(AM, AZ, BY, KG, KZ, RU, TJ, TM), EP(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), OA(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG), 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, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, 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

Fターム(参考) 4C086 AA01 AA02 BC17 BC82 CB05 CB06 CB09 MA01 MA04 NA14
ZC08 ZC42

(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

(10) International Publication Number
WO 2019/036503 A1

(51) International Patent Classification:
A61K 31/415 (2006.01) *C07D 487/04* (2006.01)
A61K 31/425 (2006.01) *A61P 5/24* (2006.01)
A61K 31/519 (2006.01)

(21) International Application Number:
PCT/US2018/046760

(22) International Filing Date:
14 August 2018 (14.08.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/545,406 14 August 2017 (14.08.2017) US

(71) Applicant: **SPRUCE BIOSCIENCES, INC.** [US/US]; 548 Market Street, Suite 74589, San Francisco, California 94105 (US).

(72) Inventors: **HOWERTON, Alexis**; 548 Market Street, Suite 74589, San Francisco, California 94105 (US). **GERBER, Hal**; 548 Market Street, Suite 74589, San Francisco, California 94105 (US). **HUANG, Michael**; 548 Market Street, Suite 74589, San Francisco, California 94105 (US).

(74) Agent: **DELANEY, Simren**; WILSON SONSINI GOODRICH & ROSATI, 650 Page Mill Road, Palo Alto, California 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

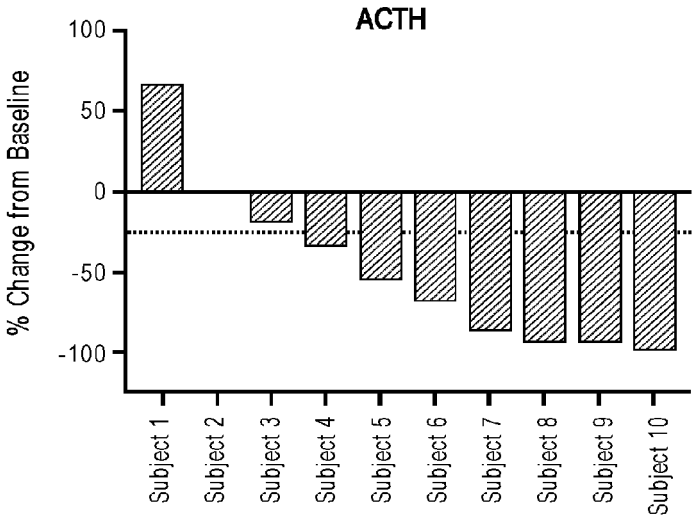


FIG. 2

(57) **Abstract:** The present invention provides novel pharmaceutical compositions comprising -(4-Chloro-2-(morpholin-4-yl)thiazol-5-yl)-7-(1-ethylpropyl)-2,5-dimethylpyrazolo(1,5-a)pyrimidine and methods of using the same for the treatment of Congenital adrenal hyperplasia (CAH).

[Continued on next page]

WO 2019/036503 A1

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. 62/545,406, filed August 14, 2017, which is incorporated herein by reference in its entirety.

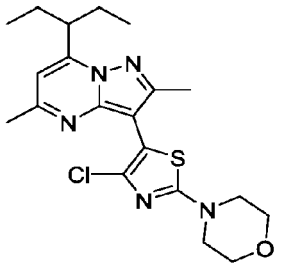
BACKGROUND OF THE INVENTION

[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 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, wherein Compound 1 is administered at a dose between about 50 mg/day and about 1600 mg/day.

[0005] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 1200 mg/day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 1000 mg/day. In some

embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 800 mg/day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 100 mg/day and about 600 mg/day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 200 mg/day and 400 mg /day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of about 200 mg /day.

[0006] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is in the form of microparticles. In some embodiments, the average size of the microparticles is between about 1 μm and about 20 μm. In some embodiments, the average size of the microparticles is between about 5 μm and about 15 μm. In some embodiments, the average size of the microparticles is less than about 10 μm.

[0007] In some embodiments, the pharmaceutical composition is in the form of a capsule or a tablet. In some embodiments, the pharmaceutical composition is in the form of a capsule. In some embodiments, the capsule is a hard gelatin capsule. In some embodiments, the capsule is a soft gelatin capsule. In some embodiments, 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.

[0008] In some embodiments, the pharmaceutical composition is free of additional excipients. In some embodiments, the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

[0009] In some embodiments, the pharmaceutical composition is in the form of a tablet. In some embodiments, the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients. In some embodiments, the pharmaceutical composition 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 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 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 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 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

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 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.

[0010] 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 80 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 30 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0011] In some embodiments, the pharmaceutical composition provides a Compound 1 T_{max} of about 2 to about 6 hours in a subject. In some embodiments, the pharmaceutical composition provides a Compound 1 T_{max} of about 3 to about 5 hours in a subject. In some embodiments, the pharmaceutical composition provides a Compound 1 T_{max} of about 6 hours in a subject. In some embodiments, the pharmaceutical composition provides a Compound 1 T_{max} of about 5 hours in a subject. In some embodiments, the pharmaceutical composition provides a Compound 1 T_{max} of about 4 hours in a subject. In some embodiments, the pharmaceutical composition provides a Compound 1 T_{max} of about 3 hours in a subject.

[0012] 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 once a day. In some embodiments, the pharmaceutical composition is administered twice a day. In some embodiments, the pharmaceutical composition is administered three times a day.

[0013] In some embodiments, the method further comprises administering 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. In some embodiments, the amount of glucocorticoid used is reduced from a supraphysiologic amount to a physiologic amount. In some embodiments, the amount of glucocorticoid 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%.

[0014] In some embodiments, the symptoms associated with high-dose glucocorticoid therapy are reduced. In some embodiments, the symptoms associated with high-dose glucocorticoid therapy are obesity, insulin resistance, metabolic abnormalities, hypertension, cardiovascular diseases, or osteoporosis. In some embodiments, the glucocorticoid is beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone.

[0015] In some embodiments, the glucocorticoid is hydrocortisone. In some embodiments, the hydrocortisone is administered at a dose less than about 15 mg/day. In some embodiments, the hydrocortisone is administered at a dose less than about 10 mg/day. In some embodiments, the hydrocortisone is administered at a dose less than about 5 mg/day.

[0016] 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 sequentially. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 24 hours. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 8 hours. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 2 hours. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 30 minutes.

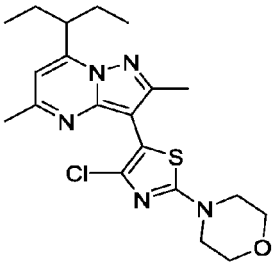
[0017] In some embodiments, the method further comprises administering a mineralocorticoid. In some embodiments, the mineralocorticoid is fludrocortisone.

[0018] 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 mins 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. In some embodiments, the pharmaceutical composition is administered at or before the expected circadian release of adrenocorticotropic hormone (ACTH). In some embodiments, the pharmaceutical composition is administered about 3-4 hours before the expected circadian release of adrenocorticotropic hormone (ACTH).

[0019] In some embodiments, CAH is classic CAH. In some embodiments, CAH is non-classic CAH.

[0020] In one aspect, the present disclosure provides 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; and

(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.

[0021] In some embodiments, the hormone is 17α-Hydroxyprogesterone (17-OHP), adrenocorticotropic hormone (ACTH), testosterone, or androstenedione. In some embodiments, the hormone is 17-OHP, and the pre-determined range is from about 200 ng/dL to about 400 ng/dL. In some embodiments, the hormone is ACTH, and the pre-determined range is below about 100 pg/mL. In some embodiments, the hormone is testosterone and the pre-determined range is from about 14 ng/dL to about 76 ng/dL. In some embodiments, the hormone is androstenedione and the pre-determined range is from about 30 ng/dL to about 150 ng/dL in males. In some embodiments, the hormone is androstenedione and the pre-determined range is from about 40 ng/dL to about 200 ng/dL in females.

[0022] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 200 mg/day and about 1600 mg/day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 200 mg/day and about 1200 mg/day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is

administered at a dose between about 200 mg/day and about 1000 mg/day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 200 mg/day and about 800 mg/day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 200 mg/day and about 600 mg/day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 200 mg/day and 400 mg /day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of about 200 mg /day.

[0023] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 1600 mg/day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 1200 mg/day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 1000 mg/day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 800 mg/day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 600 mg/day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and 400 mg /day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between 50 mg/day and 200 mg/day. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of about 50 mg /day.

[0024] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is in the form of microparticles. In some embodiments, the average size of the microparticles is between about 1 μm and about 20 μm. In some embodiments, the average size of the microparticles is between about 5 μm and about 15 μm. In some embodiments, the average size of the microparticles is less than about 10 μm.

[0025] In some embodiments, Compound 1 is formulated in a pharmaceutical composition in the form of a capsule or a tablet. In some embodiments, the pharmaceutical composition is in the form of a capsule. In some embodiments, the capsule is a hard gelatin capsule. In some embodiments, the capsule is a soft gelatin capsule. In some embodiments, 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.

[0026] In some embodiments, the pharmaceutical composition is free of additional excipients. In some embodiments, the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients. In some embodiments, the pharmaceutical composition is in the form of a tablet. In some embodiments, the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

[0027] In some embodiments, the pharmaceutical composition 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 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 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 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 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 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 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.

[0028] 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, he 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 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 30 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0029] In some embodiments, the pharmaceutical composition provides a Compound 1 T_{max} of about 2 to about 6 hours in a subject. In some embodiments, the pharmaceutical composition provides a Compound 1 T_{max} of about 3 to about 5 hours in a subject. In some embodiments, the pharmaceutical composition provides a Compound 1 T_{max} of about 6 hours in a subject. In some embodiments, the pharmaceutical composition provides a Compound 1 T_{max} of about 5 hours in a subject. In some embodiments, the pharmaceutical composition provides a Compound 1 T_{max} of about 4 hours in a subject. In some embodiments, the pharmaceutical composition provides a Compound 1 T_{max} of about 3 hours in a subject.

[0030] 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 once a day. In some embodiments, the pharmaceutical composition is administered twice a day. In some embodiments, the pharmaceutical composition is administered three times a day.

[0031] In some embodiments, the method further comprises administering 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. In some embodiments, the amount of glucocorticoid used is reduced from a supraphysiologic amount to a physiologic amount. In some embodiments, the amount of glucocorticoid 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%.

[0032] In some embodiments, the symptoms associated with high-dose glucocorticoid therapy are reduced. In some embodiments, the symptoms associated with high-dose glucocorticoid therapy are obesity, insulin resistance, metabolic abnormalities, hypertension, cardiovascular diseases, or osteoporosis. In some embodiments, the glucocorticoid is beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone. In some embodiments, the glucocorticoid is hydrocortisone.

[0033] In some embodiments, the hydrocortisone is administered at a dose less than about 15 mg/day. In some embodiments, the hydrocortisone is administered at a dose less than about 10 mg/day. In some embodiments, the hydrocortisone is administered at a dose less than about 5 mg/day.

[0034] 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 sequentially. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 24 hours. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 8 hours. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 2 hours. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 30 minutes.

[0035] In some embodiments, the method further comprises administering a mineralocorticoid. In some embodiments, the mineralocorticoid is fludrocortisone.

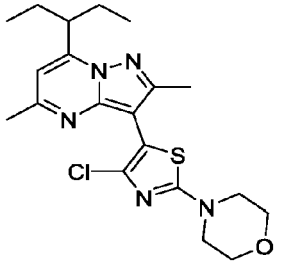
[0036] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at bedtime. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered less than about 4 hours before sleeping. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered less than about 2 hours before sleeping. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered less than about 30 mins before sleeping. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered in the evening. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at 10 pm at night.

[0037] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at or before the expected circadian release of adrenocorticotropic hormone (ACTH). In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered about 3-4 hours before the expected circadian release of adrenocorticotropic hormone (ACTH).

[0038] In some embodiments, CAH is classic CAH. In some embodiments, CAH is non-classic CAH.

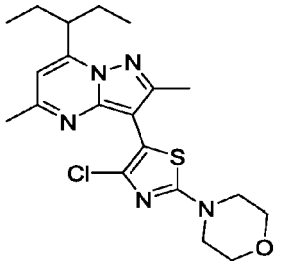
[0039] In some embodiments, the subject in need thereof is from about 12 years of age to about 20 years of age.

[0040] In one aspect, the present disclosure provides a method of improving hyperandrogenic symptoms in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 1:



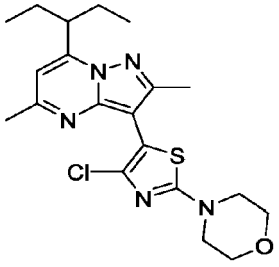
; or a pharmaceutically acceptable salt or solvate thereof, wherein Compound 1 is administered at a dose between about 50 mg/day and about 1600 mg/day. In some embodiments, the hyperandrogenic symptoms are selected from the group consisting of acne, hirsutism and alopecia.

[0041] In one aspect, the present disclosure provides a method of treating menstrual irregularity, ovulatory dysfunction or infertility, in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 1:



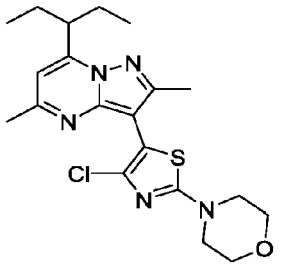
; or a pharmaceutically acceptable salt or solvate thereof, wherein Compound 1 is administered at a dose between about 50 mg/day and about 1600 mg/day.

[0042] In one aspect, the present disclosure provides a method of improving metabolic symptoms in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 1:



; or a pharmaceutically acceptable salt or solvate thereof, wherein Compound 1 is administered at a dose between about 50 mg/day and about 1600 mg/day. In some embodiments, the metabolic symptoms are selected from the group consisting of body weight, BMI, waist circumference, blood pressure and glycemic control.

[0043] In one aspect, the present disclosure provides a method of improving quality of life in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 1:



; or a pharmaceutically acceptable salt or solvate thereof, wherein Compound 1 is administered at a dose between about 50 mg/day and about 1600 mg/day, wherein treatment results in an improved quality of life.

INCORPORATION BY REFERENCE

[0044] 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

[0045] 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:

[0046] FIG. 1 shows Compound 1 in patients with CAH following 14-days of once daily dosing at each level;

[0047] FIG. 2 demonstrates the attenuation of ACTH across different subjects due to the administration of Compound 1;

[0048] FIG. 3 demonstrates the reduction in 17-OHP due to the administration of Compound 1;

[0049] FIG. 4 demonstrates the reduction of Androstenedione due to the administration of Compound.

DETAILED DESCRIPTION OF THE INVENTION

[0050] 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.

[0051] 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.

[0052] 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.

[0053] 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.

[0054] 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.

[0055] 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.

[0056] 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.

[0057] 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.

[0058] 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

[0059] 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.

[0060] 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.

[0061] 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.

[0062] 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."

[0063] 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.

[0064] "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.

[0065] 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.

[0066] 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.

[0067] The term "supraphysiologic" amount" describes hormones levels that are elevated compared to average levels found in healthy individuals.

[0068] The term "physiologic amount" describes average hormone levels found in healthy individuals.

[0069] 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).

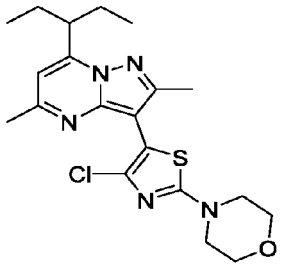
[0070] The terms "treat," "treated," "treatment," or "treating" as used herein refers to both therapeutic treatment in some embodiments and prophylactic or preventative measures in other embodiments, wherein the object is to prevent or slow (lessen) an undesired physiological condition, disorder or disease, 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.

[0071] 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

[0072] 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

[0073] Disclosed herein is a pharmaceutical composition comprising Compound 1, a pharmaceutically acceptable salt, and/or a solvate thereof.

Dosage Form

[0074] 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.

[0075] 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.

[0076] 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

[0077] 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.

[0078] 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.

[0079] In some embodiments, the capsule comprises one or more pharmaceutically acceptable excipients. In some embodiments, the capsule is free of additional excipients.

[0080] 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

[0081] Poorly soluble drugs may be difficult to formulate using standard technologies such as high shear wet granulation. Optimum delivery of poorly soluble 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 poorly soluble 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 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 and more difficult. Furthermore, at a certain drug load, it may become impossible.

[0082] In some embodiments, the pharmaceutical composition is formulated as a tablet.

[0083] 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.

[0084] 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.

[0085] In some embodiments, the tablet is produced at a commercial scale.

[0086] In some embodiments, the tablet comprises one or more pharmaceutically acceptable excipients.

[0087] 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

[0088] 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.

[0089] 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.
- 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.
- Cationic surfactants such as cetrimide, benzalkonium chloride and cetylpyridinium chloride.
- Anion surfactants such as alkyl sulphates, alkyl ethoxylate sulphates, soaps, carboxylates, sulfates, and sulfonates.
- Non-ionic surfactants such as polyoxyethylene derivatives, polyoxypropylene derivatives, polyol derivatives, polyol esters, polyoxyethylene esters, poloxamers, glyol esters, glycerol esters, sorbitan derivatives, polyethylene glycol (PEG-40, PEG-50, PEG-55), and ethers of fatty alcohols.
- Organic materials such as carbohydrate and modified carbohydrates, lactose, a-lactose monohydrate, spray dried lactose and anhydrous lactose, starch and pre-gelatinized starch, sucrose, manitol, sorbitol, cellulose, powdered cellulose and microcrystalline cellulose.
- Inorganic materials such as calcium phosphates (anhydrous dibasic calcium phosphate, dibasic calcium phosphate and tribasic calcium phosphate).
- Co-processed diluents.
- Surfactants such as sodium lauryl sulfate.
- Compression aids.
- Anti-tacking agents such as silicon dioxide and talc

Amounts

[0090] 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.

[0091] 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.

[0092] 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.

[0093] 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.

[0094] 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.

[0095] 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.

[0096] 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.

[0097] 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

[0098] 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.

[0099] 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

[0100] 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.

[0101] 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

[0102] 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.

[0103] 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.

[0104] 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.

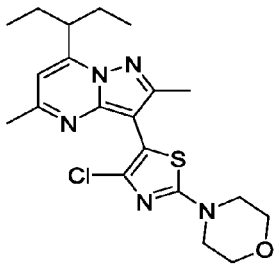
Methods of Use

[0105] 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.

[0106] 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.

[0107] In some embodiment, the hormone is 17α-Hydroxyprogesterone (17-OHP), adrenocorticotropic hormone (ACTH), testosterone, or androstenedione.

[0108] 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.

[0109] 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.

[0110] 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.

[0111] 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

[0112] 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.

[0113] 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.

[0114] 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, Compound 1 is administered at a dose between about 50 mg/day and about 1600 mg/day. In some embodiments, Compound 1 is administered at a dose between about 50 mg/day and about 1500 mg/day. In some embodiments, Compound 1 is administered at a dose between about 50 mg/day and about 1400 mg/day. In some

embodiments, Compound 1 is administered at a dose between about 50 mg/day and about 1300 mg/day. In some embodiments, Compound 1 is administered at a dose between about 50 mg/day and about 1200 mg/day. In some embodiments, Compound 1 is administered at a dose between about 50 mg/day and about 1100 mg/day. In some embodiments, Compound 1 is administered at a dose between about 50 mg/day and about 1000 mg/day. In some embodiments, Compound 1 is administered at a dose between about 50 mg/day and about 900 mg/day. In some embodiments, Compound 1 is administered at a dose between about 50 mg/day and about 800 mg/day. In some embodiments, Compound 1 is administered at a dose between about 60 mg/day and about 800 mg/day. In some embodiments, Compound 1 is administered at a dose between about 70 mg/day and about 800 mg/day. In some embodiments, Compound 1 is administered at a dose between about 80 mg/day and about 800 mg/day. In some embodiments, Compound 1 is administered at a dose between about 90 mg/day and about 800 mg/day. In some embodiments, Compound 1 is administered at a dose between about 100 mg/day and about 800 mg/day. In some embodiments, Compound 1 is administered at a dose between about 100 mg/day and about 700 mg/day. In some embodiments, Compound 1 is administered at a dose between about 100 mg/day and about 600 mg/day. In some embodiments, Compound 1 is administered at a dose between 150 mg/day and about 600 mg/day. In some embodiments, Compound 1 is administered at a dose between 200 mg/day and about 600 mg/day. In some embodiments, Compound 1 is administered at a dose between 200 mg/day and about 500 mg/day. In some embodiments, Compound 1 is administered at a dose between 200 mg/day and about 400 mg/day.

[0115] In some embodiments, Compound 1 is administered at a dose of about 500 mg/day. In some embodiments, Compound 1 is administered at a dose of about 400 mg/day. In some embodiments, Compound 1 is administered at a dose of about 300 mg/day. In some embodiments, Compound 1 is administered at a dose of about 200 mg/day. In some embodiments, Compound 1 is administered at a dose of about 100 mg/day.

[0116] 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.

[0117] 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.

[0118] 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.

[0119] In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein at bedtime.

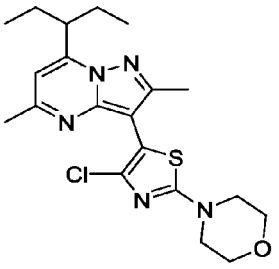
[0120] In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein less than about 4 hours before sleep. In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein less than 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.

[0121] In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein in the evening.

[0122] 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.

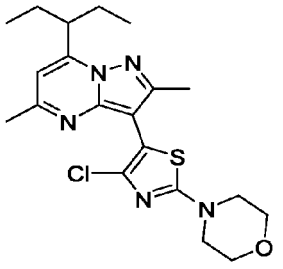
[0123] In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein at or before the expected circadian release of adrenocorticotropic hormone (ACTH). In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein about 3-4 hours before the expected circadian release of adrenocorticotropic hormone (ACTH). In some embodiments, the subject in need thereof is from about 12 years of age to about 20 years of age.

[0124] In one aspect, the present disclosure provides a method of improving hyperandrogenic symptoms in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 1:



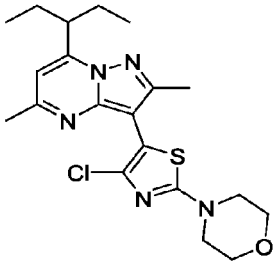
; or a pharmaceutically acceptable salt or solvate thereof, wherein Compound 1 is administered at a dose between about 50 mg/day and about 1600 mg/day. In some embodiments, the hyperandrogenic symptoms are selected from the group consisting of acne, hirsutism and alopecia.

[0125] In one aspect, the present disclosure provides a method of treating menstrual irregularity, ovulatory dysfunction or infertility, in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 1:



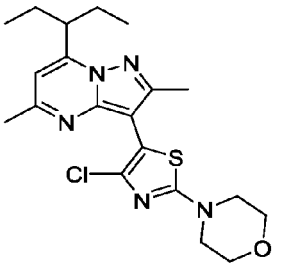
; or a pharmaceutically acceptable salt or solvate thereof, wherein Compound 1 is administered at a dose between about 50 mg/day and about 1600 mg/day.

[0126] In one aspect, the present disclosure provides a method of improving metabolic symptoms in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 1:



; or a pharmaceutically acceptable salt or solvate thereof, wherein Compound 1 is administered at a dose between about 50 mg/day and about 1600 mg/day. In some embodiments, the metabolic symptoms are selected from the group consisting of body weight, BMI, waist circumference, blood pressure and glycemic control.

[0127] In one aspect, the present disclosure provides a method of improving quality of life in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 1:



; or a pharmaceutically acceptable salt or solvate thereof, wherein Compound 1 is administered at a dose between about 50 mg/day and about 1600 mg/day, wherein treatment results in an improved quality of life.

Combination Therapy

[0128] 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.

[0129] In some embodiments, the methods described herein reduce the amount of a glucocorticoid administered from a supraphysiologic amount to a physiologic amount.

[0130] 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.

[0131] 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.

[0132] 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.

[0133] 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.

[0134] 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.

[0135] 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.

[0136] In some embodiments, the glucocorticoid is beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone. In some embodiments, the glucocorticoid is hydrocortisone.

[0137] In some embodiments, the glucocorticoid is hydrocortisone and the dose administered is less than the recommended dose of 15-25 mg/day.

[0138] In some embodiments, the glucocorticoid is prednisone and the dose administered is less than the recommended dose of 5-7.5 mg/day.

[0139] In some embodiments, the glucocorticoid is prednisolone and the dose administered is less than the recommended dose of 4-6 mg/day.

[0140] In some embodiments, the glucocorticoid is dexamethasone and the dose administered is less than the recommended dose of 0.25-0.5 mg/day.

[0141] 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

[0142] 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.

[0143] 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

[0144] 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

[0145] 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.

[0146] 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

[0147] 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

[0148] 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

[0149] Compound 1 has been investigated in 2 Phase 1 studies in healthy adult volunteers.

[0150] 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.

[0151] 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.

[0152] 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.

[0153] 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	31	29.2	44.2	41.9

	(NC)	(NC)	(10.8-53.4)	(20.0 - 41.5)	(20.2 - 101)	(24.1 - 67.8)
$AUC_{0-tlast}$ (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)

[0154] 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	288

	(26)	(48)
$AUC_{0-\infty}$ (ng•h/mL)	956 (25)	331 (44)
CL/F (L/h)	157 (25)	454 (44)
Vz/F (L)	6620 (38)	25100 (81)
Vss/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)

[0155] 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}.

[0156] 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.

[0157] 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	204	86.5	77.4

	NC NC	NC NC	NC NC	(71.3) 2.80	(41.0) 2.51	(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.

[0158] 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

[0159] 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.

[0160] Compound 1, when administered as multiple doses up to 200 mg, was generally well tolerated in the healthy subject population studied. 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.

[0161] 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 Clinical Studies

[0162] 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.

[0163] 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.

[0164] 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

6. 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).

[0165] 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.

[0166] 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.

[0167] 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: 3-Month Phase 2 Study

[0168] This is a 3-month Phase 2 study to evaluate the safety and efficacy of Compound 1 in subjects with classic CAH. This study will investigate the extended use of Compound 1 over a 3-month period in combination with replacement glucocorticoids and mineralocorticoids in the treatment of CAH.

[0169] This study will enroll up to approximately 24 eligible subjects who will receive Compound 1 daily for up to 3-months. Compound 1 will be administered as an oral daily dose. At the end of each 2-week dosing period, patients will return for morning laboratory assessments. A follow-up outpatient visit will occur 30 days after their last dose.

Study Design

- Study Type: Interventional
- Primary Purpose: Treatment
- Study Phase: Phase 2
- Interventional Study Model: Sequential Assignment
- Number of Arms: 1
- Masking: No Masking
- Allocation: Non-Randomized
- Enrollment: Up to approximately 24 [anticipated]

Outcome Measures

Primary Outcome Measure:

1. To evaluate the safety of Compound 1 in subjects with CAH.

Second Outcome Measure:

2. To evaluate the efficacy of Compound 1 in subjects with CAH in terms of changes in hormones.

Exploratory:

3. To evaluate the effect of Compound 1 on quality of life, metabolic parameters, exploratory adrenal hormones, and semen analysis in subjects with CAH.

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.

Example 6: Adolescent Study

[0170] This is a Phase 2, open-label, multiple-dose study designed to provide proof of concept for the use of Compound 1 to treat adolescents 12 to 17 years of age with classic CAH. The study will evaluate the safety, efficacy, and PK of 2 weeks of treatment with Compound 1. Each subject will undergo a 2-week diary run-in period during screening, a 2-week treatment period, and a 30-day washout and safety follow-up period.

[0171] An adaptive design with up to 3 sequential dose cohorts (Cohorts 1, 2, and 3) is planned to identify an optimal dose strength and regimen in adolescents with classic CAH. Each cohort will initially enroll 3 subjects and may later enroll 3 more subjects based on interim data from the first 3 subjects, for a total of 6 subjects per cohort. However, if safety, efficacy, and PK results from 6 subjects are inconclusive, a cohort may be expanded up to a maximum of 12 subjects. Cohort 1 will receive study drug at 200 mg QD. Cohorts 2 and 3 will be sequentially activated only if further investigation of an optimal dose strength or regimen is required; the dose strength and frequency and timing of dosing for these cohorts will be determined based on interim data from the previous cohort(s). However, the dose strength administered to each successive cohort will be capped at twice the daily dose strength administered to the previous cohort.

[0172] This study will enroll patients with inadequately controlled CAH despite an existing glucocorticoid (GC) regimen (including any currently-available regimen: hydrocortisone, prednisone/prednisolone, and/or dexamethasone). In practice, CAH patients are typically treated

with supraphysiologic doses of GCs which are both toxic and not effective in many adolescent patients. In this population, GCs are associated with multiple well-described short- and long-term toxicities including: growth retardation, weight gain, hypertension, hyperglycemia, hyperlipidemia, and Cushingoid features.

[0173] The objective of treatment of this patient population with Compound 1 is reduction (or normalization) of key hormonal outcome measures which could allow reduction of their GC replacement requirements which will improve clinical outcomes of concern, including growth velocity. Improvement of hormonal outcome measures in this population are also expected to delay premature puberty and bone maturation, ultimately leading to improvements in height in adolescents with CAH.

Outcome Measures

Primary Outcome Measures:

1. To evaluate the safety of Compound 1 in adolescent subjects with CAH.

Secondary Outcome Measures:

2. To evaluate the dose(s) of Compound 1 that cause changes in key pharmacodynamics bioamarkers in blood in adolescent subjects with CAH.
3. To investigate the effect of Compound 1 on quality of life in adolescent subjects with CAH.
4. To determine the pharmacokinetics of Compound 1 in adolescent subjects with CAH.
5. To investigate potential relationships between pharmacodynamics and pharmacokinetics in adolescent subjects with CAH.

Exploratory

6. To explore the dose(s) of Compound 1 that cause changes in exploratory pharmacodynamics biomarkers in blood and urine in adolescent subjects with CAH.

Eligibility Criteria

Inclusion Criteria:

- Male and female subjects 12 to 17 years of age, inclusive
- Documented diagnosis of classic CAH due to 21-hydroxylase deficiency
- Elevated 17-OHP at screening
- On a stable regimen of glucocorticoid replacement for 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.

Example 7: Phase 3 Clinical Studies

[0174] This is a 24-week, randomized, double-blind, placebo-controlled study of Compound 1 for the treatment of classic CAH. After screening, eligible patients will be enrolled into a 24-week treatment period followed by a 4-week safety follow-up period.

[0175] Subjects in the study will be randomized in a 1:1 ratio to either receive Compound 1 or a matching placebo for up to 24 weeks. Compound 1 or a placebo will be administered as an oral daily dose. Patients will return approximately monthly for study visits. A follow-up outpatient visit will occur 30 days after their last dose. It is initially planned that up to approximately 150 patients will be enrolled.

[0176] Consistent with the design of the Phase 2 study, the Phase 3 clinical study will seek to enroll patients with inadequately controlled CAH despite an existing glucocorticoid (GC) regimen (including any currently-available regimen: hydrocortisone, prednisone/prednisolone, and/or dexamethasone). In practice, CAH patients are typically treated with supraphysiologic doses of GCs which are both toxic and not effective in the majority of patients. GCs are associated with multiple well-described short- and long-term toxicities in adults including: excessive weight gain, hypertension, hyperglycemia, hyperlipidemia, and Cushingoid features.

Study Design:

- Study Type: Interventional
- Primary Purpose: Treatment
- Study Phase: Phase 3
- Interventional Study Model: Randomized Controlled Trial
- Allocation: Randomized
- Enrollment: 150 [Anticipated]

Outcome Measures

Primary Outcome Measures

1. Evaluate efficacy of Compound 1 in reducing A4 and 17-OHP in patients with CAH.

Secondary Outcome Measures

2. Evaluate efficacy of Compound 1 in patients with CAH in terms of changes in key hormones.
3. Evaluate efficacy of Compound 1 in improving quality of life and/or mood in patients with CAH.

4. Evaluate efficacy of Compound 1 in improving hyperandrogenic symptoms in patients with CAH.
5. Evaluate efficacy of Compound 1 in improving metabolic parameters in patients with CAH.
6. Evaluate safety of Compound 1 in patients with CAH.

Eligibility

- Minimum age: 16 Years
- Maximum Age:
- Sex: All
- Gender Based: No
- Accepts Healthy Volunteers: No
- Criteria: Inclusion

Criteria

Inclusion Criteria:

- Male and female patients age 16 or older.
- Document diagnosis of classic CAH due to 21-hydroxylase deficiency
- Elevated 17-OHP
- 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.

Example 8: Phase 3 OLE and steroid sparing studies

[0177] Upon completion of the study described in Example 7, eligible patients will then be offered the opportunity to enroll into an open label extension study lasting up to 1 year. The open label study will allow all eligible patients to receive active study drug during their participation in the Phase 3 program. Patients will undergo study assessments every 1-3 months during the open label phase, and will be evaluated for long-term safety and efficacy (including both hormonal endpoints and long-term clinical outcome measures of concern in this population).

[0178]

[0179] A further study may seek to enroll patients with inadequately controlled CAH despite an existing glucocorticoid (GC) regimen (including any currently-available regimen:

hydrocortisone, prednisone/prednisolone, and/or dexamethasone). Such CAH patients are typically treated with supraphysiologic doses of GCs which are both toxic and not effective in the majority of patients. GCs are associated with multiple well-described short- and long-term toxicities in adults including: excessive weight gain, hypertension, hyperglycemia, hyperlipidemia, and Cushingoid features.

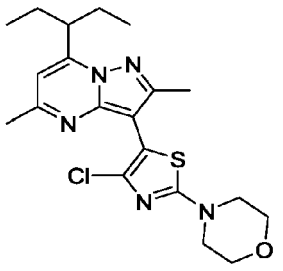
[0180] [0159] Following a period of treatment (for example 12 weeks) during the dose of GC will be kept stable, patients in this study will have their dose of GC progressively reduced from a supraphysiologic dose to a lower dose at or close to a dose equivalent to a physiologic dose of GC.

[0181] 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 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, wherein Compound 1 is administered at a dose between about 50 mg/day and about 1600 mg/day.

2. The method of claim 1, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 1200 mg/day.
3. The method of claim 1 or 2, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 1000 mg/day.
4. The method of any one of claims 1 to 3, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 800 mg/day.
5. The method of any one of claims 1 to 4, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 100 mg/day and about 600 mg/day.
6. The method of any one of claims 1 to 5, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 200 mg/day and 400 mg /day.
7. The method of any one of claims 1 to 6, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of about 200 mg /day.
8. The method of any one of claims 1 to 7, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is in the form of microparticles.
9. The method of claim 8, wherein the average size of the microparticles is between about 1 μm and about 20 μm.

10. The method of claim 8 or 9, wherein the average size of the microparticles is between about 5 μm and about 15 μm.
11. The method of any one of claims 8 to 10, wherein the average size of the microparticles is less than about 10 μm.
12. The method of any one of claims 1 to 11, wherein the pharmaceutical composition is in the form of a capsule or a tablet.
13. The method of any one of claims 1 to 12, wherein the pharmaceutical composition is in the form of a capsule.
14. The method of claim 13, wherein the capsule is a hard gelatin capsule.
15. The method of claim 13, wherein the capsule is a soft gelatin capsule.
16. The method of any one of claims 13 to 15, 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.
17. The method of any one of claims 13 to 16, wherein the pharmaceutical composition is free of additional excipients.
18. The method of any one of claims 13 to 16, wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.
19. The method of any one of claims 1 to 12, wherein the pharmaceutical composition is in the form of a tablet.
20. The method of claim 19, wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.
21. The method of any one of claims 1 to 20, wherein the pharmaceutical composition comprises between about 1 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
22. The method of any one of claims 1 to 21, wherein the pharmaceutical composition comprises between about 5 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
23. The method of any one of claims 1 to 22, wherein the pharmaceutical composition comprises between about 10 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
24. The method of any one of claims 1 to 23, wherein the pharmaceutical composition comprises between about 10 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

25. The method of any one of claims 1 to 24, wherein the pharmaceutical composition comprises between about 10 mg and about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
26. The method of any one of claims 1 to 23, wherein the pharmaceutical composition comprises between about 50 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
27. The method of any one of claims 1 to 23, wherein the pharmaceutical composition comprises between about 100 mg and about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
28. The method of any one of claims 1 to 24, wherein the pharmaceutical composition comprises between about 100 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
29. The method of any one of claims 1 to 24, wherein the pharmaceutical composition comprises between about 150 mg and about 250 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
30. The method of any one of claims 1 to 23, wherein the pharmaceutical composition comprises about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
31. The method of any one of claims 1 to 24, wherein the pharmaceutical composition comprises about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
32. The method of any one of claims 1 to 24, wherein the pharmaceutical composition comprises about 250 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
33. The method of any one of claims 1 to 24, wherein the pharmaceutical composition comprises about 200 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
34. The method of any one of claims 1 to 24, wherein the pharmaceutical composition comprises about 150 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
35. The method of any one of claims 1 to 25, wherein the pharmaceutical composition comprises about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

36. The method of any one of claims 1 to 25, wherein the pharmaceutical composition comprises about 80 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
37. The method of any one of claims 1 to 25, wherein the pharmaceutical composition comprises about 60 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
38. The method of any one of claims 1 to 25, wherein the pharmaceutical composition comprises about 50 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
39. The method of any one of claims 1 to 25, wherein the pharmaceutical composition comprises about 30 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.
40. The method of any one of claims 1 to 39, wherein the pharmaceutical composition provides a Compound 1 T_{max} of about 2 to about 6 hours in a subject.
41. The method of any one of claims 1 to 40, wherein the pharmaceutical composition provides a Compound 1 T_{max} of about 3 to about 5 hours in a subject.
42. The method of any one of claims 1 to 40, wherein the pharmaceutical composition provides a Compound 1 T_{max} of about 6 hours in a subject.
43. The method of any one of claims 1 to 41, wherein the pharmaceutical composition provides a Compound 1 T_{max} of about 5 hours in a subject.
44. The method of any one of claims 1 to 41, wherein the pharmaceutical composition provides a Compound 1 T_{max} of about 4 hours in a subject.
45. The method of any one of claims 1 to 41, wherein the pharmaceutical composition provides a Compound 1 T_{max} of about 3 hours in a subject.
46. The method of any one of claims 1 to 45, wherein the pharmaceutical composition is administered in the fed state.
47. The method of any one of claims 1 to 45, wherein the pharmaceutical composition is administered in the fasted state.
48. The method of any one of claims 1 to 47, wherein the pharmaceutical composition is administered once a day.
49. The method of any one of claims 1 to 47, wherein the pharmaceutical composition is administered twice a day.
50. The method of any one of claims 1 to 47, wherein the pharmaceutical composition is administered three times a day.
51. The method of any of claims 1 to 50, further comprising administering a glucocorticoid.

52. The method of claim 51, wherein 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.

53. The method of claim 52, wherein the amount of glucocorticoid used is reduced from a supraphysiologic amount to a physiologic amount.

54. The method of claim 52, wherein the amount of glucocorticoid 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%.

55. The method of any one of claims 52 to 54, wherein the symptoms associated with high-dose glucocorticoid therapy are reduced.

56. The method of claim 55, wherein the symptoms associated with high-dose glucocorticoid therapy are obesity, insulin resistance, metabolic abnormalities, hypertension, cardiovascular diseases, or osteoporosis.

57. The method of any one of claims 51 to 56, wherein the glucocorticoid is beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone.

58. The method of any one of claims 51 to 57, wherein the glucocorticoid is hydrocortisone.

59. The method of claim 58, wherein the hydrocortisone is administered at a dose less than about 15 mg/day.

60. The method of claim 58 or 59, wherein the hydrocortisone is administered at a dose less than about 10 mg/day.

61. The method of any one of claims 58 to 60, wherein the hydrocortisone is administered at a dose less than about 5 mg/day.

62. The method of any one of claims 51 to 61, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered concurrently.

63. The method of any one of claims 51 to 62, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially.

64. The method of any one of claims 51 to 62, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 24 hours.

65. The method of any one of claims 51 to 62, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 8 hours.

66. The method of any one of claims 51 to 62, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 2 hours.

67. The method of any one of claims 51 to 62, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 30 minutes.

68. The method of any one of claims 1 to 67, further comprising administering a mineralocorticoid.

69. The method of claim 68, wherein the mineralocorticoid is fludrocortisone.

70. The method of any one of claims 1 to 69, wherein the pharmaceutical composition is administered at bedtime.

71. The method of any one of claims 1 to 70, wherein the pharmaceutical composition is administered less than about 4 hours before sleep.

72. The method of any one of claims 1 to 70, wherein the pharmaceutical composition is administered less than about 2 hours before sleep.

73. The method of any one of claims 1 to 70, wherein the pharmaceutical composition is administered less than about 30 mins before sleep.

74. The method of any one of claims 1 to 70, wherein the pharmaceutical composition is administered in the evening.

75. The method of any one of claims 1 to 70, wherein the pharmaceutical composition is administered at about 10 pm at night.

76. The method of any one of claims 1 to 70, wherein the pharmaceutical composition is administered at or before the expected circadian release of adrenocorticotropic hormone (ACTH).

77. The method of any one of claims 1 to 70, wherein the pharmaceutical composition is administered about 3-4 hours before the expected circadian release of adrenocorticotropic hormone (ACTH).

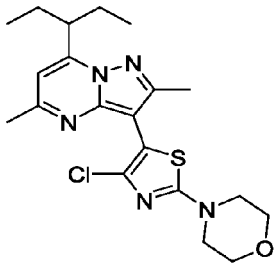
78. The method of any one of claims 1 to 77, wherein CAH is classic CAH.

79. The method of any one of claims 1 to 77, wherein CAH is non-classic CAH.

80. A method of treating congenital adrenal hyperplasia (CAH) in a subject in need thereof, the method comprising:

(iv)measuring a hormone level in the subject in need thereof;

(v) administering Compound 1:



or a pharmaceutically acceptable salt or solvate thereof;

(vi) 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.

81. The method of claim 80, wherein the hormone is 17α-Hydroxyprogesterone (17-OHP), adrenocorticotropic hormone (ACTH), testosterone, or androstenedione.

82. The method of claim 81, wherein the hormone is 17-OHP, and the pre-determined range is from about 200 ng/dL to about 400 ng/dL.

83. The method of claim 81, wherein the hormone is ACTH, and the pre-determined range is below about 100 pg/mL.

84. The method of claim 81, wherein the hormone is testosterone and the pre-determined range is from about 14 ng/dL to about 76 ng/dL.

85. The method of claim 81, wherein the hormone is androstenedione and the pre-determined range is from about 30 ng/dL to about 150 ng/dL in males.

86. The method of claim 81, wherein the hormone is androstenedione and the pre-determined range is from about 40 ng/dL to about 200 ng/dL in females.

87. The method of any one of claims 80 to 86, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 200 mg/day and about 1600 mg/day.

88. The method of any one of claims 80 to 87, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 200 mg/day and about 1200 mg/day.

89. The method of any one of claims 80 to 88, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 200 mg/day and about 1000 mg/day.

90. The method of any one of claims 80 to 89, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 200 mg/day and about 800 mg/day.

91. The method of any one of claims 80 to 90, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 200 mg/day and about 600 mg/day.

92. The method of any one of claims 80 to 91, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 200 mg/day and 400 mg /day.

93. The method of any one of claims 80 to 92, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of about 200 mg /day.

94. The method of any one of claims 80 to 86, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 1600 mg/day.

95. The method of claim 94, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 1200 mg/day.

96. The method of claims 94 or 95, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 1000 mg/day.

97. The method of any one of claims 94 to 96, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 800 mg/day.

98. The method of any one of claims 94 to 97, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and about 600 mg/day.

99. The method of any one of claims 94 to 98, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between about 50 mg/day and 400 mg /day.

100. The method of any one of claims 94 to 99, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose between 50 mg/day and 200 mg/day.

101. The method of any one of claims 94 to 99, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at a dose of about 50 mg /day.

102. The method of any one of claims 80 to 101, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is in the form of microparticles.

103. The method of claim 102, wherein the average size of the microparticles is between about 1 μm and about 20 μm.

104. The method of claim 102 or 103, wherein the average size of the microparticles is between about 5 μm and about 15 μm.

105. The method of any one of claims 102 to 104, wherein the average size of the microparticles is less than about 10 μm.

106. The method of any one of claims 80 to 105, wherein Compound 1 is formulated in a pharmaceutical composition in the form of a capsule or a tablet.

107. The method of claim 106, wherein the pharmaceutical composition is in the form of a capsule.

108. The method of claim 107, wherein the capsule is a hard gelatin capsule.

109. The method of claim 107, wherein the capsule is a soft gelatin capsule.

110. The method of any one of claims 106 to 109, 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.

111. The method of any one of claims 106 to 110, wherein the pharmaceutical composition is free of additional excipients.

112. The method of any one of claims 106 to 110, wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

113. The method of claim 106, wherein the pharmaceutical composition is in the form of a tablet.

114. The method of claim 113, wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

115. The method of any one of claims 106 to 114, wherein the pharmaceutical composition comprises between about 1 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

116. The method of any one of claims 106 to 115, wherein the pharmaceutical composition comprises between about 5 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

117. The method of any one of claims 106 to 116, wherein the pharmaceutical composition comprises between about 10 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

118. The method of any one of claims 106 to 117, wherein the pharmaceutical composition comprises between about 10 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

119. The method of any one of claims 106 to 118, wherein the pharmaceutical composition comprises between about 10 mg and about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

120. The method of any one of claims 106 to 117, wherein the pharmaceutical composition comprises between about 50 mg and about 500 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

121. The method of any one of claims 106 to 117, wherein the pharmaceutical composition comprises between about 100 mg and about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

122. The method of any one of claims 106 to 118, wherein the pharmaceutical composition comprises between about 100 mg and about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

123. The method of any one of claims 106 to 118, wherein the pharmaceutical composition comprises between about 150 mg and about 250 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

124. The method of any one of claims 106 to 117, wherein the pharmaceutical composition comprises about 400 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

125. The method of any one of claims 106 to 118, wherein the pharmaceutical composition comprises about 300 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

126. The method of any one of claims 106 to 118, wherein the pharmaceutical composition comprises about 250 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

127. The method of any one of claims 106 to 118, wherein the pharmaceutical composition comprises about 200 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

128. The method of any one of claims 106 to 118, wherein the pharmaceutical composition comprises about 150 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

129. The method of any one of claims 106 to 118, wherein the pharmaceutical composition comprises about 100 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

130. The method of any one of claims 106 to 118, wherein the pharmaceutical composition comprises about 80 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

131. The method of any one of claims 106 to 118, wherein the pharmaceutical composition comprises about 60 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

132. The method of any one of claims 106 to 118, wherein the pharmaceutical composition comprises about 50 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

133. The method of any one of claims 106 to 118, wherein the pharmaceutical composition comprises about 30 mg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

134. The method of any one of claims 106 to 133, wherein the pharmaceutical composition provides a Compound 1 T_{max} of about 2 to about 6 hours in a subject.

135. The method of any one of claims 106 to 118, wherein the pharmaceutical composition provides a Compound 1 T_{max} of about 3 to about 5 hours in a subject.

136. The method of any one of claims 106 to 118, wherein the pharmaceutical composition provides a Compound 1 T_{max} of about 6 hours in a subject.

137. The method of any one of claims 106 to 135, wherein the pharmaceutical composition provides a Compound 1 T_{max} of about 5 hours in a subject.

138. The method of any one of claims 106 to 135, wherein the pharmaceutical composition provides a Compound 1 T_{max} of about 4 hours in a subject.

139. The method of any one of claims 106 to 135, wherein the pharmaceutical composition provides a Compound 1 T_{max} of about 3 hours in a subject.

140. The method of any one of claims 106 to 139, wherein the pharmaceutical composition is administered in the fed state.

141. The method of any one of claims 106 to 140, wherein the pharmaceutical composition is administered in the fasted state.

142. The method of any one of claims 106 to 141, wherein the pharmaceutical composition is administered once a day.

143. The method of any one of claims 106 to 141, wherein the pharmaceutical composition is administered twice a day.

144. The method of any one of claims 106 to 141, wherein the pharmaceutical composition is administered three times a day.

145. The method of any of claims 80 to 144, further comprising administering a glucocorticoid.

146. The method of claim 145, wherein 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.

147. The method of claim 145, wherein the amount of glucocorticoid used is reduced from a supraphysiologic amount to a physiologic amount.

148. The method of claim 145, wherein the amount of glucocorticoid 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%.

149. The method of any one of claims 145 to 148, wherein the symptoms associated with high-dose glucocorticoid therapy are reduced.

150. The method of claim 149, wherein the symptoms associated with high-dose glucocorticoid therapy are obesity, insulin resistance, metabolic abnormalities, hypertension, cardiovascular diseases, or osteoporosis.

151. The method of any one of claims 145 to 150, wherein the glucocorticoid is beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone.

152. The method of any one of claims 145 to 151, wherein the glucocorticoid is hydrocortisone.

153. The method of claim 152, wherein the hydrocortisone is administered at a dose less than about 15 mg/day.

154. The method of claim 152 or 153, wherein the hydrocortisone is administered at a dose less than about 10 mg/day.

155. The method of any one of claims 152 to 154, wherein the hydrocortisone is administered at a dose less than about 5 mg/day.

156. The method of any one of claims 152 to 155, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered concurrently.

157. The method of any one of claims 152 to 155, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially.

158. The method of any one of claims 152 to 155, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 24 hours.

159. The method of any one of claims 152 to 155, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 8 hours.

160. The method of any one of claims 152 to 155, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 2 hours.

161. The method of any one of claims 152 to 155, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, and the glucocorticoid are administered sequentially within 30 minutes.

162. The method of any one of claims 80 to 161, further comprising administering a mineralocorticoid.

163. The method of claim 162, wherein the mineralocorticoid is fludrocortisone.

164. The method of any one of claims 80 to 163, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at bedtime.

165. The method of any one of claims 80 to 163, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered less than about 4 hours before sleeping.

166. The method of any one of claims 80 to 163, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered less than about 2 hours before sleeping.

167. The method of any one of claims 80 to 163, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered less than about 30 mins before sleeping.

168. The method of any one of claims 80 to 163, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered in the evening.

169. The method of any one of claims 80 to 163, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at 10 pm at night.

170. The method of any one of claims 80 to 163, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered at or before the expected circadian release of adrenocorticotropic hormone (ACTH).

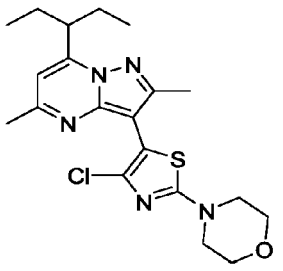
171. The method of any one of claims 80 to 163, wherein Compound 1, or a pharmaceutically acceptable salt or solvate thereof, is administered about 3-4 hours before the expected circadian release of adrenocorticotropic hormone (ACTH).

172. The method of any one of claims 80 to 171, wherein CAH is classic CAH.

173. The method of any one of claims 80 to 171, wherein CAH is non-classic CAH.

174. A method of any one of claims 1 to 173, wherein the subject in need thereof is from about 12 years of age to about 20 years of age.

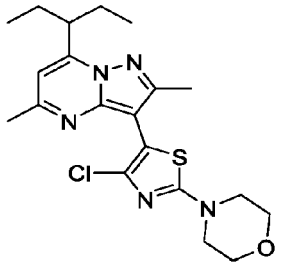
175. A method of improving hyperandrogenic symptoms in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 1:



; or a pharmaceutically acceptable salt or solvate thereof, wherein Compound 1 is administered at a dose between about 50 mg/day and about 1600 mg/day.

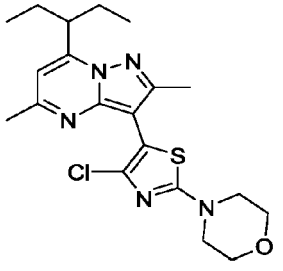
176. A method of claim 175, wherein the hyperandrogenic symptoms are selected from the group consisting of acne, hirsutism and alopecia.

177. A method of treating menstrual irregularity, ovulatory dysfunction or infertility, in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 1:



; or a pharmaceutically acceptable salt or solvate thereof, wherein Compound 1 is administered at a dose between about 50 mg/day and about 1600 mg/day.

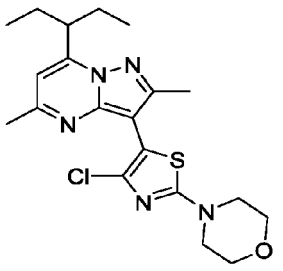
178. A method of improving metabolic symptoms in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 1:



; or a pharmaceutically acceptable salt or solvate thereof, wherein Compound 1 is administered at a dose between about 50 mg/day and about 1600 mg/day.

179. A method of claim 178, wherein the metabolic symptoms are selected from the group consisting of body weight, BMI, waist circumference, blood pressure and glycemic control.

180. A method of improving quality of life in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 1:



; or a pharmaceutically acceptable salt or solvate thereof, wherein Compound 1 is administered at a dose between about 50 mg/day and about 1600 mg/day, wherein treatment results in an improved quality of life.

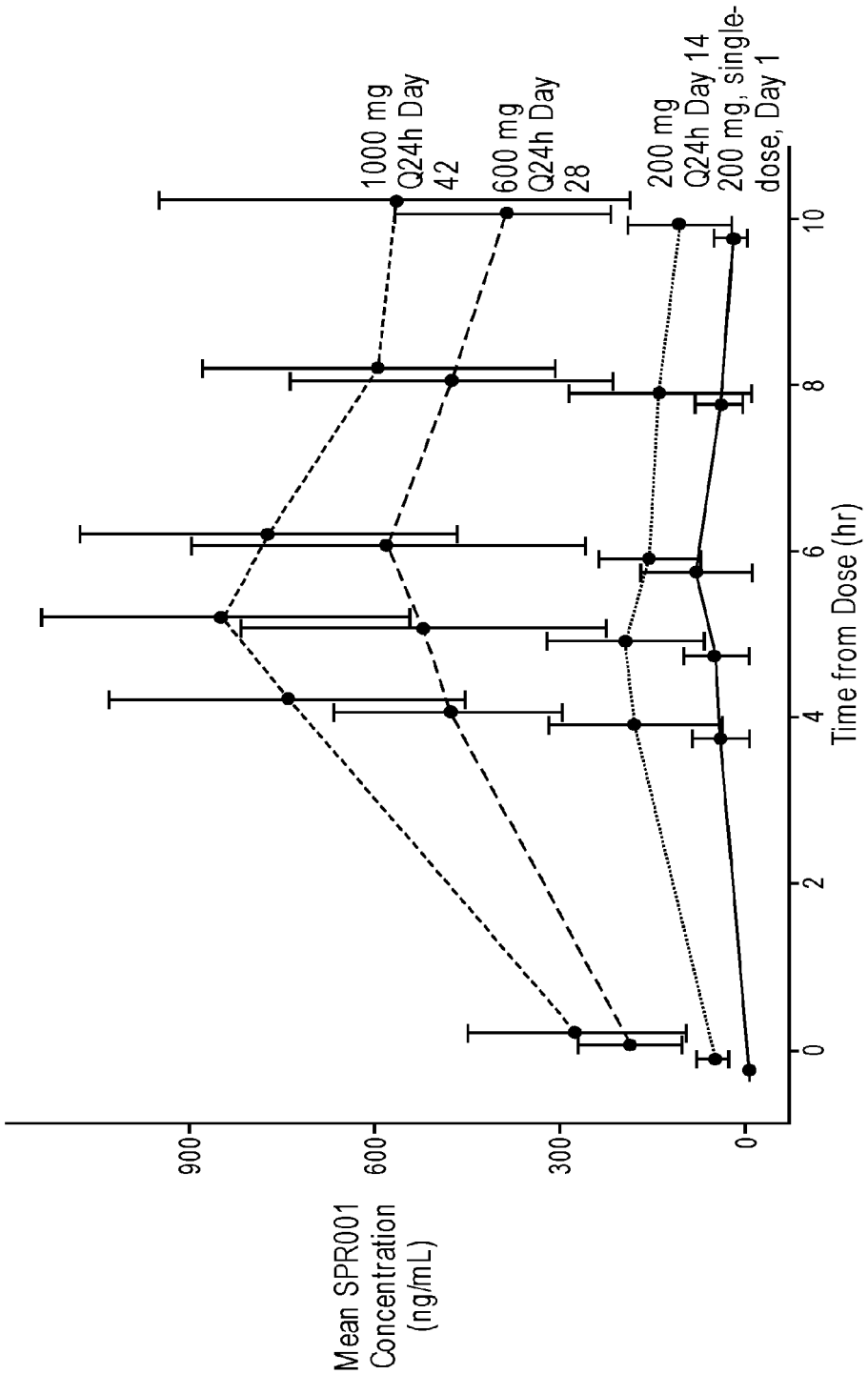

Mean SPR001 Concentration (ng/mL)
900
600
300
0
0
2
4
6
8
10
Time from Dose (hr)
1000 mg Q24h Day 42
600 mg Q24h Day 28
200 mg Q24h Day 14
200 mg, single-dose, Day 1

FIG. 1

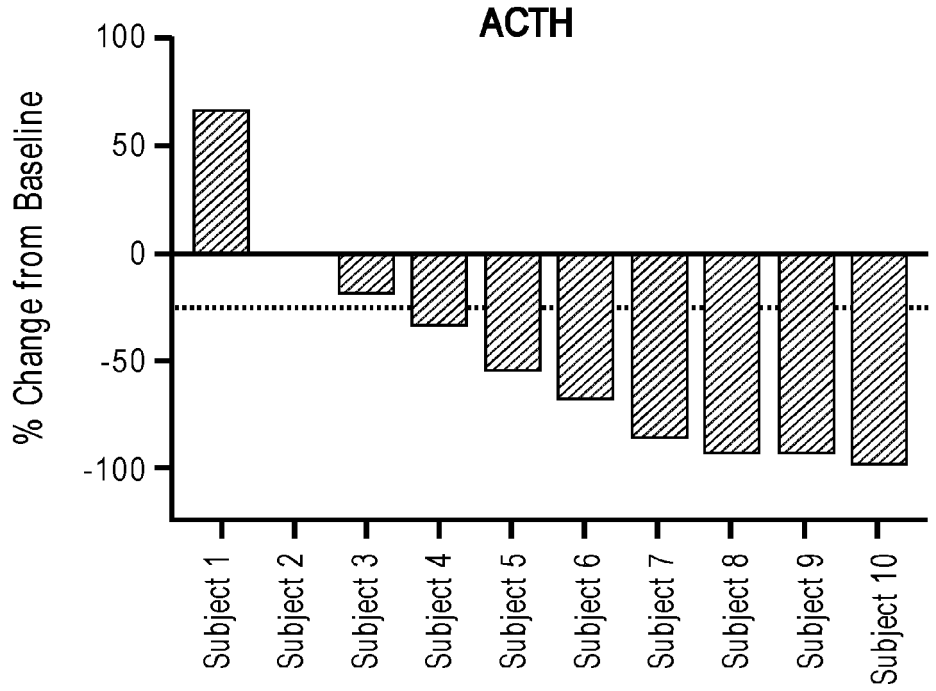

ACTH
% Change from Baseline
100
50
0
-50
-100
Subject 1
Subject 2
Subject 3
Subject 4
Subject 5
Subject 6
Subject 7
Subject 8
Subject 9
Subject 10

FIG. 2

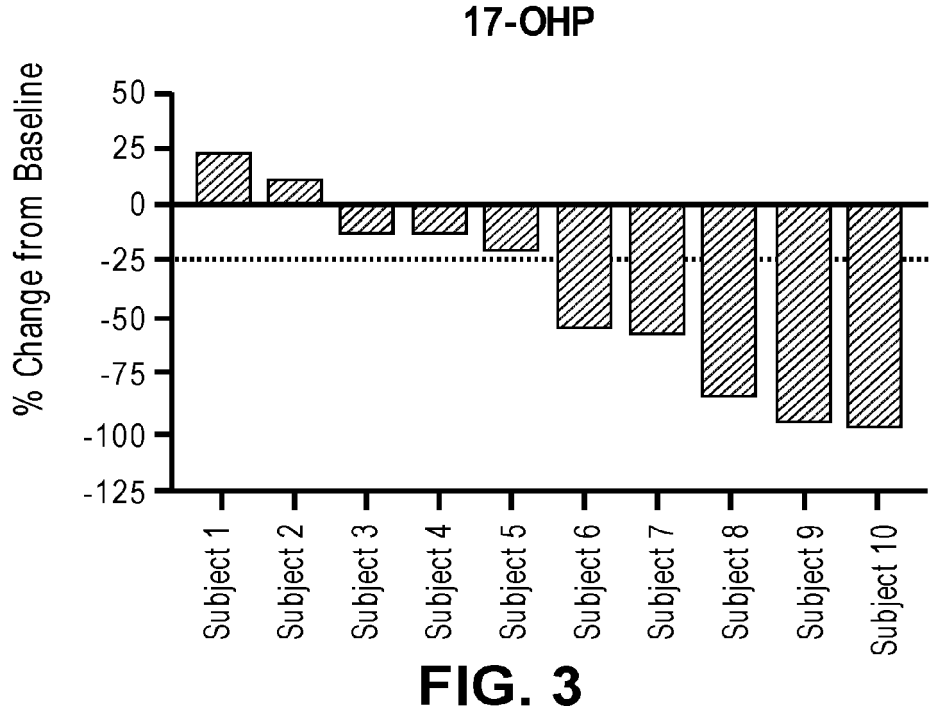

17-OHP
% Change from Baseline
50
25
0
-25
-50
-75
-100
-125
Subject 1
Subject 2
Subject 3
Subject 4
Subject 5
Subject 6
Subject 7
Subject 8
Subject 9
Subject 10

FIG. 3

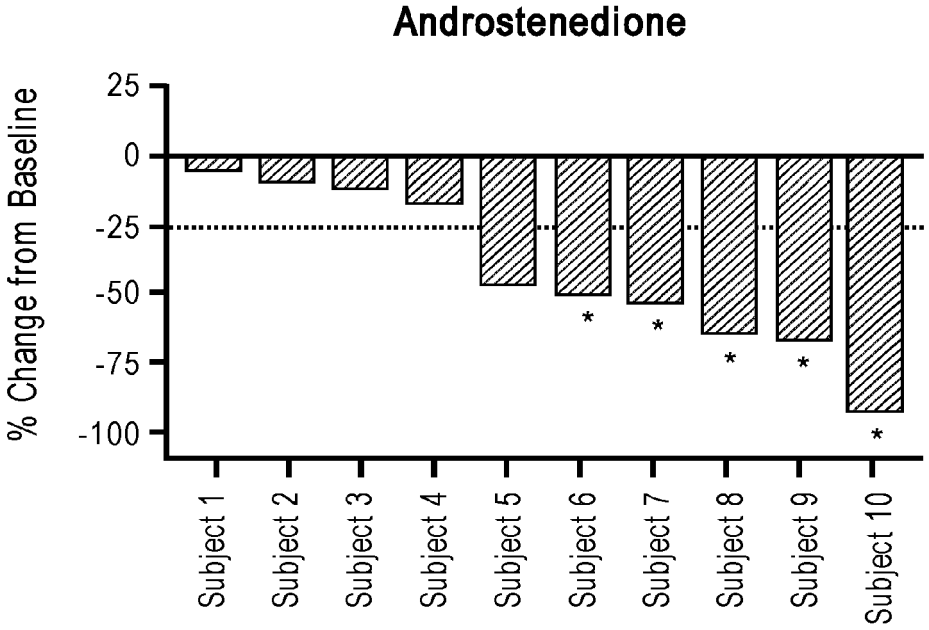

Androstenedione
% Change from Baseline
25
0
-25
-50
-75
-100
*
*
*
*
*
Subject 1
Subject 2
Subject 3
Subject 4
Subject 5
Subject 6
Subject 7
Subject 8
Subject 9
Subject 10

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/46760

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/415; 31/425; 31/519; C07D 487/04; A61P 5/24 (2018.01)
CPC - A61K 31/41; A61K 31/519; C07D 487/04; C07D 401/04

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.
Y	US 8,030,304 B2 (CHEN et al.) 04 October 2011 (04.10.2011) Col 1, ln 10-15; Col 2, ln 51-56;Col 3, ln 8-12; Col 5, ln 63 to Col 6, ln 4; Col 6, ln 11-13; Col 32, Example 16	1-3, 80-87, 94-95; 175-180
Y	TURCU et al. 'Single-Dose Study of a Corticotropin-Releasing Factor Receptor-1 Antagonist in Women With 21-Hydroxylase Deficiency', J. Clin. Endocrinol. Metab. 2016, Vol. 101(3), pp. 1174-1180. Abstract; pg. 1177, Table 2; pg. 1179, Table 3; pg 1179, Col 2, para 2 to pg. 1180, Col 1, para 2	1-3, 80-87, 94-95; 175-180
A	US 2017/0020877 A1 (NEUROCRINE BIOSCIENCES, INC.) 26 January 2017 (26.01.2017) ENTIRE DOCUMENT	1-3, 80-87, 94-95; 175-180
A	US 2010/0155595 A1 (GHOSHAL et al.) 24 June 2010 (24.06.2010) ENTIRE DOCUMENT	1-3, 80-87, 94-95; 175-180
A	Lee et al. 'Attenuated Forms of Congenital Adrenal Hyperplasia due to 21-hydroxylase Deficiency', Journal of Clin Endocrin Metab 1982, Vol. 55, pp. 866-871. ENTIRE DOCUMENT	1-3, 80-87, 94-95; 175-180

☐ 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
25 SEPTEMBER 2018	24 OCT 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: 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 18/46760

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.: 4-79, 88-93 and 96-174
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.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: SIMREN DELANEY
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing *(day/month/year)*	12 AUG 2019
Applicant's or agent's file reference 50535-710601	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US2019/029486	International filing date *(day/month/year)* 26 April 2019
Applicant SPRUCE BIOSCIENCES, INC.	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   How? Directly to the International Bureau of WIPO preferably through ePCT or on paper to, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

   For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any** protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.
   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may **submit comments on an informal basis on the written opinion of the International Searching Authority** to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

   Shortly after the expiration of **18 months from the priority date, the international application will be published** by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until **30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

   Within **22 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45*bis*.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2017)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 50535-710601	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2019/029486	International filing date *(day/month/year)* 26 April 2019	(Earliest) Priority Date *(day/month/year)* 27 April 2018
Applicant SPRUCE BIOSCIENCES, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the language, the international search was carried out on the basis of:
      ☒ the international application in the language in which it was filed.
      ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
   b. ☐ This international search report has been established taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the title,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the drawings,
   a. the figure of the drawings to be published with the abstract is Figure No. 11B
      ☒ as suggested by the applicant.
      ☐ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/029486

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.: 15-33, 35-71
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:
See extra sheet(s).

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.:
1-4, 9, 13, 14, 34

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

International application No.
PCT/US2019/029486

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/4162; A61K 31/505; A61K 31/519; C07D 231/54; C07D 233/70; C07D 247/02 (2019.01)
CPC - A61K 31/4162; A61K 31/505; A61K 31/519; C07D 231/54; C07D 233/70; C07D 247/02 (2019.05)

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
USPC - 514/228.8; 514/230.2; 514/230.5 (keyword delimited)

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.
Y	US 2017/0020877 A1 (NEUROCRINE BIOSCIENCES, INC.) 26 January 2017 (26.01.2017) entire document	1-4, 9, 13, 14, 34
Y	US 2010/0222339 A1 (CHEN et al) 02 September 2010 (02.09.2010) entire document	1-4, 9, 13, 14, 34
Y	US 2010/0196389 A1 (EVANS-FREKE) 05 August 2010 (05.08.2010) entire document	3, 14

☐ 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
22 July 2019	12 AUG 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver 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/US2019/029486

Continued from Box No. III Observations where unity of invention is lacking

Claims 1-4, 9, 13, 14, and 34 have been analyzed subject to the restriction that the claims read on 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 (CRF1) antagonist or a pharmaceutically acceptable salt thereof, wherein the CRF1 antagonist is a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is hydrogen; and R4 is hydrogen.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-14 and 34 are drawn to methods of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART).

The first invention of Group I+ is restricted to 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 (CRF1) antagonist or a pharmaceutically acceptable salt thereof, wherein the CRF1 antagonist is a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is hydrogen; R4 is hydrogen; and methods of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART). It is believed that claims 1-4, 9, 13, 14, and 34 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected formula(e) requires the selection of a single definition for each compound variable. An exemplary election would be 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 (CRF1) antagonist or a pharmaceutically acceptable salt thereof, wherein the CRF1 antagonist is a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is Br; R4 is hydrogen; and methods of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART). Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulae do not share a significant structural element requiring the selection of alternatives for the compound variables R1, R2, R3, R4, and accordingly these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features of 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 (CRF1) antagonist or a pharmaceutically acceptable salt thereof; 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):, and 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, these shared technical features do not represent a contribution over the prior art as disclosed by US 2017/0020877 A1 to Neurocrine Biosciences, Inc. and US 2010/0222339 A1 to Chen et al.

US 2017/0020877 A1 to Neurocrine Biosciences, Inc. teaches a method of treating or preventing testicular adrenal rest tumors (TART) (Para. [0044],...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.; Para. [0047], Treatment of CAH includes efforts to normalize the cortisol deficiency with glucocorticoids...; Claim 1, A method for treating Congenital Adrenal Hyperplasia (CAH) by administering to a subject in need thereof a CRF1 receptor antagonist...), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF1) antagonist (Claim 1; Para. [0007]); and a method of treating or preventing testicular adrenal rest tumors (TART) (Paras. [0044] and [0047]; Claim 1), comprising administering to a subject in need thereof a pharmaceutical composition (Paras. [0061] and [0065], The pharmaceutical compositions described herein that comprise at least one of the CRF1 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.; Claim 1).

US 2010/0222339 A1 to Chen et al., in the field of treating congenital adrenal hyperplasia (Para. [0014]), teach a corticotropin-releasing factor type-1 (CRF1) antagonist (Para. [0125], Ex. No. 2, 3-(2,4-Dibromo-thiazol-5-yl)- 7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a] pyrimidine; Para. [0057], 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.); a compound of structural Formula (I) wherein , R1 and R2 are independently ethyl; R3 is Br; and R4 is Br (Para. [0125], Ex. No. 2, 3-(2,4-Dibromo-thiazol-5-yl)- 7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a] pyrimidine); and a pharmaceutical composition, comprising a compound of structural Formula (I) (Claim 10, A pharmaceutical composition comprising: a compound according to claim 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.; (Para. [0125], Ex. No. 2, 3-(2,4-Dibromo-thiazol-5-yl)- 7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: SIMREN DELANEY
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	12 AUG 2019

Applicant's or agent's file reference	FOR FURTHER ACTION
50535-710601	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2019/029486	26 April 2019	27 April 2018

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A61K 31/4162; A61K 31/505; A61K 31/519; C07D 231/54; C07D 233/70; C07D 247/02 (2019.01)
CPC - A61K 31/4162; A61K 31/505; A61K 31/519; C07D 231/54; C07D 233/70; C07D 247/02 (2019.05)

Applicant SPRUCE BIOSCIENCES, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	22 July 2019	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (January 2015)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/029486

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a)).

3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

☐ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/029486

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 15-33, 35-71

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 15-33, 35-71 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 15-33 and 35-71 are multiple dependent claims not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 15-33, 35-71

☐ a meaningful opinion could not be formed without the sequence listing, the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/029486

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:

   ☐ paid additional fees.

   ☐ paid additional fees under protest and, where applicable, the protest fee.

   ☐ paid additional fees under protest but the applicable protest fee was not paid.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

<See Supplemental Box>

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-4, 9, 13, 14, 34

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/029486

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-4, 9, 13, 14, 34	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-4, 9, 13, 14, 34	NO
Industrial applicability (IA)	Claims	1-4, 9, 13, 14, 34	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-4, 9, 13, 14, and 34 have been analyzed subject to the restriction that the claims read on 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 (CRF1) antagonist or a pharmaceutically acceptable salt thereof, wherein the CRF1 antagonist is a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is hydrogen; and R4 is hydrogen.

Claims 1, 2, 4, 9, 13, and 34 lack an inventive step under PCT Article 33(3) as being obvious over Neurocrine Biosciences, Inc. in view of Chen et al. (hereinafter Chen).

Regarding Claim 1, Neurocrine Biosciences, Inc. discloses a method of treating or preventing testicular adrenal rest tumors (TART) (Para. [0044],...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.; Para. [0047], Treatment of CAH includes efforts to normalize the cortisol deficiency with glucocorticoids...; Claim 1, A method for treating Congenital Adrenal Hyperplasia (CAH) by administering to a subject in need thereof a CRF1 receptor antagonist...), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF1) antagonist (Claim 1; Para. [0007]). Neurocrine Biosciences, Inc. fails to explicitly disclose the CRF1 antagonist is a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is hydrogen; and R4 is hydrogen. Chen teaches a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is hydrogen; and R4 is hydrogen (Para. [0150],...7-(1-ethyl-propyl)-2,5-dimethyl-3-thiazol-5-yl-pyrazolo[1,5-a] pyrimidine□). It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Neurocrine Biosciences, Inc. with the teaching of Chen for the purpose of providing a compound with sufficient specificity to reduce the likelihood of unwanted side effects thereby treating the disease or disorder.

Regarding Claim 2, modified Neurocrine Biosciences, Inc. discloses the method of claim 1, wherein the subject has congenital adrenal hyperplasia (CAH) (Claim 1, A method for treating Congenital Adrenal Hyperplasia (CAH) by administering to a subject in need thereof a CRF1 receptor antagonist□).

Regarding Claim 4, Neurocrine Biosciences, Inc. discloses a method of treating or preventing testicular adrenal rest tumors (TART) (Para. [0044],...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.; Para. [0047], Treatment of CAH includes efforts to normalize the cortisol deficiency with glucocorticoids...; Claim 1, A method for treating Congenital Adrenal Hyperplasia (CAH) by administering to a subject in need thereof a CRF1 receptor antagonist...), comprising administering to a subject in need thereof a compound (Claim 1; Para. [0007]). Neurocrine Biosciences, Inc. fails to explicitly disclose a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is hydrogen; and R4 is hydrogen. Chen teaches a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is hydrogen; and R4 is hydrogen (Para. [0150],...7-(1-ethyl-propyl)-2,5-dimethyl-3-thiazol-5-yl-pyrazolo[1,5-a]pyrimidine□). It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Neurocrine Biosciences, Inc. with the teaching of Chen for the purpose of providing a compound with sufficient specificity to reduce the likelihood of unwanted side effects thereby treating the disease or disorder.

Regarding Claim 9, modified Neurocrine Biosciences, Inc. discloses the method of claim 4. Neurocrine Biosciences, Inc. fails to explicitly disclose wherein R4 is hydrogen. Chen teaches wherein R4 is hydrogen (Para. [0150],...7-(1-ethyl-propyl)-2,5-dimethyl-3-thiazol-5-yl-pyrazolo[1,5-a]pyrimidine□). It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Neurocrine Biosciences, Inc. with the teaching of Chen for the purpose of providing a compound with sufficient specificity to reduce the likelihood of unwanted side effects thereby treating the disease or disorder.

Regarding Claim 13, modified Neurocrine Biosciences, Inc. discloses the method of claim 4, wherein the subject has congenital adrenal hyperplasia (CAH) (Claim 1, A method for treating Congenital Adrenal Hyperplasia (CAH) by administering to a subject in need thereof a CRF1 receptor antagonist□).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/029486

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

<Box No. IV Lack of unity of invention>

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-14 and 34 are drawn to methods of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART).

The first invention of Group I+ is restricted to 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 (CRF1) antagonist or a pharmaceutically acceptable salt thereof, wherein the CRF1 antagonist is a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is hydrogen; R4 is hydrogen; and methods of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART). It is believed that claims 1-4, 9, 13, 14, and 34 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected formula(e) requires the selection of a single definition for each compound variable. An exemplary election would be 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 (CRF1) antagonist or a pharmaceutically acceptable salt thereof, wherein the CRF1 antagonist is a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is Br; R4 is hydrogen; and methods of treating or preventing testicular adrenal rest tumors (TART) or ovarian adrenal rest tumors (OART). Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulae do not share a significant structural element requiring the selection of alternatives for the compound variables R1, R2, R3, R4, and accordingly these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features of 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 (CRF1) antagonist or a pharmaceutically acceptable salt thereof; 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):, and 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, these shared technical features do not represent a contribution over the prior art as disclosed by US 2017/0020877 A1 to Neurocrine Biosciences, Inc. and US 2010/0222339 A1 to Chen et al.

US 2017/0020877 A1 to Neurocrine Biosciences, Inc. teaches a method of treating or preventing testicular adrenal rest tumors (TART) (Para. [0044],...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.; Para. [0047], Treatment of CAH includes efforts to normalize the cortisol deficiency with glucocorticoids...; Claim 1, A method for treating Congenital Adrenal Hyperplasia (CAH) by administering to a subject in need thereof a CRF1 receptor antagonist...), comprising administering to a subject in need thereof a corticotropin-releasing factor type-1 (CRF1) antagonist (Claim 1; Para. [0007]); and a method of treating or preventing testicular adrenal rest tumors (TART) (Paras. [0044] and [0047]; Claim 1), comprising administering to a subject in need thereof a pharmaceutical composition (Paras. [0061] and [0065], The pharmaceutical compositions described herein that comprise at least one of the CRF1 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.; Claim 1).

US 2010/0222339 A1 to Chen et al., in the field of treating congenital adrenal hyperplasia (Para. [0014]), teach a corticotropin-releasing factor type-1 (CRF1) antagonist (Para. [0125], Ex. No. 2, 3-(2,4-Dibromo-thiazol-5-yl)- 7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a] pyrimidine; Para. [0057], 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.); a compound of structural Formula (I) wherein , R1 and R2 are independently ethyl; R3 is Br; and R4 is Br (Para. [0125], Ex. No. 2, 3-(2,4-Dibromo-thiazol-5-yl)- 7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a] pyrimidine); and a pharmaceutical composition, comprising a compound of structural Formula (I) (Claim 10, A pharmaceutical composition comprising: a compound according to claim 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.; (Para. [0125], Ex. No. 2, 3-(2,4-Dibromo-thiazol-5-yl)- 7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/029486

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding Claim 34, Neurocrine Biosciences, Inc. discloses a method of treating or preventing testicular adrenal rest tumors (TART) (Paras. [0044] and [0047]; Claim 1), comprising administering to a subject in need thereof a pharmaceutical composition (Paras. [0061] and [0065], The pharmaceutical compositions described herein that comprise at least one of the CRF1 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.; Claim 1) and a pharmaceutically acceptable excipient (Para. [0061], 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.) Neurocrine Biosciences, Inc. fails to explicitly disclose a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is hydrogen; and R4 is hydrogen. Chen teaches a compound of structural Formula I or a pharmaceutically acceptable salt thereof, wherein: R1 and R2 are independently ethyl; R3 is hydrogen; and R4 is hydrogen (Para. [0150],...7-(1-ethyl-propyl)-2,5-dimethyl-3-thiazol-5-yl-pyrazolo[1,5-a] pyrimidine). It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Neurocrine Biosciences, Inc. with the teaching of Chen for the purpose of providing a compound with sufficient specificity to reduce the likelihood of unwanted side effects thereby treating the disease or disorder.

Claims 3 and 14 lack an inventive step under PCT Article 33(3) as being obvious over Neurocrine Biosciences, Inc. in view of Chen et al. (hereinafter Chen) and Evans-Freke.

Regarding Claim 3, modified Neurocrine Biosciences, Inc. discloses the method of claim 1. Neurocrine Biosciences, Inc. fails to explicitly disclose wherein the size and/or number of the tumors is decreased or reduced. Evans-Freke, in the field of methods for treating cancer in a human by administering a high dose of corticotropin-releasing factor (CRF) (Abstract; Claim 2,...testicular cancer...), teaches the size and/or number of the tumors is decreased or reduced (Para. [0004],...CRF, when used in combination with one or more agents...is effective preventing the development or growth of a tumor and may even reduce the size of a tumor.; Para. [0006],...the methods of the disclosure can be used to prevent tumor progression...the tumor growth is inhibited, stopped or reversed, that the size or volume of the tumor remains the same or decreases). It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Neurocrine Biosciences, Inc. with the teaching of Evans-Freke for the purpose of controlling the growth of tumors to allow for further anti-cancer intervention thereby prolonging survival rates of the patient.

Regarding Claim 14, modified Neurocrine Biosciences, Inc. discloses the method of claim 4. Neurocrine Biosciences, Inc. fails to explicitly disclose wherein the size and/or number of the tumors is decreased or reduced. Evans-Freke, in the field of methods for treating cancer in a human by administering a high dose of corticotropin-releasing factor (CRF) (Abstract; Claim 2,...testicular cancer...), teaches the size and/or number of the tumors is decreased or reduced (Para. [0004],...CRF, when used in combination with one or more agents...is effective preventing the development or growth of a tumor and may even reduce the size of a tumor.; Para. [0006],...the methods of the disclosure can be used to prevent tumor progression...the tumor growth is inhibited, stopped or reversed, that the size or volume of the tumor remains the same or decreases). It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Neurocrine Biosciences, Inc. with the teaching of Evans-Freke for the purpose of controlling the growth of tumors to allow for further anti-cancer intervention thereby prolonging survival rates of the patient.

Claims 1-4, 9, 13, 14, and 34 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

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

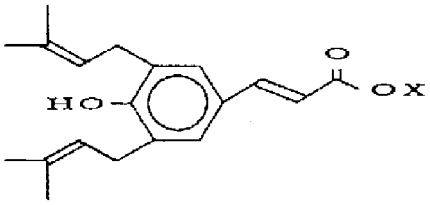
(72)発明者　木本　哲夫
岡山県岡山市山崎311番地の９

(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、ブ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、ブ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	６０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。

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

(22) **International Filing Date:** 23 September 1997 (23.09.97)

(30) **Priority Data:**
96402033.3 25 September 1996 (25.09.96) EP
(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).

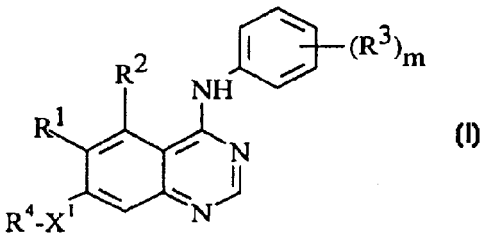
(74) **Agent:** MACK, John, Richard; Zeneca Pharmaceuticals, Intellectual Property Dept., Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).

(81) **Designated States:** AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) **Title:** QUINAZOLINE DERIVATIVES AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM

(57) **Abstract**



(I)

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.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

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 varicty 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.

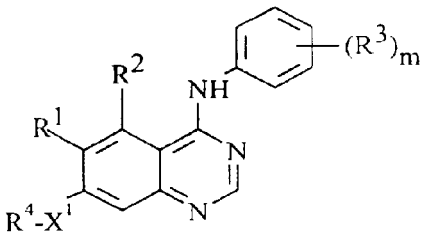
European Patent Publication No. 0566226 discloses anilinoquinazolines which have activity against epidermal growth factor (EGF) receptor tyrosine kinase. EP 0566226 contains no teaching whatsoever concerning angiogenesis and/or increased vascular permeability mediated by growth factors such as VEGF. 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.

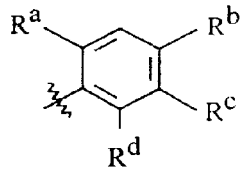
R^1 is preferably hydrogen, hydroxy, cyano, nitro, trifluoromethyl, methyl, ethyl, methoxy or ethoxy, more preferably hydrogen, methyl or methoxy, most preferably hydrogen or methoxy, but especially methoxy.

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}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 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}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 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^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}.

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

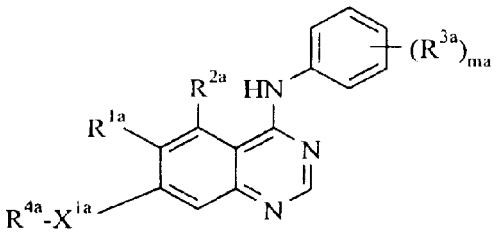
8) C_{2-3}alkylR^{43} (wherein R^{43} is morpholino which may bear one or two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl) with the proviso that when R^4 is C_{2-3}alkylR^{43}, X^1 is -S-, -SO-, $-SO_2-$, $-SO_2NR^9-$ or $-NR^{10}SO_2-$.

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}

$_2$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.

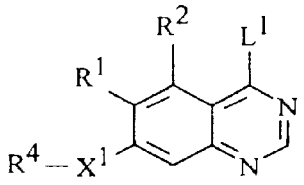
A compound of the formula I, or salt thereof, and other compounds of the invention (as hereinafter defined) may be prepared by any process known to be applicable to the

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.

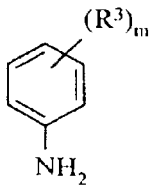
Synthesis of Compounds of Formula I

(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:



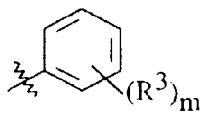
(IV)

(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.

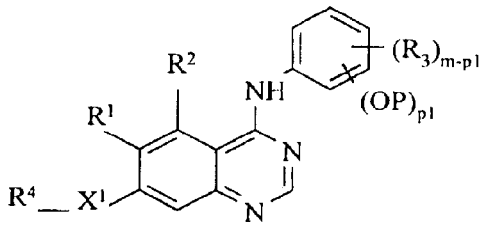
The compound of the invention may be obtained from this process in the form of the free base or alternatively it may be obtained in the form of a salt with the acid of the formula H-L^1 wherein L^1 has the meaning defined hereinbefore. When it is desired to obtain the free base from the salt, the salt 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:

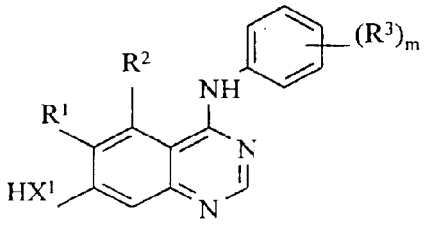


(V)

(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:



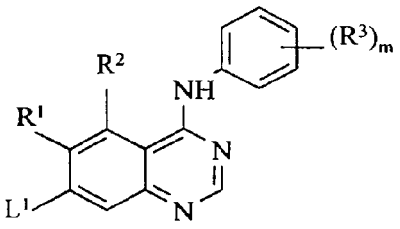
(VI)

(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:

$$R^4\text{-}X^1\text{-}H \qquad (IX)$$

(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.

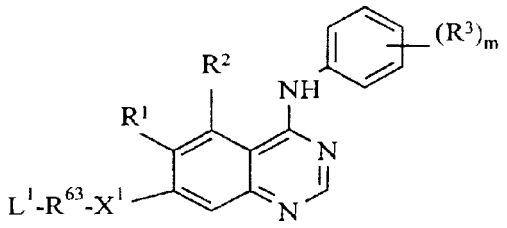
(e) Compounds of the formula I and salts thereof wherein R^4 is C_{1-5}alkylR^{53}, [wherein R^{53} is selected from one of the following three groups:

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.

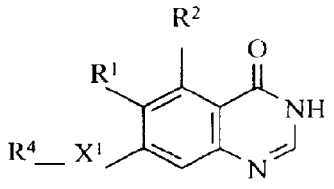
(f) The production of those compounds of the formula I and salts thereof wherein the substituent R^1 is represented by $-NR^5R^6$, where one or both of R^5 and R^6 are C_{1-3}alkyl, may be effected by the reaction of compounds of formula I wherein the substituent R^1 is an amino group and an alkylating 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:



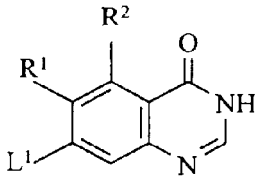
(XII)

(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.

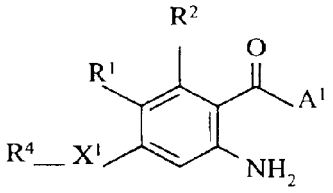
The compounds of formula XII and salts thereof which constitute a further feature of the present invention may for example be prepared by reacting a compound of the formula XIII:



(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:

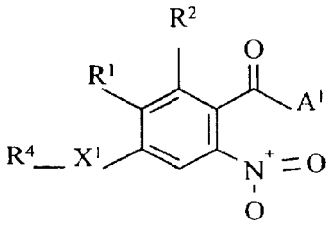


(XIV)

(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:

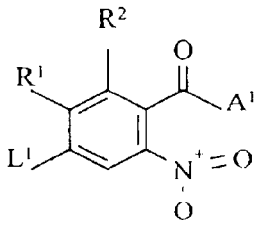


(XV)

(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.

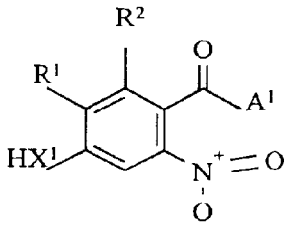
Compounds of the formula XV and salts thereof which constitute a further feature of the present invention, may for example be prepared by the reaction of a compound of the formula XVI:



(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.

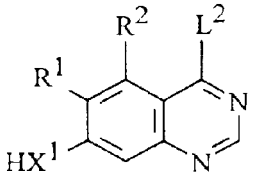
Compounds of formula XV and salts thereof, may for example also be prepared by the reaction of a compound of the formula XVII:



(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:

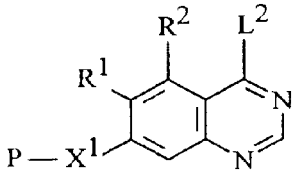


(XVIII)

(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:

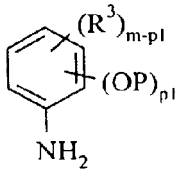


(XIX)

(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.

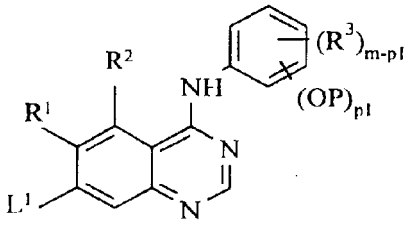
(ii) The compounds of formula V and salts thereof, constitute a further feature of the present invention, and may for example be prepared by the reaction of a compound of formula III as hereinbefore defined with a compound of the formula XX:



(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.

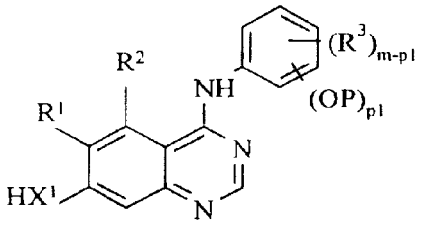
The compounds of formula V and salts thereof may also be prepared by reacting a compound of formula XXI:



(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.

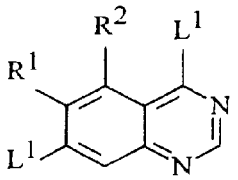
The compounds of formula V and salts thereof may also be prepared by reacting a compound of formula XXII:



(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.

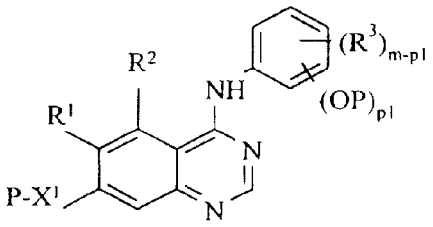
The compounds of formula XXI and salts thereof may for example be prepared by reaction of a compound of formula XXIII:



(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.

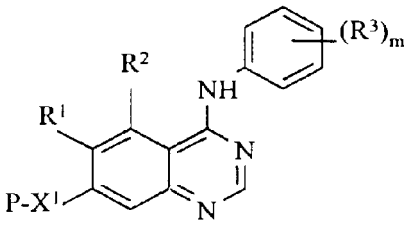
Compounds of the formula XXII and salts thereof may be made by reacting compounds of the formulae XIX and XX as hereinbefore defined, under conditions described in (a) hereinbefore, to give a compound of formula XXIV:



(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.

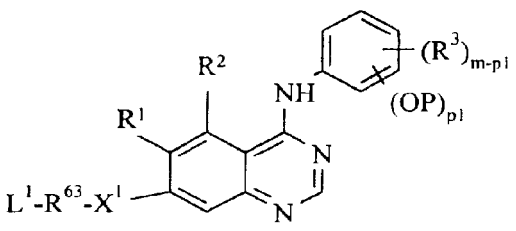
(v) Compounds of the formula X as defined hereinbefore and salts thereof may for example be made by the reaction of a compound of formula VI as defined hereinbefore with a compound of the formula XXVI:

$$L^1\text{-}R^{63}\text{-}L^1$$

(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
$C_{23}H_{27}N_4O_3ClF\ 1H_2O\ 2HCl$	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.
^{1}H 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
$C_{20}H_{21}N_3O_4ClFS\ 0.5H_2O$	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 **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 - ES1: 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)

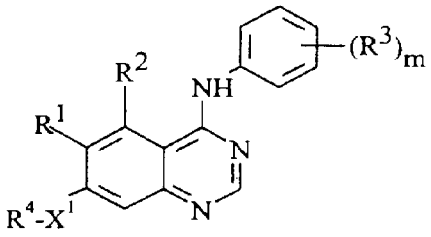
Compound X	0.1% w/v
Sodium phosphate BP	2.26% w/v
Citric acid	0.38% w/v
Polyethylene glycol 400	3.5% w/v
Water for injection to	100%

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

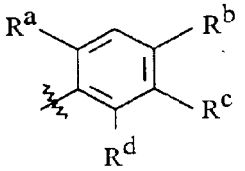
13) C_{1-5}alkylR^{44} (wherein R^{44} is morpholino which bears at least one and optionally two substituents selected from oxo, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl); with the further proviso that 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 X^1 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-;

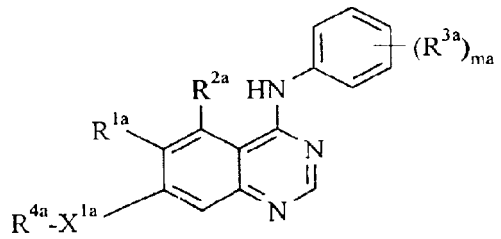
6) 2-(3,3-dimethylureido)ethyl, 3-(3,3-dimethylureido)propyl, 2-(3-methylureido)ethyl, 3-(3-methylureido)propyl, 2-ureidoethyl, 3-ureidopropyl, 2-(N,N-dimethylcarbamoyloxy)ethyl, 3-(N,N-dimethylcarbamoyloxy)propyl, 2-(N-methylcarbamoyloxy)ethyl, 3-(N-methylcarbamoyloxy)propyl, 2-(carbamoyloxy)ethyl, 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.

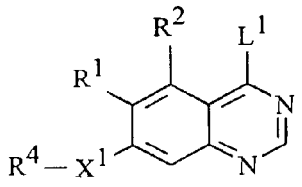
11. A quinazoline derivative as claimed in claim 1 selected from:-
(E)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-(pyrrolidin-1-yl)but-2-en-1-yloxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(methylsulphonyl)propoxy)quinazoline,
(S)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-yl)methoxyquinazoline 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.

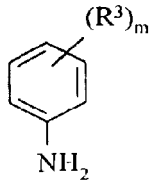
14. A process for the preparation of a quinazoline derivative of formula I or salt thereof (as defined in claim 1) which comprises:-

(a) the reaction of a compound of the formula III:



(III)

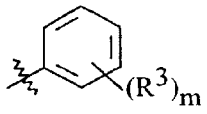
(wherein R^1, R^2, X^1 and R^4 are as defined in claim 1 and L^1 is a displaceable moiety), with a compound of the formula IV:



(IV)

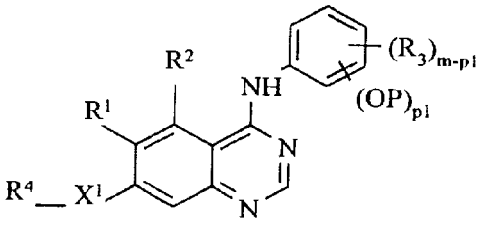
(wherein R^3 and m are as defined in claim 1) whereby to obtain compounds of the formula I and salts thereof;

(b) for the preparation of compounds of formula I and salts thereof in which the group of formula IIa:



(IIa)

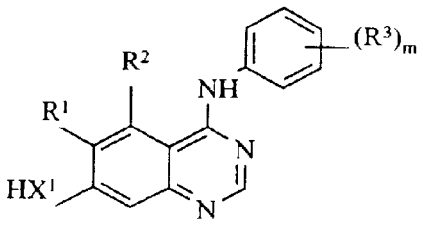
(wherein R^3 and m are as defined in claim 1) represents a phenyl group carrying one or more hydroxy groups, the deprotection of a compound of formula V:



(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:



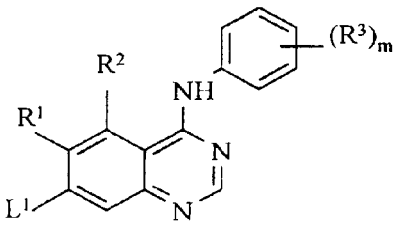
(VI)

(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);

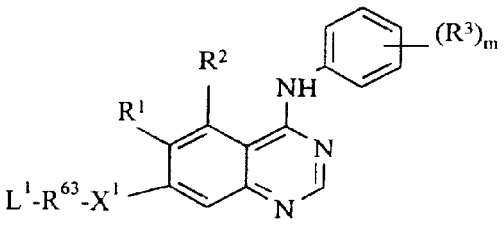
(e) for the preparation of compounds of formula I and salts thereof wherein R^4 is $C_{1-5}alkylR^{53}$, [wherein R^{53} is selected from one of the following three groups:

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
EP 566226 A	20-10-93	AT 130000 T	15-11-95
		AU 661533 B	27-07-95
		AU 3101093 A	22-07-93
		CA 2086968 A	21-07-93
		CZ 282038 B	16-04-97
		DE 69300754 D	14-12-95
		DE 69300754 T	28-03-96
		ES 2078798 T	16-12-95
		HK 36497 A	04-04-97
		HU 9500185 A	28-07-95
		MX 9300277 A	30-06-94
		NO 301541 B	10-11-97
		NZ 245662 A	26-09-95
		SK 1693 A	09-09-93
		US 5457105 A	10-10-95
		US 5616582 A	01-04-97
		ZA 9300015 A	20-07-93
		JP 6073025 A	15-03-94
WO 9722596 A	26-06-97	AU 1106197 A	14-07-97
WO 9732856 A	12-09-97	AU 1866497 A	22-09-97

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 December 2001 (06.12.2001)

PCT

(10) International Publication Number
WO 01/92224 A1

(51) International Patent Classification[7]: C07D 209/42, A61K 31/40, A61P 43/00

(21) Interuational Application Number: PCT/GB01/02335

(22) International Filing Date: 25 May 2001 (25.05.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
00401551.7 31 May 2000 (31.05.2000) EP
00402956.7 25 October 2000 (25.10.2000) EP

(71) Applicant *(for all designated States except MG, US)*: **ASTRAZENECA AB** [SE/SE]; S-151 85 Södertalje (SE).

(71) Applicant *(for MG only)*: **ASTRAZENECA UK LIMITED** [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **ARNOULD, Jean-Claude** [FR/FR]; Z.I. la Pompelle, Boîte postale 1050, F-51689 Reims Cedex 2 (FR). **BIRD, Thomas, Geoffrey** [GB/FR]; 7, rue Pierre Boileau, F-51420 Witry-les-Reims (FR). **BOYLE, Francis, Thomas** [GB/GB]; Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). **BLAKEY, David, Charles** [GB/GB]; Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).

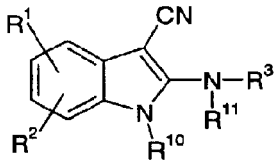
(74) Agent: **BRYANT, Tracey**; AstraZeneca, Global Intellectual Property, P.O. Box 272, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4GR (GB).

(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.

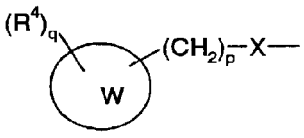
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian

[Continued on next page]

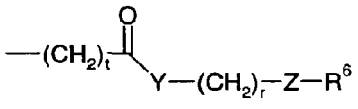
(54) Title: INDOLE DERIVATIVES WITH VASCULAR DAMAGING ACTIVITY



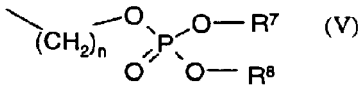
(I)



(II)



(IV)



(V)

(57) Abstract: The invention provides a compound of Formula (I) wherein: R^1 and R^2 are independently selected from hydrogen, halogen, -CN, a hydrocarbyl group or a group of Formula (II); wherein W is aryl or a heterocyclic group, R^4 is independently selected from hydrogen, halogen, -OH, amino, alkanoylamino, $-OPO_3H_2$, or a hydrocarbyl group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified or two R^4 groups together form an optionally substituted cyclic or heterocyclic group; X is selected from $M(y) S+-, +-O-+, -+S(O)+-, -+S(O_2)+- and -+NH--; p is an integer from 0 to 4; and q is an integer from 1 to 4; R^3 and R^{10} are independently selected from hydrogen, lower alkyl or a group of Formula (IV): wherein Y is selected from + NH+-, - $M(Y)O$M(Y)- or a bond; Z is selected from + NH+-, -+Ou-, -+C(O)+- or a bond; r is an integer from 0 to 4; t is an integer from 0 to 1; R^6 is hydrogen, a hydrocarbyl group or a group of Formula (V): wherein n is an integer of from 1 to 6, and; R^7 and R^8 are independently selected from hydrogen or a hydrocarbyl group; and R^{11} is hydrogen or lower alkyl; or a salt or solvate thereof; provided that: I) when R^1 is an unsubstituted phenylthio group (Ph-S-), R^2 is H, R^{10} is H and R^{11} is H then R^3 is neither H nor- $C(O)-O-CH_2CH_3$; and ii) R^1, R^2 and R^3 are not all hydrogen. Such compounds are predicted to cause the selective destruction of tumour vasculature. They may therefore be used to inhibit and/or reverse, and/or alleviate symptoms of angiogenesis and/or any disease state associated with angiogenesis.

WO 01/92224 A1

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).

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.

Published:

— *with international search report*

INDOLE DERIVATIVES WITH VASCULAR DAMAGING ACTIVITY

This invention relates to vascular damaging agents and their uses. In particular it relates to certain novel compounds which may be of use as vascular damaging agents, to methods for preparing the compounds, to their use as medicaments (including in methods for the treatment of angiogenesis or disease states associated with angiogenesis) and to pharmaceutical compositions containing them. The invention also relates to the use of such compounds, and of certain analogous, known compounds in the manufacture of medicaments for the production of anti-angiogenic and/or anti-vascular effects.

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 therefore expected to have a beneficial therapeutic effect.

The present invention is based on the discovery of compounds that show surprising activity in *in vitro* cell detachment assays, in particular by inducing changes in the shape of proliferating endothelial cells and by causing rapid cell detachment, which activity is a likely indicator of vascular damaging activity. The compounds are expected to cause damage to newly formed vasculature, for example the vasculature of tumours, without affecting the normal, established vascular endothelium of the host species. This vascular damage would effectively reverse the process of angiogenesis, as compared to known anti-angiogenic agents

which can prevent vasculature formation but which tend to be less effective once the vasculature has formed.

Such vascular-damaging activity would clearly be 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.

A compound of Formula (I) in which R^1 is Ph-S- and both R^2 and R^3 are H (2-amino-3-cyano-5-(phenylthio)indole, also known as amphethinile) is disclosed in US-4,533,672 as an anti-cancer agent, as is a compound of Formula (I) in which R^1 is Ph-S-, R^2 is H and R^3 is –C(O)-O-CH_2CH_3 (ethyl 3-cyano-5-(phenylthio)indol-2-ylcarbamate). However, anti-cancer activity, in this case an apparent inhibition of tumour growth in mice, is not in itself an indicator of vascular damaging activity, for which the compounds of the present invention are of interest. Amphethinile has also been disclosed as a tubulin-binding agent (McGowan and Fox , *British Journal of Cancer* **59**, 865-868, 1989).

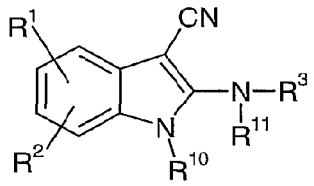
Several anticancer agents have been described which bind to tubulin, these include Taxol™ and Taxotere™ . However, these agents do not act as selective vascular targeting agents. For example, Taxol does not cause cell detachment in the HUVEC detachment assay, described herein. Other tubulin-binding agents such as colchicine and vinca alkaloids such as vincristine only elicit vascular damaging activity at doses approaching the maximum tolerated dose (MTD) (see Dark et al 1997 *Cancer Research* **57**, 1829-1834). More recently Combretastatin A4 has been described which is a tubulin binding agent which acts as a selective vascular targeting agent, producing anti-vascular effects at doses greater than 10-times less than the MTD (Dark et al 1997 *Cancer Research* **57**, 1829-1834). Thus, tubulin-binding activity is not in itself an indicator of compounds that have selective vascular damaging activity.

Tubulin binding agents that do cause selective destruction of tumour vasculature such as combretastatin and ZD6126 (N-acetylcolchinol phosphate) have been reported *in vitro* at non-cytotoxic concentrations to cause selective effects on the cell shape of proliferating endothelial cells leading to endothelial cell detachment[Dark et al, *Cancer Research* **57**, 1829-34, 1997; Galbraith et al, **21(1A)**, 93-102, 2001; Blakey D C et al, *Proceedings of the American Association for Cancer Research*, **41**, 329, 2000 abstract 2086, Davis P D et al,

Proceedings of the American Association for Cancer Research, **41**, 329, 2000 abstract 2085; Chaplin D J & Dougherty G J, *Br J Cancer*, **80**, Suppl 1, 57-64, 1999] .

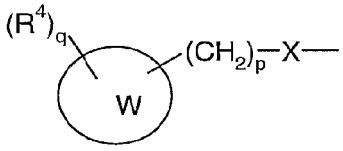
The compounds of the present in invention are tubulin binding agents that have similar activity to combretastatin and ZD6126 on proliferating endothelial cells. It can therefore be expected that the compounds of the present invention will have damaging effects on newly-formed vasculature, for example the vasculature of tumours. It can reasonably be predicted, for example, that they will be capable of causing selective destruction of tumour vasculature, both *in vitro* and *in vivo*. Destruction of tumour vasculature in turn leads to a reduction in tumour blood flow and to tumour cell death due to starvation of oxygen and nutrients, ie, to anti-tumour activity [Davis P D et al; Chaplin D J & Dougherty G J; Blakey D C et al, all *supra*].

Thus, according to the first aspect of the invention there is provided a compound of Formula (I).



Formula (I)

wherein R^1 and R^2 are independently selected from hydrogen, halogen, -CN, a hydrocarbyl group or a group of Formula (II);



Formula (II)

wherein W is aryl or a heterocyclic group,

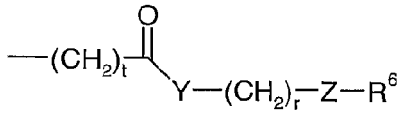
R^4 is independently selected from hydrogen, halogen, -OH, amino, alkanoylamino, —OPO_3H_2, or a hydrocarbyl group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified or two R^4 groups together form an optionally substituted cyclic or heterocyclic group;

X is selected from —S—, —O—, —S(O)—, —$S(O_2)$— and —NH—;

p is an integer from 0 to 4; and

q is an integer from 1 to 4;

$R^3$and R^{10} are independently selected from hydrogen, lower alkyl or a group of Formula (IV):



Formula (IV)

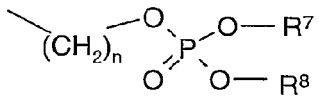
wherein Y is selected from —NH—, —O— or a bond;

Z is selected from —NH—, —O—, —C(O)— or a bond;

r is an integer from 0 to 4;

t is an integer from 0 to 1;

R^6 is hydrogen, a hydrocarbyl group or a group of Formula (V):



Formula (V)

wherein n is an integer of from 1 to 6, and;

R^7 and R^8 are independently selected from hydrogen or a hydrocarbyl group; and

R^{11} is hydrogen or lower alkyl;

or a salt or solvate thereof;

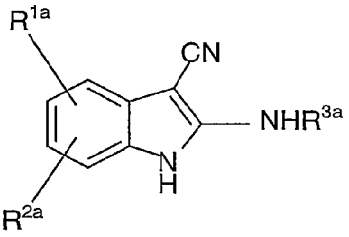
provided that:

i) when R^1 is an unsubstituted phenylthio group (Ph-S-), R^2 is H, R^{10} is H and R^{11} is H then R^3 is neither H nor –C(O)-O-CH_2CH_3; and

ii) R^1, R^2 and R^3 are not all hydrogen.

Whilst pharmaceutically acceptable salts of compounds of the invention are preferred, other non-pharmaceutically acceptable salts of the invention may be useful in the preparation of pharmaceutically-acceptable salts of compounds of the invention.

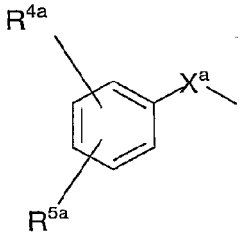
According to a further feature of the first aspect of the present invention there is provided a compound of the general Formula (Ia):



Formula (Ia)

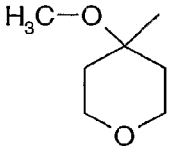
or a derivative thereof,

wherein R^{1a} and R^{2a} are independently selected from hydrogen, halogen (such as fluorine, chlorine or bromine), -OH, -CN, a hydrocarbyl group (such as a lower alkyl group) or a group of Formula (IIa):



Formula (IIa)

wherein R^{4a} and R^{5a} are independently selected from hydrogen, halogen (such as fluorine, chlorine or bromine), -OH or a hydrocarbyl group (such as a C_1-C_{10} alkyl group, an alkoxy group such as –O-alkyl or a group of Formula (IIIa);

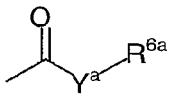


Formula (IIIa)

or a carbonyl group of the Formula –C(O)-R^{9a} where R^{9a} is hydrogen, -OH, a hydrocarbyl group such as a lower alkyl group or an alkoxy group), or R^{4a} and R^{5a} together form a cyclic or heterocyclic group, for instance to make R^{1a} into –X-naphthyl;

X^a is selected from —S—, —O—, — S(O)—, —$S(O_2)$— and —NH—;

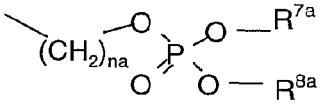
R^{3a} is either hydrogen or a group of Formula (IVa):



Formula (IVa)

wherein Y^a is selected from —NH—, —O— or a bond; and

R^{6a} is hydrogen or a hydrocarbyl group (such as an alkyl, aryl, alkoxy or phenoxy group, preferably –Ph or a lower alkyl group) or a group of Formula (Va):



Formula (Va)

wherein na is an integer of from 1 to 6, preferably 2 or 3, and R^{7a} and R^{8a} are independently selected from hydrogen or a hydrocarbyl group (in particular an alkaryl group such as $-(CH_2)_{ma}$-Ph where ma is an integer of from 1 to 6, preferably 1);

provided that:

i) when R^1 is an unsubstituted phenylthio group (Ph-S-) and R^{2a} is H, R^{10a} is H and R^{11a} is H then R^{3a} is neither H nor $-C(O)-O-CH_2CH_3$; and

ii) R^{1a}, R^{2a} and R^{3a} are not all hydrogen.

In this specification , the term **"alkyl"** refers to straight or branched chain alkyl groups, suitably containing up to 20 and preferably up to 10 or up to 6 carbon atoms, which may be substituted with one or more functional groups. The terms "**C_{1-4}alkyl**" and "**C_{1-6}alkyl**" refer to both straight-chain and branched alkyl groups containing between 1 and 4 carbon atoms and between 1 and 6 carbon atoms respectively. The terms **"alkenyl"** and **"alkynyl"** refer to optionally substituted unsaturated straight or branched chains which include for example from 2 to 20 carbon atoms, in particular from 2 to 10 or from 2 to 6 carbon atoms; these chains may include one or more double or triple bonds respectively. The term **"alkoxy"** refers to alkyl, alkenyl or alkynyl groups containing the group —O—, for example methoxy, ethoxy and propoxy. The term **"alkanoyl"** refers to alkyl, alkenyl or alkynyl groups containing the group —C(O)—, for example acetyl, propanoyl and butanoyl. The term **"alkanoylamino"** refers to alkyl, alkenyl or alkynyl groups containing the group —C(O)— followed by —N(H)—, for example acetylamino, propanoylamino and butanoylamino. The term **"carbamoyl"** refers to the group $-C(O)-NH_2$. The term **"carbamoylalkyl"** refers to an alkyl chain terminating in a

carbamoyl group, for example carbamoylmethyl which has the formula -CH_2-C(O)-NH_2. The term **"halogen"** refers to chloro, bromo, fluoro or iodo.

The term **"cyclic group"** refers to an optionally substituted carbon ring structure, which may contain one or more double or triple bonds, the term encompassing aryl and aralkyl groups.

The term **"aryl"** refers to wholly or partially aromatic groups such as phenyl (for which the abbreviation "Ph" is used) or naphthyl, which may be substituted with one or more functional groups. The term **"alkaryl"** means an alkyl group having at least one (optionally substituted) aryl substituent, and the term **"aralkyl"** an aryl group having at least one (optionally substituted) alkyl substituent.

The term **"lower"**, applied to the terms alkyl, alkenyl, alkynyl, alkoxy, alkanoyl, alkanoylamino, alkaryl and aralkyl, means a group containing from 1 to 6 carbon atoms, preferably from 1 to 4.

The term **"hydrocarbyl"** refers to any structure comprising carbon and hydrogen atoms. For example, these may be alkyl, alkenyl, alkynyl, alkoxy, aryl such as phenyl or naphthyl, alkaryl, aralkyl, cycloalkyl, cycloalkenyl or cycloalkynyl. Suitably they will contain up to 20 and preferably up to 10 carbon atoms. They may be substituted with other functional groups, or an alkyl chain optionally substituted with other functional groups and they may include heteroatoms such as oxygen, nitrogen, sulphur and/or phosphorous within a carbon chain or ring.

The term **"heterocyclic group"** includes optionally substituted aromatic, partially aromatic or non-aromatic rings or mixtures thereof, for example containing from 3 to 20, suitably from 5 to 10 ring atoms, at least one of which is a heteroatom such as oxygen, sulphur or nitrogen. Examples of such groups include morpholinyl, morpholino, piperazinyl, piperidino, furyl, thienyl, pyrrolyl, pyrrolidinyl, imidazolyl, triazolyl, thiazolyl, tetrazolyl, oxazolyl, isoxazolyl, pyrazolyl, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, quinolinyl, iosquinolinyl, quinoxalinyl, benzthiazolyl, benzoxazolyl, benzothienyl or benzofuryl.

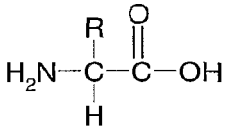
The term **"functional group"** refers to reactive groups such as halogen, cyano, nitro, oxo, $C(O)_yR^a$, OR^a, $S(O)_zR^a$, NR^bR^c, $OC(O)NR^bR^c$, $C(O)NR^bR^c$, $-NR^7C(O)_yR^6$, $-NR^aCONR^bR^c$, $-C{=}NOR^a$, $-N{=}CR^bR^c$, $S(O)_zNR^bR^c$, $C(S)_yR^a$, $C(S)OR^a$, $C(S)NR^bR^c$ or $-NR^bS(O)_zR^a$ where R^a, R^b and R^c are independently selected from hydrogen or optionally

substituted hydrocarbyl, or R^b and R^c together form an optionally substituted ring which optionally contains further heteroatoms such as $S(O)_z$, oxygen and nitrogen. In this paragraph, y is an integer of 1 or 2 and z is 0 or an integer from 1 to 3.

The term **"heteroatom"** as used herein refers to non-carbon atoms such as oxygen, nitrogen, sulphur or phosphorous atoms. Where nitrogen atoms are present, they will generally be present as part of an amino or amido residue so that they will be substituted for example by hydrogen, alkyl or carbonyl.

The term **"derivative"** as used herein refers to salts, solvates and pro-drugs of compounds of the invention.

An **"amino acid residue"** is defined as that derived from the coupling of an L-amino acid with an amino group via an amide bond. This bond can either be formed via a carboxylate group on the amino acid backbone or via a side chain carboxylate group, preferably via a carboxylate group on the amino acid backbone. Amino acid residues include those derived from natural and non-natural amino acids, preferably natural amino acids and include α-amino acid residues β-amino acid residues and γ-amino acid residues. For the avoidance of doubt amino acids include those with the generic structure:



where R is the amino acid side chain. The definition of amino acid also includes amino acid analogues which have additional methylene groups within the amino acid backbone, for example β-alanine and amino acids which are not naturally occurring such as cyclohexylalanine. Preferred amino acid residues are those derived from naturally occurring amino acids. For avoidance of doubt the term **'naturally occurring amino acids'** is defined as those amino acids which occur in the proteins of animals.

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, and glycine.

"Esterifying groups at R_4" are esterifying groups which increase the solubility of the molecule in water at a pH of approximately pH=7. Such groups include groups with ionisable groups, such as acidic functions or basic functions and groups containing a hydrophilic function. Basic functions include: amino, morpholino, piperidino, piperazinyl, pyrrolidino, amino acids and imidazolino. Acidic functions include: carboxy, sulphonic acid, phosphate, sulphate and acid mimetics such as tetrazolyl. Hydrophilic groups include hydroxyl.

Suitable R_4 groups wherein hydroxy is esterfied include: C_{1-6}alkanoyloxy, arylcarbonyloxy, heterocyclylcarbonyloxy, heteroarylcarbonyloxy wherein the R_1 group is optionally substituted with between 1 and 3 groups selected from C_{1-4}alkyl, C_{1-4}alkanoyl, C_{1-4}alkanoylC_{1-4}alkyl, C_{1-4}alkanoylheterocyclyl, hydroxy, hydroxyC_{1-4}alkyl, carboxy, carboxyphenyl, phosphono, phosphonoC_{1-4}alkyl, amino, aminoC_{1-4}alkyl, N-C_{1-4}alkylamino, N,N-diC_{1-4}alkylamino, carbamoyl, carbamoylC_{1-4}alkyl, heterocyclyl, heterocyclylC_{1-4}alkyl, heterocyclylcarbonyl, heterocyclC_{1-4}alkanoylamino, carbamoylheterocyclyl, [wherein optional substituents comprising heterocyclyl are optionally further substituted by C_{1-4}alkyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkanoyl and formyl, wherein the carbamoyl and amino optional substituents are optionally further N-substituted by C_{1-4}alkyl, di-C_{1-4}alkyl, hydroxyC_{1-4}alkyl, di-(hydroxyC_{1-4}alkyl), carboxyC_{1-4}alkyl, and wherein the amino group is optionally substituted by an amino acid residue] with the proviso that when R_1 is C_{1-6}alkanoyloxy or arylcarbonyloxy R_1 is not unsubstituted and R_1 is not substituted by C_{1-4}alkyl.

More preferred R_4 groups wherein hydroxy is esterfied include: carboxypentanoyloxy, 4-carboxyphenylpropanoyloxy; 4-(N-methylpiperazin-1-ylethyl)phenylcarbonyloxy, 4-(piperazin-1-ylethyl)phenylcarbonyloxy, 4-[N-di-(hydroxyethyl)aminomethyl]phenylcarbonyloxy, 3-(N-acetylpiperazin-1-ylethyl)phenylcarbonyloxy, 3-[N-di-(hydroxyethyl)aminomethyl]phenylcarbonyloxy, 4-(N-methylpiperazin-1-ylpropanoylamino)phenylcarbonyloxy, N-methylpiperazin-1-ylcarbonylpropanoyloxy, N-di-(hydroxyethyl)aminocarbonylpropanoyloxy, piperazin-1-ylcarbonylpropanoyloxy, (N-acetylpiperazin-1-yl)carbonylpropanoyloxy, (N-di-(hydroxyethyl)aminocarbonylpropanoyloxy, and 4-(piperazin-1-ylmethyl)phenylcarbonyloxy.

Further preferred R_1 groups wherein hydroxy is esterfied include:
4-(N-methylpiperazin-1-ylpropanoylamino)phenylcarbonyloxy,
N-methylpiperazin-1-ylcarbonylpropanoyloxy and
N-di-(hydroxyethyl)aminocarbonylpropanoyloxy.

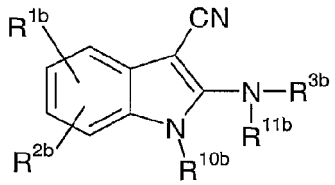
A suitable pharmaceutically-acceptable salt of a compound of the invention is, for example, an acid-addition salt of a compound of the invention which is sufficiently basic, for example, an acid-addition salt with, for example, an inorganic or organic acid, for example hydrochloric, hydrobromic, sulphuric, phosphoric, trifluoroacetic, citric or maleic acid. In addition a suitable pharmaceutically-acceptable salt of a carbazole derivative of the invention which is sufficiently acidic is an alkali metal salt, for example a sodium or potassium salt, an alkaline earth metal salt, for example a calcium or magnesium salt, an ammonium salt or a salt with an organic base which affords a physiologically acceptable cation, for example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

It is to be understood that, insofar as certain of the compounds in the different features of the invention 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 the property of inhibiting and/or reversing and/or alleviating the symptoms of angiogenesis and/or any disease states associated with angiogenesis. 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, activity of these compounds may be evaluated using the standard laboratory techniques referred to hereinafter.

The invention also relates to any and all tautomeric forms of the compounds of the different aspects of the invention that possess the property of inhibiting and/or reversing and/or alleviating the symptoms of angiogenesis and/or any disease states associated with angiogenesis.

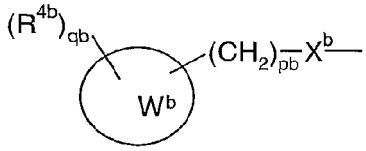
It will also be understood that certain compounds of the present invention may exist in solvated, for example hydrated, as well as unsolvated forms. It is to be understood that the present invention encompasses all such solvated forms which possess the property of the property of inhibiting and/or reversing and/or alleviating the symptoms of angiogenesis and/or any disease states associated with angiogenesis.

According to the second aspect of the invention there is provided the use of a compound of Formula (Ib) as a medicament:



Formula (Ib)

wherein R^{1b} and R^{2b} are independently selected from hydrogen, halogen, -CN, a hydrocarbyl group or a group of Formula (IIb);



Formula (IIb)

wherein W^b is aryl or a heterocyclic group,

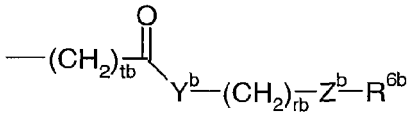
R^{4b} is independently selected from hydrogen, halogen, -OH, amino, alkanoylamino, —OPO_3H_2, or a hydrocarbyl group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified or two R^{4b} groups together form an optionally substituted cyclic or heterocyclic group;

X^b is selected from —S—, —O—, —S(O)—, —$S(O_2)$— and —NH—;

pb is an integer from 0 to 4; and

qb is an integer from 1 to 4;

$R^{3b}$and R^{10b} are independently selected from hydrogen, lower alkyl or a group of Formula (IVb):



Formula (IVb)

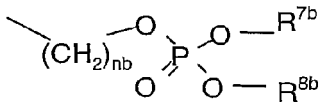
wherein Y^b is selected from —NH—, —O— or a bond;

Z^b is selected from —NH—, —O—, —C(O)— or a bond;

rb is an integer from 0 to 4;

tb is an integer from 0 to 1;

R^{6b} is hydrogen, a hydrocarbyl group or a group of Formula (Vb):



Formula (Vb)

wherein nb is an integer of from 1 to 6, and;

R^{7b} and R^{8b} are independently selected from hydrogen or a hydrocarbyl group; and

R^{11b} is hydrogen or lower alkyl;

or a salt or solvate thereof;

provided that:

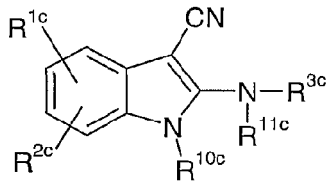
i) when R^{1b} is an unsubstituted phenylthio group (Ph-S-), R^{2b} is H, R^{10b} is H and R^{11b} is H then R^{3b} is neither H nor $-C(O)-O-CH_2CH_3$.

According to a further feature of the second aspect of the invention there is provided a compound of Formula (Ib) for use in any surgical, therapeutic or diagnostic method practised on a human or animal patient.

According to a further feature of the second aspect of the invention there is provided a compound of Formula (Ib) for use in the preparation of a medicament for use in any surgical, therapeutic or diagnostic method practiced on a human or animal patient.

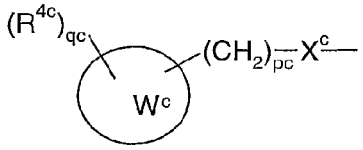
For each feature of the second aspect of the invention, the surgical, therapeutic or diagnostic method preferably involves the treatment of a human or animal patient to inhibit and/or reverse, and/or to alleviate a symptom of, angiogenesis and/or any disease state associated with angiogenesis. In other words, compounds and derivatives according to the second aspect of the invention are preferably used as vascular damaging and/or anti-angiogenic agents.

Thus, according to the third aspect of the present invention there is provided the use of a compound of Formula (Ic) or a pharmaceutically-acceptable salt or solvate thereof, for the manufacture of a medicament to inhibit and/or reverse and/or alleviate symptoms of angiogenesis and/or any disease state associated with angiogenesis:



Formula (Ic)

wherein R^{1c} and R^{2c} are independently selected from hydrogen, halogen, -CN, a hydrocarbyl group or a group of Formula (IIc);



Formula (IIc)

wherein W^c is aryl or a heterocyclic group,

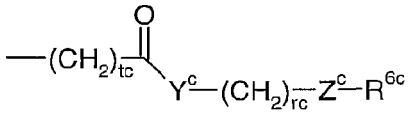
R^{4c} is independently selected from hydrogen, halogen, -OH, amino, alkanoylamino, —OPO_3H_2, or a hydrocarbyl group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified or two R^{4c} groups together form an optionally substituted cyclic or heterocyclic group;

X^c is selected from —S—, —O—, —S(O)—, —$S(O_2)$— and —NH—;

pc is an integer from 0 to 4; and

qc is an integer from 1 to 4;

R^{3c} and R^{10c} are independently selected from hydrogen, lower alkyl or a group of Formula (IVc):



Formula (IVc)

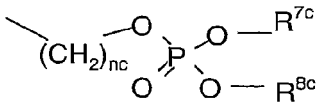
wherein Y^c is selected from —NH—, —O— or a bond;

Z^c is selected from —NH—, —O—, —C(O)— or a bond;

rc is an integer from 0 to 4;

tc is an integer from 0 to 1;

R^{6c} is hydrogen, a hydrocarbyl group or a group of Formula (Vc):



Formula (Vc)

wherein nc is an integer of from 1 to 6, and;

R^{7c} and R^{8c} are independently selected from hydrogen or a hydrocarbyl group; and

R^{11c} is hydrogen or lower alkyl;

According to a further feature of the third aspect of the invention there is provided a method of treatment, in an animal, to inhibit and/or reverse and/or alleviate symptoms of angiogenesis and/or any disease state associated with angiogenesis, comprising administering to said animal a therapeutically effective amount of a compound of Formula (Ic), or a pharmaceutically-acceptable salt or solvate thereof.

An animal is preferably a warm-blooded animal, more preferably a mammal, most preferably a human.

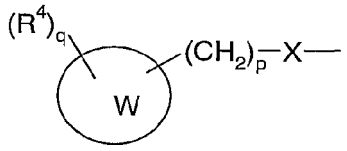
According to a further feature of the third aspect of the invention provides a method of treatment of a patient to inhibit and/or reverse, and/or to alleviate a symptom of, angiogenesis and/or any disease state associated with angiogenesis, the method involving administering to the patient a therapeutically (including prophylactically) effective amount of a compound of Formula (Ia) or derivative thereof , or of a compound of Formula (Ia) (or derivative thereof) in which R^1 is an unsubstituted phenylthio group (Ph-S-), R^2 is H and R^3 is either H or –C(O)-O-CH_2CH_3.

A patient is preferably a warm-blooded animal, more preferably a mammal, most preferably a human.

The term **"therapy"** and **"therapeutically effective"** as used here includes prophylaxis.

A series of preferences for groups of values and for groups of compounds of the invention follow, these relate to compounds of Formula (I) and also relate to the respective groups in compounds of Formula (Ia), Formula (Ib) and Formula (Ic). For example, preferences for R^1 also relate to R^{1a}, R^{1b}, and R^{1c}, and preferences for R^4 also relate to R^{4a}, R^{5a}, R^{4b} and R^{4c}.

A preferred group of values for R^1 in each aspect of the invention is hydrogen, halogen, -CN, lower alkyl or a group of Formula (II);



Formula (II)

wherein W, R^4, X, p and q are as defined above. More preferably R^1 is hydrogen, halogen, lower alkyl or a group of Formula (II) wherein W, R^4, X, p and q are as defined above. More preferably R^1 is fluoro, chloro, methyl, or a group of Formula (II) wherein W, R^4, X, p and q are as defined above. Most preferably R^1 is a group of Formula (II) wherein W, R^4, X, p and q are as defined above.

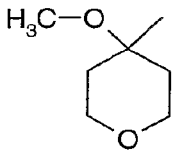
A preferred group of values for R^2 in each aspect of the invention is hydrogen, halogen, preferably fluoro or chloro, a lower alkyl group, preferably methyl, ethyl, propyl, isopropyl, butyl or *t*-butyl. More preferably R^2 is hydrogen, fluoro, chloro or methyl. Most preferably R^2 is hydrogen.

A preferred group of values for W in each aspect of the invention is aryl or a heterocyclic group. More preferably W is phenyl, naphthyl or pyridyl. Most preferably W is phenyl.

A preferred group of values for X in each feature of the invention is —O—, —NH—, —S— or —$S(O_2)$—. More preferably X is —O— or —S—. Most prefcrably X is —O—.

A preferred group of values for p in each aspect of the invention is when p is 0 or 1. Most preferably p is 0.

A preferred group of values for R^4 , when q is an integer between 1 and 4, in each aspect of the invention is hydrogen, halogen, -OH, amino, —OPO_3H_2,or C_1-C_{10} alkyl group, a lower alkoxy group, a lower alkanoyl group, a lower alkanoylamino group, a group of Formula (III),

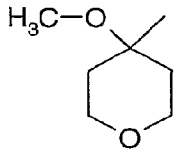


Formula (III)

or a carbonyl group of the Formula –C(O)-R^9 where R^9 is hydrogen, -OH, a hydrocarbyl group (such as a lower alkyl group or an alkoxy group) , wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified. More preferably R^4 is hydrogen, fluoro, -OH, amino, —OPO_3H_2, a lower alkanoylamino group, or a

lower alkoxy group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified. Further preferably R^4 is hydrogen, -OH, amino, —OPO_3H_2, acetylamino, methoxy, serylamino, glutamylamino preferably α-glutamylamino, glycylamino, alanylamino and 4-methylpiperazin-1-ylcarbonylpropanoyloxy. Most preferably R^4 is amino, -OH, acetylamino or methoxy.

A preferred group of values for R^4, when q is 1, in each aspect of the invention is hydrogen, halogen, -OH, amino, —OPO_3H_2, or C_1-C_{10} alkyl group, a lower alkoxy group, a lower alkanoyl group, a lower alkanoylamino group, a group of Formula (III),



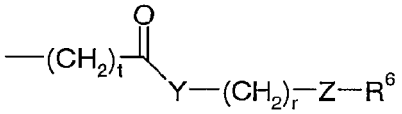
Formula (III)

or a carbonyl group of the Formula –C(O)-R^9 where R^9 is hydrogen, -OH, a hydrocarbyl group such as a lower alkyl group or an alkoxy group) , wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified. More preferably R^4 is hydrogen, fluoro, -OH, amino, —OPO_3H_2, a lower alkanoylamino group, or a lower alkoxy group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified. Further preferably R^4 is hydrogen, -OH, amino, —OPO_3H_2, acetylamino, methoxy, serylamino, glutamylamino preferably α-glutamylamino, glycylamino, alanylamino and 4-methylpiperazin-1-ylcarbonylpropanoyloxy. Most preferably serylamino, α-glutamylamino, glycylamino, —OPO_3H_2, amino, -OH, acetylamino or methoxy.

A preferred group of values for R^4, when q is 2 or 3, in each aspect of the invention is when R^4 is independently selected from hydrogen, halogen, -OH, —OPO_3H_2,or a C_{1-10} alkyl group, or a lower alkoxy group, a lower alkanoyl group, or a carbonyl group of the Formula -C(O)-R^9 where R^9 is hydrogen, lower alkyl or alkoxy. More preferably R^4 is independently selected from hydrogen, fluoro, -OH, —OPO_3H_2, or a lower alkoxy group. Further preferably R^4 is hydrogen, -OH, —OPO_3H_2 or methoxy. Most preferably R^4 is —OPO_3H_2, -OH or methoxy.

A preferred group of values for q is 2 or 3. More preferably q is 3.

A preferred group of values for R^3 in each aspect of the invention is when R^3 is hydrogen, lower alkyl or a group of Formula (IV):



Formula (IV)

wherein t, Y, r, Z and R^6 are as defined above. More preferably R^3 is hydrogen or a group of Formula (IV). Most preferably R^3 is hydrogen.

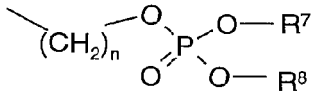
A preferred group of values for t in each aspect of the invention is 0 or 1. Preferably t is 0.

A preferred group of values for Y in each aspect of the invention is —O— or—NH—. More preferably Y is —O— or a bond. Most preferably —Y— is —O—.

A preferred group of values for r in each aspect of the invention is 2 or 3. Preferably r is 3.

A preferred group of values for Z in each aspect of the invention is —C(O)— or a bond. Preferably Z is a bond.

A preferred group of values for R^6 in each aspect of the invention is hydrogen, lower alkyl, a lower alkoxy group, an aryl group, a heterocyclic group or a group of Formula (V), wherein the aryl group and heterocyclic group are optionally substituted by lower alkyl or a lower alkanoyl group:



Formula (V).

More preferably R^6 is a non-aromatic heterocyclic group, an aryl group or a group of Formula (V), wherein the aryl group and non-aromatic heterocyclic group are optionally substituted by lower alkyl or a lower alkanoyl group. Further preferably R^6 is –Ph, morpholino, piperazinyl or a group of Formula (V), wherein the –Ph, morpholino and piperazinyl group are optionally substituted by methyl or acetyl and R^7 and R^8 are both hydrogen. Most preferably R^6 is –Ph, morpholino or 4-methyl-piperazin-1-yl.

A preferred group of values for n in each aspect of the invention is 2 or 3. Preferably n is 2.

A preferred group of values for R^7 and R^8 in each aspect of the invention is R^7 and R^8 are independently selected from hydrogen or an alkaryl group, preferably $-(CH_2)_m$-Ph where m is an integer of from 1 to 6, preferably m is 1. More preferably R^7 and R^8 are independently selected from hydrogen or benzyl (-CH_2-Ph). Preferably R^7 and R^8 are both hydrogen.

A preferred group of values for R^{10} in each aspect of the invention is hydrogen, lower alkyl or a group of Formula (IV), wherein t, Y, r, Z and R^6 are as defined above. More preferably R^{10} is hydrogen, lower alkyl or lower carbamoylalkyl group. Most preferably R^{10} is hydrogen, methyl or carbamoylmethyl.

A preferred group of values for a group of Formula (IV) is phenoxycarbonyl, phosphonooxyethylaminocarbonyl, 4-methylpiperazin-1-ylpropoxycarbonyl, carbamoylmethyl, 4-acetyl-piperazin-1-ylethoxycarbonyl, 4-methyl-piperazin-1-ylcarbonylpropanoyl and morpholinoethoxycarbonyl. Preferably phenoxycarbonyl, 4-methylpiperazin-1-ylpropoxycarbonyl or carbamoylmethyl.

Preferably only one of R^3 and R^{10} is a group of Formula (IV).

A preferred group of values for R^{11} in each aspect of the invention is hydrogen or lower alkyl. More preferably R^{11} is hydrogen or methyl. Most preferably R^{11} is hydrogen.

In the following preferred groups of compounds of each aspect of the invention values for R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, n, m, p, q, r, t, W, X, Y and Z are as hereinbefore defined, unless specifically defined with a preferred group of compounds.

A further preferred group of compounds of each aspect of the invention described herein, comprise compounds wherein:

R^1 is a group of Formula (II);

X is —O—;

p is 0 or 1; and

W is phenyl;

or a salt or solvate thereof.

A further preferred group of compounds of each aspect of the invention described herein, comprise compounds wherein:

R^1 is a group of Formula (II);

X is —NH—;

p is 0 or 1; and

W is phenyl;

or a salt or solvate thereof.

A further preferred group of compounds of each aspect of the invention described herein, comprise compounds wherein:

R^1 is a group of Formula (II);

X is —S—, —SO— or —$S(O_2)$—, preferably X is —S— or —$S(O_2)$—, most preferably X is —S—;

p is 0 or 1; and

W is phenyl;

or a salt or solvate thereof.

A further preferred group of compounds of each aspect of the invention described herein, comprise compounds wherein:

R^1 is a group of Formula (II);

X is —S—, —$S(O_2)$—, —O— or —NH—; preferably X is —O— or —S—; most preferably X is —O—;

p is 0 or 1; and

W is phenyl;

R^4 is hydrogen, -OH, amino, —OPO_3H_2, or a C_{1-4}alkoxy group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified;

q is an integer between 1 and 3;

or a salt or solvate thereof.

A further preferred group of compounds of each aspect of the invention described herein, comprise compounds wherein:

R^1 is a group of Formula (II);

X is —S—, —$S(O_2)$—, —O— or —NH—; preferably X is —O— or —S—, most preferably X is —O—;

p is 0 or 1;

W is phenyl;

R^4 is an amino group or —OPO_3H_2, wherein the amino group is substituted by an amino acid residue. Preferably R^4 is serylamino, glutamylamino, glycylamino, or alanylamino. Most preferably R^4 is serylamino, α-glutamylamino, glycylamino, or alanylamino; and

q is 1;

or a salt or solvate thereof.

A further preferred group of compounds of each aspect of the invention described herein, comprise compounds wherein:

R^1 is a group of Formula (II);

X is —S—, —S(O_2)—, —O— or —NH—; preferably X is —O— or —S—; most preferably X is —O—;

p is 0 or 1; and

W is phenyl;

R^4 is independently selected from hydrogen, -OH, —OPO_3H_2, or a C_{1-4}alkoxy group, preferably R^4 is independently selected from —OPO_3H_2, methoxy or ethoxy, most preferably R^4 is methoxy;

q is an integer between 1 and 4, preferably q is 2 or 3, most preferably q is 3;

or a salt or solvate thereof.

A further preferred group of compounds of each aspect of the invention described herein, comprise compounds wherein:

R^3 and R^{10} are independently selected from hydrogen, methyl , ethyl or a group of Formula (IV), preferably methyl or a group of Formula (IV);

or a salt or solvate thereof.

A further preferred groups of compounds of each aspect of the invention described herein comprise compounds wherein:

R^3 is a group of Formula (IV) and R^{10} is hydrogen or lower alkyl or R^3 is hydrogen or lower alkyl and R^{10} is a group of Formula (IV);

t is 0 or 1, preferably 0;

Y is —NH— or —O—, preferably Y is —O—;

r is an integer between 2 and 4, preferably 2 or 3;

Z is a bond; and

R^6 is a –Ph or a non-aromatic heterocyclic group, wherein –Ph and the non-aromatic heterocyclic group are optionally substituted by lower alkyl or a lower alkanoyl group, preferably R^6 is a non-aromatic heterocyclic group optionally substituted by lower alkyl or a lower alkanoyl group;

or a salt or solvate thereof.

Preferred compounds of the invention include:

2-phenoxycarbonylamino-5-phenylsulphanyl-1*H*-indole-3-carbonitrile;

5-(4-hydroxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile;

5-(3,6-dimethoxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile;

5-phenylsulphanyl-2-(4-methylpiperazin-1-ylpropoxycarbonylamino)-1*H*-indole-3-carbonitrile;

5-phenylsulphanyl-1-(4-methylpiperazin-1-ylpropoxycarbonyl)-2-amino-1*H*-indole-3-carbonitrile;

6-benzyloxy-2-amino-1*H*-indole-3-carbonitrile;

5-[4-(4-methylpiperazin-1-yl-4-oxobutanoyloxy)phenoxy]-2-amino-1*H*-indole-3-carbonitrile;

5-(4-glycylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(4-alanylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(4-glutamylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(3-glycylbenzyloxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(4-hydroxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(4-phosponooxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(3-serylbenzyloxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(4-acetylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile; and

5-(4-serylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile;

or salt or solvate thereof.

More preferred compounds of the invention include:

5-(4-aminophenoxy)-2-amino-1*H*-indole-3-carbonitrile; and

5-(3,4,5-trimethoxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile,

or salt or solvate thereof.

Most preferred compounds of the invention include:

5-(3,4-dimethoxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile; and (Example 3)

5-(3,4,5-trimethoxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile; (Example 11)

or salt or solvate thereof.

In any aspect of the invention it is desirable to include in the compound of the invention, at any appropriate position, a water solubilising group such as (but not limited to) a phosphate,

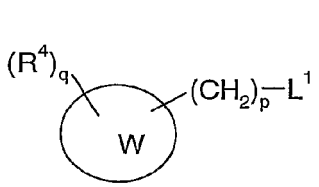
sulphate or sugar group, to enhance the bioavailability of the compound on administration to a patient.

The first aspect of the invention also encompasses derivatives of compounds of Formula (I), such as pharmaceutically acceptable salts and/or ionic forms (for instance where a terminal hydroxyl group is actually present as $-O^-$ or a terminal amine is in it's protonated form), co-ordination complexes for example with metal ions, hydrated forms, labelled forms for instance for use in diagnostic methods and pharmaceutical precursors which are convertible, either *in vitro* or *in vivo*, into the relevant compound.

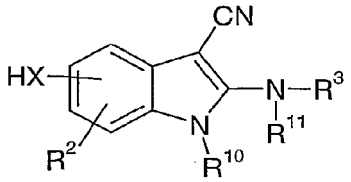
A compound of the invention or a pharmaceutically-acceptable salt, or solvate thereof, may be prepared by any process known to be applicable to the preparation of chemically related compounds. Such processes, when used to prepare a compound of the invention or a pharmaceutically-acceptable salt, or solvate thereof, are provided as a further feature of the invention and are illustrated by the following representative examples in which R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, m, n, p, q, r, t, W, X, Y and Z have the same meaning as herein before defined. The reader is referred to Advanced Organic Chemistry, 4th Edition, by Jerry March, published by John Wiley & Sons 1992, for general guidance on reaction conditions and reagents. The reader is referred to Protective Groups in Organic Synthesis 2nd Edition, by Green et al, published by John Wiley & Sons for general guidance on protecting groups.

Thus, according to the fourth aspect of the invention there is provided a process for preparing a compound of Formula (I), or salt, or solvate thereof, which process (wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, m, n, p, q, r, t, W, X, Y and Z are unless otherwise specified as defined in Formula (I)) comprises:

a) for compounds of Formula (I) wherein R^1 is of the Formula (II) and X is —O—, —S— or —NH—, reacting a compound of Formula (A) with a compound of Formula (B),



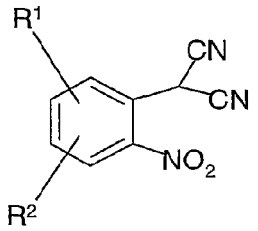
Formula (A)



Formula (B)

wherein L^1 is a leaving group;

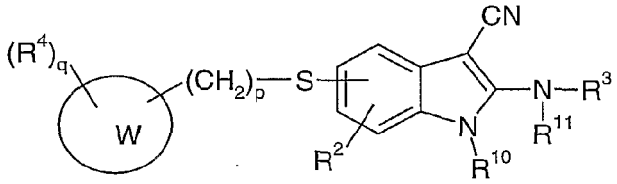
b) for compounds of Formula (I) in which R^3 is hydrogen and R^{11} is hydrogen may be prepared by reduction followed by ring closure of compounds of Formula (C):



Formula (C)

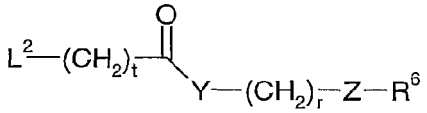
c) for compounds of Formula (I) wherein R^{10} is lower alkyl, reacting a compound of Formula (I) wherein R^{10} is hydrogen with a suitable alkylhalide,

d) for compounds of Formula (I) wherein R^1 is of the Formula (II) and X is —S(O)—, —$S(O_2)$—, oxidising a compound of Formula (D),



Formula (D)

e) for compounds of Formula (I) in which R^3 is a group of Formula (IV) may be prepared by the reaction of compounds of Formula (I) in which R^3 is hydrogen with compounds of Formula (F) below, in which L^2 is a leaving group.:



Formula (F)

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 salt, or solvate.

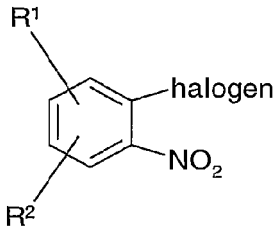
According to a further feature of the fourth aspect of the invention there is provided the processes a), b), c), d) and e) described above for the preparation of compounds of the Formula (I), or a salt, or solvate thereof.

Specific reaction conditions for the above reactions are as follows:

Process a) Compounds of Formula (A) and compound of Formula (B) can be reacted together in an organic solvent, at a temperature between room temperature and about 80°C, optionally in the presence of a base such as sodium hydride, potassium carbonate or triethylamine.

Process b) The conditions for reduction of a compound of Formula (C) are well known in the art. Examples of reducing agents include hydrogen and a hydrogenation catalyst (for example palladium on carbon), iron and acetic acid, and zinc and hydrochloric acid. The reaction is preferably carried out in the presence of a suitable solvent such as an alcohol, for example methanol or ethanol, and at a temperature in the range of 0-80°C, preferably at or near room temperature. Further examples of reducing conditions include sodium dithionite in the presence of a base, preferably sodium bicarbonate in a suitable solvent such as DMF or N-methyl-pyrrolidone.

Compounds of Formula (C) can be prepared by displacement of a halogen atom, preferably F or Cl , from compounds of Formula (E), using a salt of malononitrile, preferably the sodium salt:



Formula (E)

A method for reduction and ring closure of a compound of Formula (C) can be found in US-4,533,672.

Process c) Compounds of Formula (I) wherein R_2 is hydrogen and a suitable alkyl halide may be reacted together in a suitable organic solvent such as DMF or DMSO, in the presence of a base, such as sodium hydride or potassium carbonate at a temperature between about room temperature and about 80°C.

Process d) The oxidization of a compound of Formula (D) can be carried out, for example, by reaction with metachloroperbenzoic acid (MCPBA) is the presence of a suitable solvent such as dichloromethane at ambient temperature. If an excess of MCPBA is used a compound of Formula (I) wherein X is —$S(O_2)$— is obtained.

Process e) Compounds of Formula (I) in which R^3 is hydrogen can be reacted with compounds of Formula (F) under conditions well known in the art. For example L^2 may be -Cl or p-nitrophenoxy. When L^2 is Cl this is carried out in the presence of a base, preferably pyridine

The synthesis of a compound of Formula (I) in which R^1, R^2 and R^3 are all hydrogen (2-aminoindole-3-carbonitrile) is described by Eger et al in *Liebigs Ann. Chem.* **1993**, 465-470.

The compounds used as starting points for the reactions described above are commercially available or they are known compounds or they are prepared by processes known in the art.

It will also be appreciated that in some of the reactions mentioned herein it may be necessary/desirable to protect any sensitive groups in the compounds. The instances where protection is necessary or desirable and suitable methods for protection are known to those skilled in the art. Conventional protecting groups may be used in accordance with standard practice (for illustration see T.W. Green, Protective Groups in Organic Synthesis, John Wiley and Sons, 1991). Thus, if reactants include groups such as amino, carboxy or hydroxy it may be desirable to protect the group in some of the reactions mentioned herein.

A suitable protecting group for an amino or alkylamino group is, for example, an acyl group, for example an alkanoyl group such as acetyl, an alkoxycarbonyl group, for example a methoxycarbonyl, ethoxycarbonyl or *t*-butoxycarbonyl group, an arylmethoxycarbonyl group, for example benzyloxycarbonyl, or an aroyl group, for example 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 or alkoxycarbonyl group 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. Alternatively an acyl group such as a *t*-butoxycarbonyl group may be removed, for example, by treatment with a suitable acid 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-carbon, or by treatment with a Lewis acid for example boron tris(trifluoroacetate). A suitable alternative protecting group for a primary amino group is, for example, a phthaloyl group which may be removed by treatment with an alkylamine, for example dimethylaminopropylamine, or with hydrazine.

A suitable protecting group for a hydroxy group is, for example, an acyl group, for example an alkanoyl group such as acetyl, an aroyl group, for example benzoyl, or an arylmethyl group, for example benzyl. 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. Alternatively an arylmethyl group such as a benzyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-carbon.

A suitable protecting group for a carboxy group is, for example, an esterifying group, for example a methyl or an ethyl group which may be removed, for example, by hydrolysis with a base such as sodium hydroxide, or for example a *t*-butyl group which may be removed, for example, by treatment with an acid, for example an organic acid such as trifluoroacetic acid, or for example a benzyl group which may be removed, for example, by hydrogenation over a catalyst such as palladium-on-carbon.

The protecting groups may be removed at any convenient stage in the synthesis using conventional techniques well known in the chemical art.

According to the fifth aspect of the invention there is provided a pharmaceutical composition comprising a compound of Formula (I) or pharmaceutically acceptable salt or solvate thereof or a compound of Formula (Ia) or pharmaceutically-acceptable derivative thereof.

According to a further feature of the fifth aspect of the invention there is provided a pharmaceutical composition comprising a compound of Formula (I) or pharmaceutically acceptable salt or solvate thereof or a compound of Formula (Ia) or pharmaceutically-acceptable derivative thereof, in admixture with a pharmaceutically-acceptable diluent or carrier.

In order to use a compound of Formula (I) or pharmaceutically acceptable salt or solvate thereof or a compound of Formula (Ia) or pharmaceutically-acceptable derivative thereof, for the therapeutic treatment (including prophylactic treatment) of mammals including humans, it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

The compositions of the invention may be in a form suitable for oral use (for example as tablets, lozenges, hard or soft capsules, aqueous or oily suspensions, emulsions, dispersible

powders or granules, syrups or elixirs), for topical use (for example as creams, ointments, gels, or aqueous or oily solutions or suspensions), for administration by inhalation (for example as a finely divided powder or a liquid aerosol), for administration by insufflation (for example as a finely divided powder) or for parenteral administration (for example as a sterile aqueous or oily solution for intravenous, subcutaneous, intramuscular or intramuscular dosing or as a suppository for rectal dosing).

The compositions of the invention may be obtained by conventional procedures using conventional pharmaceutical excipients, well known in the art. Thus, compositions intended for oral use may contain, for example, one or more colouring, sweetening, flavouring and/or preservative agents.

Suitable pharmaceutically-acceptable excipients for a tablet formulation include, for example, inert diluents such as lactose, sodium carbonate, calcium phosphate or calcium carbonate, granulating and disintegrating agents such as corn starch or algenic acid; binding agents such as starch; lubricating agents such as magnesium stearate, stearic acid or talc; preservative agents such as ethyl or propyl p-hydroxybenzoate, and anti-oxidants, such as ascorbic acid. Tablet formulations may be uncoated or coated either to modify their disintegration and the subsequent absorption of the active ingredient within the gastrointestinal tract, or to improve their stability and/or appearance, in either case, using conventional coating agents and procedures well known in the art.

Compositions for oral use may be in the form of hard gelatin capsules in which the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules in which the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions generally contain the active ingredient in finely powdered form together with one or more suspending agents, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents such as lecithin or condensation products of an alkylene oxide with fatty acids (for example polyoxethylene stearate), or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and

hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives (such as ethyl or propyl p-hydroxybenzoate), anti-oxidants (such as ascorbic acid), colouring agents, flavouring agents, and/or sweetening agents (such as sucrose, saccharine or aspartame).

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil (such as arachis oil, olive oil, sesame oil or coconut oil) or in a mineral oil (such as liquid paraffin). The oily suspensions may also contain a thickening agent such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set out above, and flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water generally contain the active ingredient together with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients such as sweetening, flavouring and colouring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis oil, or a mineral oil, such as for example liquid paraffin or a mixture of any of these. Suitable emulsifying agents may be, for example, naturally-occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soya bean, lecithin, an esters or partial esters derived from fatty acids and hexitol anhydrides (for example sorbitan monooleate) and condensation products of the said partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening, flavouring and preservative agents.

Syrups and elixirs may be formulated with sweetening agents such as glycerol, propylene glycol, sorbitol, aspartame or sucrose, and may also contain a demulcent, preservative, flavouring and/or colouring agent.

The pharmaceutical compositions may also be in the form of a sterile injectable aqueous or oily suspension, which may be formulated according to known procedures using one or more of the appropriate dispersing or wetting agents and suspending agents, which have been mentioned above. A sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example a

solution in 1,3-butanediol.

Suppository formulations may be prepared by mixing the active ingredient 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. Suitable excipients include, for example, cocoa butter and polyethylene glycols.

Topical formulations, such as creams, ointments, gels and aqueous or oily solutions or suspensions, may generally be obtained by formulating an active ingredient with a conventional, topically acceptable, vehicle or diluent using conventional procedures well known in the art.

Compositions for administration by insufflation may be in the form of a finely divided powder containing particles of average diameter of, for example, 30μm or much less, the powder itself comprising either active ingredient alone or diluted with one or more physiologically acceptable carriers such as lactose. The powder for insufflation is then conveniently retained in a capsule containing, for example, 1 to 50mg of active ingredient for use with a turbo-inhaler device, such as is used for insufflation of the known agent sodium cromoglycate.

Compositions for administration by inhalation may be in the form of a conventional pressurised aerosol arranged to dispense the active ingredient either as an aerosol containing finely divided solid or liquid droplets. Conventional aerosol propellants such as volatile fluorinated hydrocarbons or hydrocarbons may be used and the aerosol device is conveniently arranged to dispense a metered quantity of active ingredient.

For further information on formulation the reader is referred to Chapter 25.2 in Volume 5 of Comprehensive Medicinal Chemistry (Corwin Hansch; Chairman of Editorial Board), Pergamon Press 1990.

The amount of active ingredient that is combined with one or more excipients to produce a single dosage form will necessarily vary depending upon the host treated and the particular route of administration. For example, a formulation intended for oral administration to humans will generally contain, for example, from 0.5 mg to 2 g of active agent compounded with an appropriate and convenient amount of excipients which may vary from about 5 to about 98 percent by weight of the total composition. Dosage unit forms will generally contain about 1 mg to about 500 mg of an active ingredient. For further information on Routes of Administration and Dosage Regimes the reader is referred to Chapter 25.3 in

Volume 5 of Comprehensive Medicinal Chemistry (Corwin Hansch; Chairman of Editorial Board), Pergamon Press 1990.

The size of the dose for therapeutic or prophylactic purposes of a compound of the Formula (I) will naturally vary according to the nature and severity of the conditions, the age and sex of the animal or patient and the route of administration, according to well known principles of medicine.

In using a compound of the Formula (I) for therapeutic or prophylactic purposes it will generally be administered so that a daily dose in the range, for example, 0.5 mg to 50 mg per kg body weight is received, given if required in divided doses. In general lower doses will be administered when a parenteral route is employed. Thus, for example, for intravenous administration, a dose in the range, for example, 0.2 mg to 10 mg per kg body weight will generally be used. Typically, intravenous doses of about 10 mg to 500 mg per patient of a compound of this invention will be used..

The compounds of this invention may be used in combination with other drugs and therapies used to inhibit and/or reverse and/or alleviate symptom of angiogenesis and/or any disease state associated with angiogenesis. Examples of such disease states include: 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.

If formulated as a fixed dose such combination products employ the compounds of this invention within the dosage range described herein and the other pharmaceutically-active agent within its approved dosage range. Sequential use is contemplated when a combination formulation is inappropriate.

Possible uses for the pharmaceutical composition include both therapeutic and diagnostic uses. In particular, it may be used to treat and/or diagnose any condition which is related to (ie, which is or can be caused or mediated, directly or indirectly, by, or which is in any way associated with) angiogenesis or vasculature formation, especially of tumours.

A therapeutic treatment method in which a compound or derivative according to the invention may be used involves the administration to a patient suffering from a relevant condition of a therapeutically (which includes prophylactically) effective amount of the compound or derivative, preferably in the form of a pharmaceutical composition according to

the fifth aspect of the invention. **"Effective amount"** means an amount sufficient to cause a benefit (which may be prophylactic) to the patient or at least to cause a change in the patient's condition, eg, to cause a medically significant inhibition of tumour development. The actual amount administered to the patient, and the rate and time-course of administration, will depend on the nature of the patient, the nature and severity of the condition, the administration method used, etc. Appropriate values can be selected by the trained medical practitioner. The compound or derivative may be administered alone or in combination with other treatments, either simultaneously or sequentially. It may be administered by any suitable route, preferably orally. It may be administered directly to a suitable site or in a manner in which it targets a particular site - suitable targeting methods are already known.

A diagnostic method according to the invention might involve the use of the compound or derivative to determine, either qualitatively or quantitatively, the existence of a particular medical condition or change in condition. Such a method may be carried out either *in vitro* or *in vivo*. One or more of the materials used in the method may be appropriately labelled.

According to a sixth aspect of the present invention there is provided a method for producing a pharmaceutical composition according to the fifth aspect of the invention, said method comprising providing (for example by synthesising) a compound of Formula (I) or pharmaceutically-acceptable salt or solvate thereof or a compound of Formula (Ia) or pharmaceutically-acceptable derivative thereof, and then mixing it with one or more pharmaceutically acceptable adjuvants, and/or with one or more other therapeutically or diagnostically active agents.

According to a seventh aspect of the present invention there is provided a compound of Formula (I) or pharmaceutically-acceptable salt or solvate thereof or a compound of Formula (Ia) or pharmaceutically-acceptable derivative thereof, preferably in the form of a pharmaceutical composition according to the fifth aspect of the invention, 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 regrowth 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 feature of the seventh 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 or solvate thereof or a compound of Formula (Ia) or pharmaceutically-acceptable derivative thereof, preferably in the form of a pharmaceutical composition according to the fifth aspect of the present invention.

According to a further feature of the seventh 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 or a compound of Formula (Ia) or pharmaceutically-acceptable derivative thereof, preferably in the form of a pharmaceutical composition according to the fifth aspect of the present invention.

According to a further feature of the seventh 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 or solvate thereof or a compound of Formula (Ia) or pharmaceutically-acceptable derivative thereof, preferably in the form of a pharmaceutical composition according to the fifth aspect of the present invention, 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 feature of the seventh 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 or solvate thereof or a compound of Formula (Ia) or pharmaceutically-acceptable derivative thereof, preferably in the form of a pharmaceutical composition according to the fifth aspect of the present invention, which together add up to a total daily dose, for administration in divided doses.

According to a further feature of the seventh 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 or solvate thereof or a compound of Formula (Ia) or pharmaceutically-acceptable derivative 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 feature of the seventh 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 or solvate thereof or a compound of Formula (Ia) or pharmaceutically-acceptable derivative 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 feature of the seventh aspect of the present invention there is provided the use of compound of Formula (I) or pharmaceutically-acceptable salt or solvate thereof or a compound of Formula (Ia) or pharmaceutically-acceptable derivative 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 feature of the seventh aspect of the present invention there is provided the use of a compound of Formula (I) or pharmaceutically-acceptable salt or solvate thereof or a compound of Formula (Ia) or pharmaceutically-acceptable derivative thereof in the manufacture of a medicament for administraton 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 feature of the seventh aspect of the present invention there is provided the use of a compound of Formula (I) or pharmaceutically-acceptable salt or solvate thereof or a compound of Formula (Ia) or pharmaceutically-acceptable derivative 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.

An anti-vascular/anti-angiogenic treatment in accordance with the present invention may be applied as a sole therapy or may involve, in addition to the compound of Formula (I) or pharmaceutically-acceptable salt or solvate thereof or a compound of Formula (Ia) or pharmaceutically-acceptable derivative thereof, 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 anti-vascular/anti-angiogenic treatment according to the invention may be, for example, surgery, radiotherapy or chemotherapy. Such chemotherapy may include the following categories of therapeutic agent:

i) anti-angiogenic agents that work by different mechanisms from the compounds of Formula (I) (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 anti-oestrogens (for example tamoxifen, toremifene, raloxifene, droloxifene, iodoxyfene), progestogens (for example megestrol acetate), aromatase inhibitors (for example anastrozole, letrazole, vorazole, exemestane), anti-progestogens, anti-androgens (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) anti-proliferative/anti-neoplastic drugs and combinations thereof, as used in medical oncology, such as anti-metabolites (for example anti-folates like methotrexate, fluoropyrimidines like 5-fluorouracil, purine and adenosine analogues, cytosine arabinoside); anti-tumour 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); anti-mitotic

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 compounds of Formula (I) or Formula (Ia) are of interest for their potential vascular damaging effects. They 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 the compounds 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, compounds of Formula (I) and their pharmaceutically acceptable derivatives may also be 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.

Compounds of Formula (I) and compounds of Formula (Ia) encompass vascular damaging agents and pro-drugs of vascular damaging agents. Pro-drugs of vascular damaging agents are believed to be cleaved *in-vivo*. Without being bound by theoretical considerations these pro-drugs may have lower activity in the in-vitro colchicine binding site competitive assay, than would be anticipated when the activity of these compounds is measured in cell based assays or *in-vivo*.

Biological Assays

(i) Colchicine Binding site competitive assay kit.

The ability of a ligand to bind specifically to the colchicine binding site on tubulin, an indicator of the vascular damaging activity, was assessed using a size exclusion chromatography assay kit from "Cytoskeleton" (1650 Fillmore St. #240, Denver, CO 80206, U.S.A.) Catalogue number of kit: BK023.

The following reagents were used:

tubulin buffer, to give 0.1mM GTP, 0.5mM $MgCl_2$, 0.5mM EGTA, 40mM PIPES buffer at pH6.9 in the final reaction mix;

purified tubulin protein from bovine brain at 1mg/ml in tubulin buffer;

0.02mM fluorescent colchicine in tubulin buffer [FITC (fluorescein isothiocyanate)-labelled];

2mM colchicine in tubulin buffer;

0.2mM vinblastine in tubulin buffer; and

G-25 Sephadex™ Fine - particle size 34-138μm.

The reaction was performed as follows:

8μl of test compound (dissolved in DMSO) was gently mixed with 150μl of tubulin. This was then incubated at 37°C for 30 minutes. Then 4μl of the fluorescent colchicine was added, the incubation mix vortexed for 5 seconds and then incubated for a further 30 minutes at 37°C. At the end of the reaction incubation size exclusion chromatography was performed to separate the tubulin with fluorescent colchicine bound from the free, unbound colchicine. If a test compound inhibited fluorescent colchicine binding then a reduced signal is measured and the compound is confirmed as a colchicine site binding moiety.

Chromatography was performed as follows, using chromatography columns filled with 3mls of G-25 Sephadex™ Fine slurry. The incubation mixture was pipetted onto the column and up to 12 elutions of 160μl were collected. The fluorescence of the tubulin-containing fractions was detected on a spectrophotometer which excites at 485nm and emits at 535nm. Control incubations were also performed, 8μl DMSO (negative control) and 8μl colchicine stock (positive competition control), instead of the 8μl of test compound in the incubation mixture.

The degree of competition of colchicine binding by either unlabelled colchicine or test compound was calculated relative to the DMSO negative control.

(ii) HUVEC detachment assay

Compounds according to the invention are expected to be able to damage vasculature, such as tumour vasculature, that has been newly formed whilst leaving normal, mature vasculature unaffected. The identification of compounds which can 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 the procedure set out below:

This assay examined the effects of compounds on the adherence of HUVECs (Human umbilical cord vein endothelial cells) to tissue culture plasticware.

HUVECs (supplied by TCS Biologicals Ltd, Buckingham, United Kingdom) 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, they were dosed with a compound under test 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.

Although the pharmacological properties of the compounds of the Formula (I) vary with structural change as expected, in general compounds of the Formula (I) possess an activity in the colchicine binding site competitive assay of greater than 20% competition at a dose of compound of 10µM and an activity in the HUVEC detachment assay of between 15-70% detachment at a dose of compound of 100µM. For example 5-(4-hydroxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile (Example 2) has an activity of 36% in the colchicine binding site competitive assay at 10µM and 55% in the cell detachment assay at 100µM and 6-methyl-5-fluoro-2-amino-1*H*-indole-3-carbonitrile (Example 8) has an activity of 31% in the colchicine binding site competitive assay at 10µM and 34% in the cell detachment assay at 100µM.

The invention will now be illustrated by 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 (MS or LCMS) 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;

(vi) flash chromatography was performed on silica (Merck Keiselgel: Art.9385);

(vii) concentrations of solvent in the elemental analyses are quoted in molar (M);

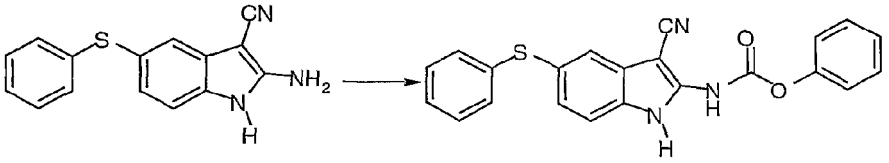
(viii) HP20SS resin (DIAION® HP20SS) was obtained from Mitsubishi Chemical America Inc.

Abbreviations

Dimethylacetamide	DMA
4-Dimethylaminopyridine	DMAP
Dimethylformamide	DMF
Dimethyl sulphoxide	DMSO
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride	EDCI
Liquid chromatographic mass spectroscopy	LCMS
Mass spectroscopy	MS
N-(9-fluorenylmethoxycarbonyl)	N-FMOC
N-methyl-pyrrolidone	NMP
Tetrahydrofuran	THF

Example 1

2-Phenoxycarbonylamino-5-phenylsulphanyl-1*H*-indole-3-carbonitrile



Example 1

A solution of 5-phenylsulphanyl-2-amino-1*H*-indole-3-carbonitrile (0.43 g ; 1.62 mmol) [Amphethinile, US 4,533,672] in THF (5 ml) and pyridine (0.17 ml ; 2.1 mmol) under argon was cooled to 0°C and treated dropwise with phenyl chloroformate (0.25 ml ; 2.0 mmol). The mixture was allowed to warm to room temperature overnight. After dilution with CH_2Cl_2 the organic phase was washed with water, then brine and dried over $MgSO_4$. The residue was purified by flash chromatography eluting with increasingly polar mixtures of Et_2O/CH_2Cl_2 (5 to 10% Et_2O) to give 2-phenoxycarbonylamino-5-phenylsulphanyl-1*H*-indole-3-carbonitrile as a beige solid (310 mg).

Yield : 50%

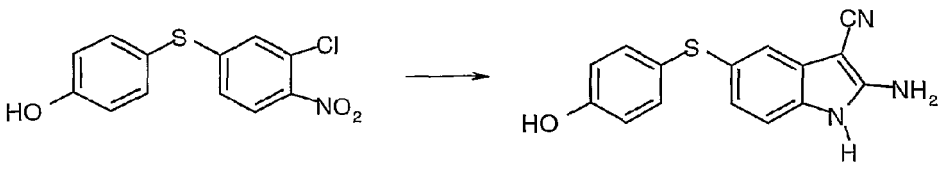
^{1}H NMR spectrum ($CDCl_3$) : 7.14-7.35 (m, 10H) ; 7.44 (t, 2H) ; 7.71 (d, 1H) ; 10.83 (s, 1H) ; 10.99 (br s, 1H).

MS-ESI : 408 $[MNa]^+$

Elemental analysis :	Found	C 67.14	H 3.94	N 10.76	S 7.79
$C_{22}H_{15}N_3O_2S$; 0.4 H_2O	Requires	C 67.30	H 4.06	N 10.7	S 8.17

Example 2

5-(4-Hydroxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile



Example 2

A stirred solution of 4-(4-hydroxyphenylsulphanyl)-2-chloro-nitrobenzene (42 g ; 150 mmol) and malononitrile (9.9 g ; 150 mmol) in N-methyl-2-pyrrolidinone (150 ml) was

treated dropwise with a 10 M aqueous solution of sodium hydroxide (45 ml ; 450 mmol). The mixture was stirred at room temperature for 50 hours.

The mixture was diluted with DMF (300 ml) and a solution of $NaHCO_3$ (105 g ; 1.25 mol) in water (420 ml) was added. To this solution was added sodium dithionite (74 g ; 0.425 mol) followed by further solid $NaHCO_3$ (105 g) and sodium dithionite (74 g). The resulting suspension was well stirred at room temperature until the purple colouration had disappeared (at least one night). Mineral salts were filtered and the filtrate was poured into water (4 l). The mixture was extracted with EtOAc (2x) and the combined extracts washed well with water, then brine and dried over $MgSO_4$.

The residue was purified by flash chromatography eluting with increasingly polar mixtures of EtOAc/hexanes (60 to 75% EtOAc). The appropriate fractions were evaporated to give 5-(4-hydroxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile as a beige solid (26.2 g).

Yield : 62%.

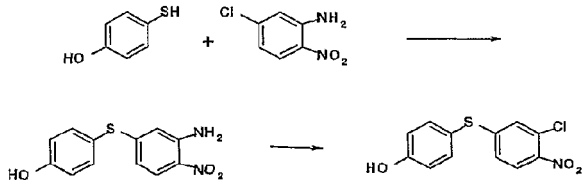
Further flash chromatography of a small sample eluting with increasingly polar mixtures of EtOAc / hexanes (60-70% EtOAc) gave colourless crystals, Melting Point 241-242°C.

^{1}H NMR spectrum (DMSO d_6) : 6.76 (d, 2H) ; 6.85 (s, 2H) ; 6.87 (dd, 1H) ; 6.98 (d, 1H) ; 7.09 (d, 1H) ; 7.2 (d, 2H) ; 9.66 (br s, 1H) ; 10.79 (br s, 1H).

MS-ESI : 282 $[MH]^+$

Elemental analysis :	Found	C 64.22	H 4.19	N 14.84	S 11.11
$C_{15}H_{11}N_3OS$	Requires	C 64.04	H 3.94	N 14.94	S 11.4

4-(4-Hydroxyphenylsulphanyl)-2-chloro-nitrobenzene was prepared as follows:-



4-(4-Hydroxyphenylsulphanyl)-2-amino-nitrobenzene

A solution of 4-hydroxybenzenethiol (46 g ; 328 mmol) in DMA (500 ml) under argon was cooled to 0°C and treated portionwise with NaH (28.9 g ; 722 mmol ; 60 % dispersion in oil). The mixture was stirred at room temperature for 2 hours and then solid 4-chloro-2-

amino-nitrobenzene (56.6 g ; 328 mmol) was added rapidly portionwise. The mixture was stirred and heated at 80°C for 30 minutes and allowed to cool overnight. The mixture was poured into aqueous HCl (2N) and the precipitate was filtered, washed with water, then pentane and dried *in vacuo* to give 4-(4-hydroxyphenylsulphanyl)-2-amino-nitrobenzene as a beige solid (86.6 g).

Yield : 100%

Purification of a small sample by flash chromatography eluting with increasingly polar mixtures of EtOAc/hexanes (40 to 60% EtOAc) gave analytically pure 4-(4-hydroxyphenylsulphanyl)-2-amino-nitrobenzene as a white solid.

^{1}H NMR spectrum (DMSO d_6) : 6.27 (dd, 1H) ; 6.5 (d, 1H) ; 6.92 (d, 2H) ; 7.41 (d, 2H) ; 7.44 (s, 2H) ; 7.86 (d, 1H) ; 10.08 (br s, 1H).

MS-ESI : 262 $[M]^+$

Elemental analysis :	Found	C 54.83	H 3.95	N 10.52	S 11.99
$C_{21}H_{26}NO_8P$; 0.7 H_2O	Requires	C 54.95	H 3.84	N 10.68	S 12.22

4-(4-Hydroxyphenylsulphanyl)-2-chloro-nitrobenzene

4-(4-Hydroxyphenylsulphanyl)-2-amino-nitrobenzene (85.9 g ; 327 mmol) was dissolved in a mixture of AcOH (1.0 l) and concentrated HCl (400 ml) by stirring and warming to 60°C. The cooled solution was added slowly to a mechanically stirred mixture of ice (1.6 kg) and concentrated HCl (400 ml) to obtain a fine suspension which was further stirred overnight. The suspension was cooled to 5°C and a chilled solution of $NaNO_2$ (22.6 g ; 327 mmol) in water (80 ml) was added dropwise, while maintaining the temperature of the mixture between 0°C and 5°C. The mixture was stirred for a further 3 hours and was poured slowly into a solution of CuCl (32.3 g ; 327 mmol) in a mixture of concentrated HCl (160 ml) and water (120 ml) at 35°C. After the addition was complete, the mixture was warmed at 50°C for 1 hour and then allowed to cool.

The solid which separated was filtered and digested in EtOAc (4 l). The organic phase was washed with sat. aqueous $NaHCO_3$, then brine and dried over $MgSO_4$.

The residue (85 g) was purified by flash chromatography eluting with CH_2Cl_2. The appropriate fractions were evaporated to give 4-(4-hydroxyphenylsulphanyl)-2-chloro-nitrobenzene as a yellow solid (45.1 g).

Yield : 49%

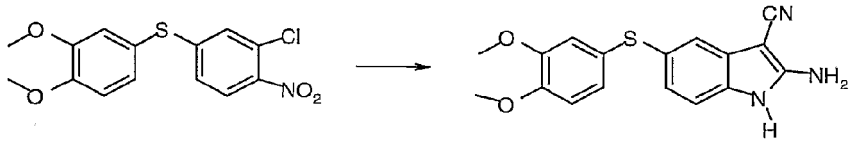
^{1}H NMR spectrum ($CDCl_3$) : 5.18 (s, 1H) ; 6.93 - 6.99 (m, 3H) ; 7.09 (d, 1H) ; 7.44 (d, 2H) ; 7.79 (d, 1H).

MS-ESI : 280 $[M-H]^-$

Elemental analysis :	Found	C 51.1	H 2.85	N 5.03	S 11.16
$C_{12}H_8ClNO_3S$	Requires	C 51.16	H 2.86	N 4.97	S 11.38

Example 3

5-(3,4-Dimethoxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile



Example 3

Using the general method described for Example 2, except that two equivalents of aqueous NaOH were employed, 5-(3,4-dimethoxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile was obtained from 4-(3,4-dimethoxyphenylsulphanyl)-2-chloro-nitrobenzene (1.7 g; 5.2 mmol) after purification by flash chromatography eluting with increasingly polar mixtures of EtOAc/hexanes (50 to 60% EtOAc). The product was a beige solid (745 mg), Melting Point 169-173°C.

Yield : 44%

^{1}H NMR spectrum (DMSO d_6) : 3.71 (s, 3H) ; 3.75 (s, 3H) ; 6.82 (dd, 1H) ; 6.88 (s, 2H) ; 6.93 (m, 3H) ; 7.08 (d, 1H) ; 7.12 (d, 1H) ; 10.85 (br s, 1H).

MS-ESI : 325 $[M]^+$

4-(3,4-Dimethoxyphenylsulphanyl)-2-chloro-nitrobenzene was prepared using the general method described for 4-(4-hydroxyphenylsulphanyl)-2-chloro-nitrobenzene in Example 2, except that one equivalent of NaH was used in the first step.

4-(3,4-Dimethoxyphenylsulphanyl)-2-amino-nitrobenzene

In the first step 4-(3,4-dimethoxyphenylsulphanyl)-2-amino-nitrobenzene was prepared (15.45 g):

Yield : 100%

^{1}H NMR spectrum (DMSO d_6) : 3.78 (s, 3H) ; 3.82 (s, 3H) ; 6.31 (dd, 1H) ; 6.54 (d, 1H) ; 7.08 - 7.41 (m, 3H) ; 7.45 (s, 2H) ; 7.86 (d, 1H).

MS-ESI : 306 $[M]^+$

Elemental analysis :	Found	C 54.57	H 4.59	N 9.15	S 9.87
$C_{14}H_{14}N_2O_4S$	Requires	C 54.89	H 4.61	N 9.14	S 10.47

4-(3,4-Dimethoxyphenylsulphanyl)-2-chloro-nitrobenzene

In the second step 4-(3,4-dimethoxyphenylsulphanyl)-2-chloro-nitrobenzene was prepared (2.27 g):

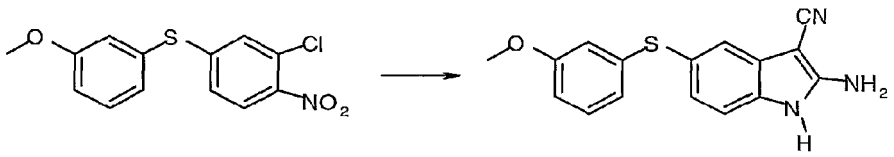
Yield : 56%

^{1}H NMR spectrum ($CDCl_3$) : 3.89 (s, 3H) ; 3.95 (s, 3H) ; 6.85 - 6.99 (m, 2H) ; 7.02 (d, 1H) ; 7.11 (d, 1H) ; 7.17 (dd, 2H) ; 7.79 (d, 1H).

Elemental analysis :	Found	C 51.45	H 3.78	N 4.38	S 9.77
$C_{14}H_{12}ClNO_4S$	Requires	C 51.62	H 3.71	N 4.3	S 9.84

Example 4

5-(3-Methoxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile



Example 4

This compound was prepared by the use of a Zymate (trade mark) Laboratory Automation System XP2 Robot (Zymark Corp.).

Thus, 4-(3-methoxyphenylsulphanyl)-2-chloro-nitrobenzene (2.45 g ; 8.28 mmol) was introduced into a robot tube. A solution of malononitrile (2M, 8.3 mmol) in N-methyl-2-pyrrolidinone was pumped into the tube and the mixture vortexed. Aqueous sodium hydroxide (10M, 16.6 mmol) was pumped in and the mixture was then vortexed and stirred for 3 days at room temperature. The contents of the tube were divided equally into a further five tubes. To each was added DMF (3 ml), water (4 ml), $NaHCO_3$ (1 g), sodium dithionate (700 mg) followed by further $NaHCO_3$ (1 g) and sodium dithionate (700 mg) with vortexing between each addition. The mixture was stirred overnight at room temperature.

Each tube was diluted with water (4 ml) and extracted with EtOAc (2x 7 ml). The organic phase was evaporated, the residues were combined in CH_2Cl_2, treated SiO_2 (5 g) and evaporated to dryness. The residue was purified by flash chromatography eluting automatically with CH_2Cl_2 and then EtOAc/CH_2Cl_2 (50/50). The appropriate fractions were evaporated, triturated with EtOAc/hexanes, filtered, rinsed and dried to give 5-(3-methoxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile as a cream solid (473 mg).

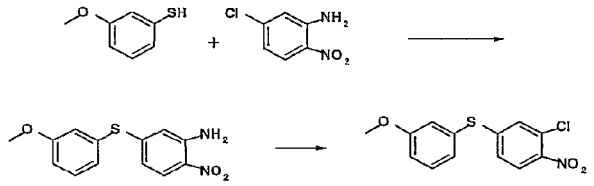
Yield : 19%

^{1}H NMR spectrum (DMSO d_6) : 3.68 (s, 3H) ; 6.62 - 6.66 (m, 2H) ; 6.73 (dd, 1H) ; 6.95 (s, 2H) ; 7.05 (dd, 1H) ; 7.15 - 7.2 (m, 3H) ; 10.93 (br s, 1H).

MS-ESI : 296 $[M]^+$

All samples prepared by the Robot contained small quantities of DMF and N-methyl-2-pyrrolidinone and were not analysed for elemental composition.

The starting material was prepared as follows:-



Using the general method described in Example 2, except that one equivalent of NaH was used in the first step, 3-methoxybenzenethiol (2.1 g ; 15 mmol) was used to prepare:

4-(3-Methoxyphenylsulphanyl)-2-amino-nitrobenzene (4.0 g):

Yield : 97%

^{1}H NMR spectrum ($CDCl_3$) : 3.82 (s, 3H) ; 6.03 (br s, 2H) ; 6.39 (d, 1H) ; 6.45 (dd, 1H) ; 6.98 (dt, 1H) ; 7.06 (t, 1H) ; 7.13 (dt, 1H) ; 7.35 (t, 1H) ; 7.98 (d, 1H).

MS-ESI : 277 $[MH]^+$

And then:

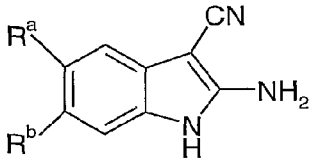
4-(3-Methoxyphenylsulphanyl)-2-chloro-nitrobenzene (2.27 g):

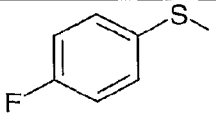
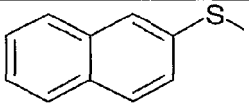
Yield : 53%

^{1}H NMR spectrum ($CDCl_3$) : 3.85 (s, 3H) ; 7.03 (dd, 1H) ; 7.07 - 7.1 (m, 2H) ; 7.14 (dd, 1H) ; 7.23 (d, 1H) ; 7.39 (t, 1H) ; 7.82 (d, 1H).

Examples 5-8

The examples in the following table were prepared using the Zymate (trade mark) Robot as described in Example 4, from the corresponding substituted 2-chloro-nitrobenzene.



Example	R^a	R^b	Footnote
5		H	a
6		H	b
7	H	F	c
8	F	CH_3-	d

Footnotes

a) Starting material: 4-(4-fluorophenylsulphanyl)-2-chloro-nitrobenzene 2.3 g8.11 mmol
Yield: 289 mg 13%
^{1}H NMR spectrum (DMSO d_6) : 6.94 (s, 2H) ; 7.03 (dd, 1H) ; 7.12 - 7.23 (m, 6H) ; 10.91 (br s, 1H).
MS-ESI : 284 $[MH]^+$

b) Starting material: 4-(2-naphthylsulphanyl)-2-chloro-nitrobenzene 1.7 g 5.4 mmol
Yield: 366 mg 21%
^{1}H NMR spectrum (DMSO d_6) : 6.95 (s, 2H) ; 7.08 (dd, 1H) ; 7.2 - 7.27 (m, 3H) ; 7.47 (m, 2H) ; 7.65 (s, 1H) ; 7.76 (d, 1H) ; 7.84 (t, 2H) ; 10.94 (br s, 1H).
MS-ESI : 314 $[M-H]^-$

c) Starting material: 5-fluoro-2-chloro-nitrobenzene 1.75 g 9.97 mmol
Yield: 18 mg 1%
^{1}H NMR spectrum (DMSO d_6) : 6.78 (s, 2H) ; 6.79 (ddd, 1H) ; 6.96 (dd, 1H) 7.07 (dd, 1H) ; 10.76 (br s, 1H).

MS-ESI : 175 $[M]^+$

d) Starting material: 4-fluoro-5-methyl-2-chloro-nitrobenzene 1.89 g 9.97 mmol

Yield: 53 mg 3%

^{1}H NMR spectrum (DMSO d_6) : 6.74 (s, 2H) ; 6.81 (d, 1H) ; 6.96 (d, 1H) ; 10.62 (br s, 1H).

MS-ESI : 188 $[M-H]^-$

Starting Materials – Example 5

Starting material 4-(4-fluorophenylsulphanyl)-2-chloro-nitrobenzene was prepared as described in Example 4.

4-(4-Fluorophenylsulphanyl)-2-amino-nitrobenzene:

Starting material: 4-fluorobenzenethiol 1.92 g 15 mmol

Yield: 3.69 g 93%

^{1}H NMR spectrum ($CDCl_3$) : 6.03 (br s, 2H) ; 6.31 (d, 1H) ; 6.38 (dd, 1H) ; 7.15 (t, 2H) ; 7.54 (dd, 2H) ; 7.98 (d, 1H).

MS-ESI : 265 $[MH]^+$.

4-(4-Fluorophenylsulphanyl)-2-chloro-nitrobenzene:

Yield: 1.72 g 44%

^{1}H NMR spectrum (DMSO d_6) : 7.0 (dd, 1H) ; 7.13 - 7.2 (m, 3H) ; 7.55 (m, 2H) ; 7.81 (d, 1H).

MS-EI : 283 $[M]^+$

Starting Materials – Example 6

Starting material 4-(2-naphthylsulphanyl)-2-chloro-nitrobenzene was prepared as described in Example 4.

4-(2-Naphthylsulphanyl)-2-amino-nitrobenzene:

Starting material: naphthalene-2-thiol 2.4 g 15 mmol

Yield: 4.45 g 100%

^{1}H NMR spectrum (DMSO d_6) : 6.40 (dd, 1H) ; 6.66 (s, 1H) ; 7.43 (br s, 2H) ; 7.54 (dd, 1H) ; 7.63 (m, 2H) ; 7.89 (d, 1H) ; 8.03 (m, 3H) ; 8.24 (s, 1H).

MS-ESI : 295 $[M-H]^-$

4-(2-Naphthylsulphanyl)-2-chloro-nitrobenzene:

Yield: 1.75 g 37%

^{1}H NMR spectrum ($CDCl_3$) : 7.07 (dd, 1H) ; 7.23 (d, 1H) ; 7.5 (dd, 1H) ; 7.59 (m, 2H) ; 7.79 (d, 1H) ; 7.83 - 7.93 (m, 3H) ; 8.1 (s, 1H).

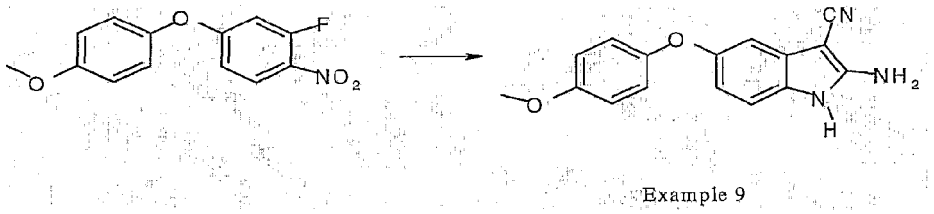
MS-EI : 315 $[M]^+$

Starting Materials – Examples 7 and 8

Starting materials 5-fluoro-2-chloro-nitrobenzene and 5-methyl-4-fluoro-2-chloro-nitrobenzene are commercially available.

Example 9

5-(4-Methoxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile



Example 9

Using the general method described for Example 2, Example 9 was prepared from a 1:2 mixture of 4-(4-methoxyphenoxy)-2-fluoro-nitrobenzene (**a**) and its isomer 2-(4-methoxyphenoxy)-4-fluoro-nitrobenzene was used (**b**) (see below) (5.97 g ; 22.7 mmol), except that two equivalents of aqueous NaOH were employed, after purification by flash chromatography eluting with increasingly polar mixtures of EtOAc/hexanes (40 to 100% EtOAc), 5-(4-methoxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile was obtained as a light beige solid (1.32 g), Melting Point 216-217°C.

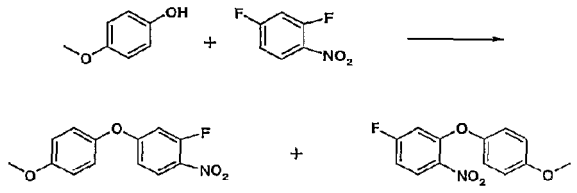
Yield : 21%

^{1}H NMR spectrum (DMSO d_6) : 3.75 (s, 3H) ; 6.58 (dd, 1H) ; 6.63 (d, 1H) ; 6.80 (s, 2H) ; 6.93 (s, 4H) ; 7.09 (d, 1H) ; 10.69 (br s, 1H).

MS-ESI : 278 $[M-H]^-$

Elemental analysis :	Found	C 65.78	H 4.52	N 14.68
$C_{16}H_{13}N_3O_2$; 0.7 H_2O	Requires	C 65.83	H 4.97	N 14.39

The 1:2 mixture of 4-(4-methoxyphenoxy)-2-fluoronitrobenzene (**a**) and its isomer 2-(4-methoxyphenoxy)-4-fluoronitrobenzene (**b**) was prepared as follows:-



a b

A solution of 4-methoxyphenol (3.75 g ; 30 mmol) in DMA (40 ml) under argon was cooled to 0°C and treated portionwise with NaH (1.35 g ; 33 mmol ; 60 % dispersion in oil). The mixture was stirred at room temperature for 1 hour. This mixture was added dropwise to a solution of 2,4-difluoro-nitrobenzene(3.3 ml ; 30 mmol) in DMA (10 ml) over 1 hour. The mixture was allowed to stir overnight and was then poured into aqueous HCl (10 %) and extracted with Et_2O (3x). The organic phase was washed with water, then brine, and dried over $MgSO_4$.

The residue (9.14 g) was purified by flash chromatography eluting with increasingly polar mixtures of EtOAc/hexanes (0 to 20% EtOAc). The appropriate fractions were evaporated to give a mixture of 4-(4-methoxyphenoxy)-2-fluoronitrobenzene (**a**) and its isomer 2-(4-methoxyphenoxy)-4-fluoronitrobenzene (**b**) (5.97 g ; 1:2 as determined by ^1H NMR).

Yield : 76%

^1H NMR spectrum ($CDCl_3$) : 3.83 (s, 3H, **b**) ; 3.84 (s, 3H, **a**) ; 6.56 (dd, 1H, **b**) ; 6.71 (dd, 1H, **a**) ; 6.76-6.82 (m, 1H, **b** and 1H, **a**) ; 6.94-6.97 (m, 2H, **b** and 2H, **a**) ; 7.02-7.06 (m, 2H, **b** and 2H, **a**) ; 8.02 (dd, 1H, **b**); 8.07 (dd, 1H, **a**).

Example 10

5-(3,6-Dimethoxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile



Example 10

5-(3,6-Dimethoxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile (1.22 g) was prepared using the general method described in Example 2, except that two equivalents of aqueous NaOH were employed, it was obtained as a white solid from 4-(3,6-dimethoxyphenylsulphanyl)-2-chloro-nitrobenzene (2.1 g ; 6.45 mmol) after purification by flash chromatography eluting with increasingly polar mixtures of CH_2Cl_2/EtOAc (0 to 50% EtOAc).

Yield : 58%

^{1}H NMR spectrum (DMSO d_6) : 3.54 (s, 3H) ; 3.80 (s, 3H) ; 6.08 (d, 1H) ; 6.68 (dd, 1H) ; 6.93 (d, 1H) ; 6.98 (s, 2H) ; 7.05 (dd, 1H) ; 7.20 (d, 1H) ; 7.24 (d, 1H) ; 10.98 (br s, 1H).

LCMS-ESI : 326 $[M+H]^+$

Elemental analysis :	Found	C 61.8	H 5.07	N 12.1	S 8.78
$C_{17}H_{15}N_3O_2S$; 0.35 EtOAc	Requires	C 62.04	H 5.04	N 11.8	S 9.0

4-(3,6-Dimethoxyphenylsulphanyl)-2-amino-nitrobenzene

Using the general method described for 4-(4-hydroxyphenylsulphanyl)–2-amino-nitrobenzene in Example 2, except that one equivalent of NaH was used, 4-(3,6-dimethoxyphenylsulphanyl)-2-amino-nitrobenzene (4.19g) was prepared from 3,6-dimethoxybenzenethiol (2.55 g ; 15 mmol).

Yield : 91%

^{1}H NMR spectrum ($CDCl_3$) : 3.78 (s, 3H) ; 3.79 (s, 3H) ; 6.02 (s, 2H) ; 6.37 (d, 1H) ; 6.41 (dd, 1H) ; 6.90 - 7.07 (m, 3H) ; 7.96 (d, 1H).

MS-ESI : 307 $[M+H]^+$

4-(3,6-dimethoxyphenylsulphanyl)-2-chloro-nitrobenzene

Similarly, using the general method described for 4-(4-hydroxyphenylsulphanyl)–2-chloro-nitrobenzene in Example 2, 4-(3,6-dimethoxyphenylsulphanyl)-2-chloro-nitrobenzene

(2.22 g) was prepared from 4-(3,6-dimethoxyphenylsulphanyl)-2-amino-nitrobenzene (4.15 g; 15 mmol) after purification by flash chromatography eluting with increasingly polar mixtures of EtOAc/hexanes (10 to 20% EtOAc).

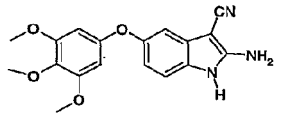
Yield : 50%

^{1}H NMR spectrum ($CDCl_3$) : 3.78 (s, 3H) ; 3.79 (s, 3H) ; 6.96 (d, 1H) ; 7.02 (m, 2H) ; 7.08 (d, 1H) ; 7.15 (d, 2H) ; 7.79 (d, 1H).

MS-ESI : 326 $[M]^+$

Example 11

5-(3,4,5-Trimethoxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile



Example 11

5-(3,4,5-Trimethoxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile (1.49 g) was prepared using the general method described in Example 2, except that two equivalents of aqueous NaOH were employed, it was obtained as a beige solid, melting point 208-209°C, from a 1:2 mixture of 4-(3,4,5-trimethoxyphenoxy)-2-fluoro-nitrobenzene (**c**) and its isomer 2-(3,4,5-trimethoxyphenoxy)-4-fluoro-nitrobenzene (**d**) (8.01 g ; 25 mmol; present in a 1:2 mixture of isomers) after purification by flash chromatography eluting with increasingly polar mixtures of EtOAc/hexanes (40 to 100% EtOAc).

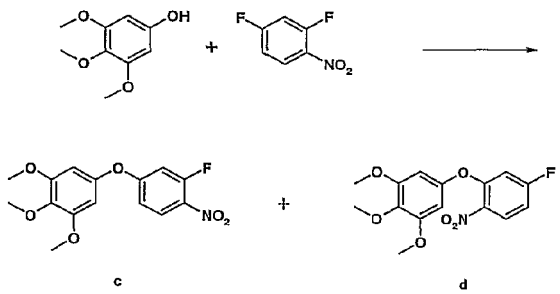
Yield : 18%

^{1}H NMR spectrum (DMSO d_6) : 3.64 (s, 3H) ; 3.70 (s, 6H) ; 6.28 (s, 2H) ; 6.64 (dd, 1H) ; 6.74 (s, 1H) ; 6.83 (s, 2H) ; 7.13 (d, 1H) ; 10.72 (br s, 1H).

LCMS-ESI : 340 $[M+H]^+$

Elemental analysis :	Found	C 62.66	H 5.12	N 12.22
$C_{18}H_{17}N_3O_4$; 0.3 H_2O	Requires	C 62.71	H 5.15	N 12.19

The starting material was prepared as follows:-



c d

Using the general method described in Example 9, a mixture of 4-(3,4,5-trimethoxyphenoxy)-2-fluoro-nitrobenzene (**c**) and its isomer 2-(3,4,5-trimethoxyphenoxy)-4-fluoro-nitrobenzene (**d**) (8.01 g ; proportions of b/bi 1:2 as determined by ^{1}H NMR) was obtained from 3,4,5-trimethoxyphenol (5.52 g ; 30 mmol) after purification by flash chromatography eluting with increasingly polar mixtures of EtOAc/hexanes (0 to 50% EtOAc).

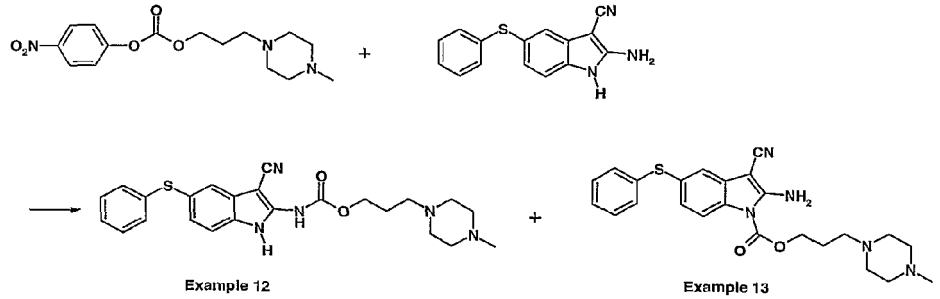
Yield : 83%

^{1}H NMR spectrum ($CDCl_3$) : 3.8 - 3.84 (m, 9H, **c** and 9H, **d**) ; 6.31 (s, 2H, **c**) ; 6.33 (s, 2H, **d**) ; 6.63 (dd, 1H, **d**) ; 6.75 (dd, 1H, **c**) ; 6.77 - 6.84 (m, 1H, **c** and 1H, **d**) ; 7.99 - 8.09 (m, 1H, **c** and 1H, **d**).

Examples 12 and Example 13

5-Phenylsulphanyl-2-(4-methylpiperazin-1-ylpropoxycarbonylamino)-1*H*-indole-3-carbonitrile, and

5-Phenylsulphanyl-1-(4-methylpiperazin-1-ylpropoxycarbonyl)-2-amino-1*H*-indole-3-carbonitrile respectively



Example 12 Example 13

A solution of 5-phenylsulphanyl-2-amino-1*H*-indole-3-carbonitrile (265 mg ; 1.0 mmol) in DMF (40 ml) under argon at room temperature was treated portionwise with NaH (200 g ; 5 mmol ; 60 % dispersion in oil). The mixture was stirred at room temperature for 30 minutes and 3-(4-methylpiperazin-1-yl)propyl 4-nitrophenyl carbonate (627 mg ; 1.5 mmol) was added

portionwise. The mixture was stirred for 1 hour, poured into water and extracted with EtOAc. The organic phase was washed with H_2O, brine and dried over $MgSO_4$. The residue was triturated with Et_2O to give5-phenylsulphanyl-1-(4-methylpiperazin-1-ylpropoxycarbonyl)-2-amino-1*H*-indole-3-carbonitrile as a beige solid (162 mg).

Yield : 36%

^{1}H NMR spectrum (DMSO d_6) : 1.95 (t, 2H) ; 2.05 (s, 3H) ; 2.05 - 2.55 (m, 10H) ; 4.52 (t, 2H) ; 7.11 (m, 2H) ; 7.26 (m, 3H) ; 7.34 (m, 2H) ; 7.82 (d, 1H) ; 7.89 (s, 2H).

LCMS-ESI : 450 $[M+H]^+$

Elemental analysis :	Found	C 53.93	H 5.48	N 13.10	S 5.55
$C_{24}H_{27}N_5O_2S$ 2.35 HCl	Requires	C 53.86	H 5.53	N 13.08	S 5.99

The filtrate was purified by flash chromatography eluting with MeOH/CH_2Cl_2 (10 % MeOH) and the appropriate fractions were evaporated to give 5-phenylsulphanyl-2-(4-methylpiperazin-1-ylpropoxycarbonylamino)-1*H*-indole-3-carbonitrile (40 mg).

Yield : 9%

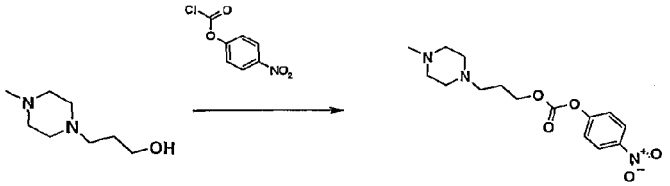
^{1}H NMR spectrum (DMSO d_6) : 1.81 (t, 2H) ; 2.15 (s, 3H) ; 2.2 - 2.25 (m, 10H) ; 4.21 (t, 2H) ; 7.16 - 7.25 (m, 4H) ; 7.3 (m, 2H) ; 7.46 (d, 1H) ; 7.55 (d, 1H) ; 11.2 (br s, 1H) ; 12.1 (br s, 1H).

LCMS-ESI : 450 $[M+H]^+$.

Elemental analysis :	Found	C 62.04	H 5.99	N 14.30	S 5.98
$C_{24}H_{27}N_5O_2S$; 0.4 HCl 0.2 EtOAc	Requires	C 61.83	H 6.07	N 14.54	S 6.66

3-(4-Methylpiperazin-1-yl)-propyl-4-nitrophenyl carbonate

This starting material was prepared as follows :



4-Nitrophenyl chloroformate (636 mg ; 3.16 mmol) was added to a solution of 3-(4-methylpiperazin-1-yl) propanol [Synthesis (1997), 6, 643-648] (500 mg ; 3.16 mmol) and triethylamine (440 ul ; 3.16 mmol) in dichloromethane (20 ml) under argon atmosphere at

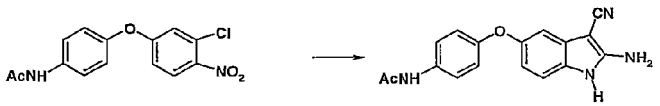
0°C. The mixture was stirred at ambient temperature for 3 hours, evaporated to dryness and triturated with Et_2O/aq. HCl (2N). The precipitate was filtered, washed with Et_2O and dried to give the dihydrochloride of 3-(4-methylpiperazin-1-yl)-propyl 4-nitrophenyl carbonate (1.22g).

Yield : 98 %.

^{1}H NMR (DMSO d_6) : 2.16 (m, 2H) ; 2.81 (s, 3H) ; 3.1-3.8 (m, 10H) ; 4.33 (t, 2H) ; 7.57 (d, 2H) ; 8.31 (d, 2H).

Example 14

5-(4-Acetylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile



Example 14

5-(4-Acetylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile (4.82 g) was prepared using the general method described in Example 2, except that two equivalents of aqueous NaOH were employed, it was obtained as a beige solid from 4-(4-acetylaminophenoxy)-2-chloro-nitrobenzene (6.13 g ; 20 mmol) after purification by trituration with a mixture of EtOAc/CH_2Cl_2.

Yield : 79%

^{1}H NMR spectrum (DMSO d_6) : 2.02 (s, 3H) ; 6.59 (dd, 1H) ; 6.66 (d, 1H) ; 6.79 (s, 2H) ; 6.89 (d, 2H) ; 7.10 (d, 1H) ; 7.53 (d, 2H) ; 9.88 (s, 1H) ; 10.69 (br s, 1H).

LCMS-ESI : 307 $[M+H]^+$

Elemental analysis :	Found	C 66.39	H 4.77	N 17.67
$C_{17}H_{14}N_4O_2$ 0.1 EtOAc	Requires	C 66.32	H 4.73	N 17.78

The starting material was prepared as follows:-

4-(4-Acetylaminophenoxy)-2-amino-nitrobenzene

4-(4-Acetylaminophenoxy)-2-amino-nitrobenzene (46.57g) was prepared from 4-acetylaminophenol (30.2 g ; 0.2 mol) using the general method described for

4-(4-hydroxyphenylsulphanyl)-2-amino-nitrobenzene in Example 2, except that one equivalent of NaH was used.

Yield : 81%

^{1}H NMR spectrum (DMSO d_6) : 2.07 (s, 3H) ; 6.3 (m, 2H) ; 7.12 (d, 2H) ; 7.48 (s, 2H) ; 7.68 (d, 2H) ; 8.01 (d, 1H) ; 10.06 (s, 1H).

4-(4-Acetylaminophenoxy)-2-chloro-nitrobenzene

Similarly, using the general method described for 4-(4-hydroxyphenylsulphanyl)-2-chloro-nitrobenzene in Example 2, 4-(4-acetylaminophenoxy)-2-chloro-nitrobenzene (11.05 g) was prepared from 4-(4-acetylaminophenoxy)-2-amino-nitrobenzene (14.35 g ; 0.5 mol) after purification by flash chromatography eluting with increasingly polar mixtures of EtOAc/hexanes (60 to 100% EtOAc).

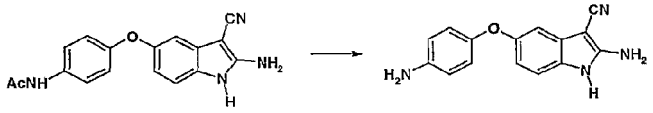
Yield : 72%

^{1}H NMR spectrum (DMSO d_6) : 2.07 (s, 3H) ; 7.05 (dd, 1H) ; 7.17 (d, 2H) ; 7.27 (d, 1H) ; 7.70 (d, 2H) ; 8.14 (d, 1H) ; 10.09 (s, 1H).

LCMS-ESI : 307 $[M+H]^+$

Example 15

5-(4-Aminophenoxy)-2-amino-1*H*-indole-3-carbonitrile



Example 15

A suspension of 5-(4-acetylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile (Example 14)(306 mg ; 1 mmol) in MeOH (10 ml) was treated with water (2 ml) and conc. HCl (1 ml) and the mixture heated under reflux for 30 hours. After cooling the mixture was poured into sat. aqueous $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with brine and dried over $MgSO_4$. The residue was purified by flash chromatography eluting with increasingly polar mixtures of EtOAc/hexanes (80 to 100% EtOAc) to give 5-(4-aminophenoxy)-2-amino-1*H*-indole-3-carbonitrile as a pale beige solid (251 mg).

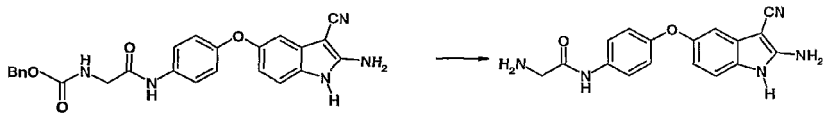
Yield : 95%

^{1}H NMR spectrum (DMSO d_6) : 4.89 (s, 2H) ; 6.55 (m, 4H) ; 6.73 (m, 4H) ; 7.06 (d, 1H) ; 10.61 (br s, 1H).

LCMS-ESI : 265 $[M+H]^+$				
Elemental analysis :	Found	C 67.40	H 4.72	N 20.38
$C_{15}H_{12}N_4O$; 0.1 H_2O 0.1 EtOAc	Requires	C 67.29	H 4.77	N 20.38

Example 16

5-(4-Glycylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile



Example 16

A solution of 5-[4-({N-benzyoxycarbonylglycyl}amino)phenoxy]-2-amino-1*H*-indole-3-carbonitrile (368 mg ; 0.8 mmol) in EtOAc (10 ml) and MeOH (10 ml) was hydrogenated over Pd/C (100 mg) at 1 Atmosphere at room temperature for 1 hour. After filtration and evaporation to dryness, the residue was purified by flash chromatography eluting with MeOH/CH_2Cl_2 (10 % MeOH) and the appropriate fractions were evaporated to give 5-(4-glycylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile (163 mg).

Yield : 63%

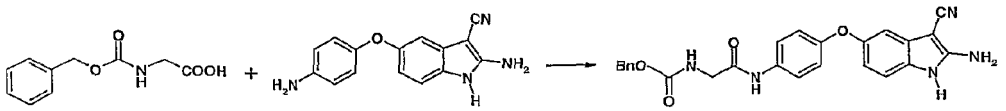
^{1}H NMR spectrum (DMSO d_6) : 2.03 (s, 2H) ; 3.24 (m, 2H) ; 6.59 (dd, 1H) ; 6.66 (d, 1H) ; 6.79 (s, 2H) ; 6.91 (d, 2H) ; 7.10 (d, 1H) ; 7.59 (d, 2H) ; 9.72 (br s, 1H) ; 10.69 (br s, 1H).

LCMS-ESI : 322 $[M+H]^+$

Elemental analysis :	Found	C 62.29	H 5.03	N 20.83
$C_{17}H_{15}N_5O_2$; 0.2 H_2O 0.2 MeOH	Requires	C 62.35	H 4.93	N 21.14

5-[4-({N-Benzyoxycarbonylglycyl}amino)phenoxy]-2-amino-1*H*-indole-3-carbonitrile

This was prepared as follows:-



A solution of carbobenzyloxyglycine (480 mg ; 2.26 mmol) and 5-(4-aminophenoxy)-2-amino-1*H*-indole-3-carbonitrile (Example 15) (300 mg ; 1.13 mmol) in CH_2Cl_2 (10 ml) was treated at room temperature with EDCI (546 mg ; 2.8 mmol) and a catalytic quantity of DMAP (20 mg). The mixture was stirred for 1 hour and was directly purified by flash

chromatography eluting with CH_2Cl_2/EtOAc (50/50). The appropriate fractions were evaporated to give 5-[4-({N-benzyoxycarbonylglycyl}amino)phenoxy]-2-amino-1*H*-indole-3-carbonitrile as a white solid (425 mg).

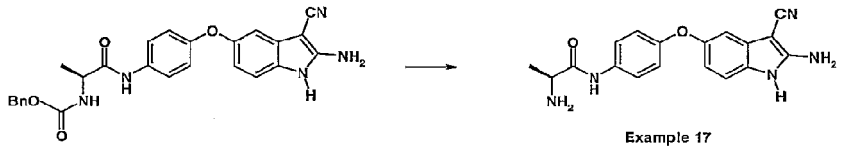
Yield : 83%

^{1}H NMR spectrum (DMSO d_6) : 3.79 (d, 2H) ; 5.05 (s, 2H) ; 6.59 (dd, 1H) ; 6.67 (d, 1H) ; 6.80 (s, 2H) ; 6.91 (d, 2H) ; 7.10 (d, 1H) ; 7.2 - 7.45 (m, 5H) ; 7.5 - 7.6 (m, 3H) ; 9.92 (s, 1H); 10.69 (s, 1H).

LCMS-ESI : 454 $[M-H]^-$

Example 17

5-(4-Alanylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile



Example 17

Using the general method of hydrogenolysis described in Example 16, 5-(4-alanylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile (213 mg) was obtained as a white solid from 5-[4-({N-benzyloxycarbonylalanyl}amino)phenoxy]-2-amino-1*H*-indole-3-carbonitrile (514 mg ; 1.1 mmol) after purification by flash chromatography eluting with MeOH/CH_2Cl_2 (10 % MeOH).

Yield : 58%

^{1}H NMR spectrum (DMSO d_6) : 1.20 (d, 3H) ; 1.98 (m, 2H) ; 3.40 (q, 1H) ; 6.59 (dd, 1H) ; 6.65 (d, 1H) ; 6.80 (s, 2H) ; 6.90 (m, 2H) ; 7.09 (d, 1H) ; 7.59 (m, 2H) ; 9.7 (br s, 1H) ; 10.6 (br s, 1H).

LCMS-ESI : 336 $[M+H]^+$

Elemental analysis :	Found	C 63.79	H 5.21	N 20.52
$C_{18}H_{17}N_5O_2$; 0.1 H_2O 0.1 MeOH	Requires	C 63.87	H 5.21	N 20.58

5-[4-({N-Benzyloxycarbonylalanyl}amino)phenoxy]-2-amino-1*H*-indole-3-carbonitrile

This was prepared using the general coupling method described in Example 16, 5-[4-({N-benzyloxycarbonylalanyl}amino)phenoxy]-2-amino-1*H*-indole-3-carbonitrile (514 mg) was obtained as a white solid from 5-(4-aminophenoxy)-2-amino-1*H*-indole-3-

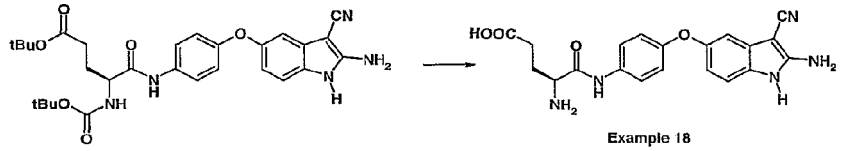
carbonitrile (Example 15)(400 mg ; 1.51 mmol) after purification by flash chromatography eluting with EtOAc/CH_2Cl_2 (50/50).

Yield : 72%

^{1}H NMR spectrum (DMSO d_6) : 1.29 (d, 3H) ; 4.18 (m, 1H) ; 5.03 (d, 2H) ; 6.58 (dd, 1H) ; 6.67 (d, 1H) ; 6.80 (s, 2H) ; 6.91 (d, 2H) ; 7.10 (d, 1H) ; 7.25 - 7.45 (m, 5H) ; 7.5 - 7.6 (m, 3H) ; 9.94 (s, 1H) ; 10.71 (s, 1H).

Example 18

5-(4-α-Glutamylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile



Example 18

A suspension of a tertiary butoxycarbonyl protected derivative of 5-(4-α-glutamylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile (1.21 g ; 2.2 mmol) in CH_2Cl_2 (100 ml) was treated with a rapid stream of gaseous HCl for 2 hours at room temperature. The mixture was evaporated to dryness, taken up in water, filtered and the filtrate freeze-dried to give the hydrochloride salt of 5-(4-α-glutamylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile as a white powder (975 mg).

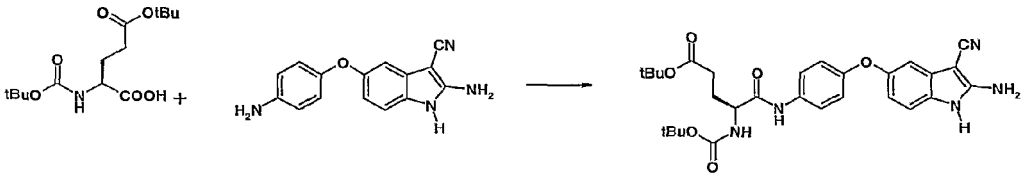
Yield : 100%

^{1}H NMR spectrum (DMSO d_6) : 2.0 - 2.15 (m, 2H) ; 2.40 (m, 2H) ; 3.8 (br s, 1H) ; 4.04 (m, 1H) ; 6.60 (dd, 1H) ; 6.69 (d, 1H) ; 6.75 (br s, 1H) ; 6.95 (d, 2H) ; 7.12 (d, 1H) ; 7.59 (d, 2H) ; 8.43 (m, 3H) ; 10.79 (br s, 1H) ; 10.81 (br s, 1H) 12.2 (br s, 1H).

LCMS-ESI : 394 $[M+H]^+$

Elemental analysis :	Found	C 52.88	H 4.83	N 15.06	Cl 8.97
$C_{17}H_{15}N_3O_2S$; 1.0 H_2O 1.15 HCl	Requires	C 52.97	H 4.93	N 15.44	Cl 8.99

The tertiary butoxycarbonyl protected derivative of 5-(4-α-glutamylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile (1.49g) was prepared using the general coupling method described in



Example 16, as a white solid from 5-(4-aminophenoxy)-2-amino-1*H*-indole-3-carbonitrile (Example 15) (800 mg ; 3.00 mmol) after purification by flash chromatography eluting with increasingly polar mixtures of EtOAc/hexanes (70 to 100% EtOAc).

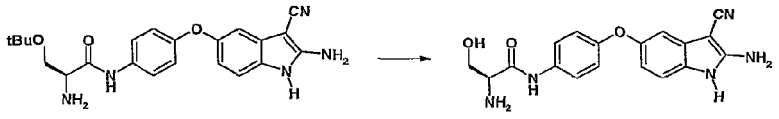
Yield : 91%

^{1}H NMR spectrum (DMSO d_6) : 1.38 (s, 18H) ; 1.80 (m, 1H) ; 1.90 (m, 1H) ; 2.26 (m, 2H); 4.05 (m, 1H) ; 6.59 (dd, 1H) ; 6.68 (d, 1H) ; 6.80 (s, 2H) ; 6.91 (d, 2H) ; 7.01 (d, 1H) ; 7.10 (d, 1H) ; 7.56 (d, 2H) ; 9.91 (s, 1H) ; 10.69 (s, 1H).

MS-ESI : 548 $[M-H]^-$

Example 19

5-(4-Serylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile



Example 19

Using the general method of deprotection described in Example 18, 5-(4-serylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile (554 mg) was obtained as its hydrochloride as a white solid from 5-[4-(O-*t*-butoxycarbonylserylamino)phenoxy]-2-amino-1*H*-indole-3-carbonitrile (800 mg ; 2.0 mmol) after trituration with water, filtration and drying.

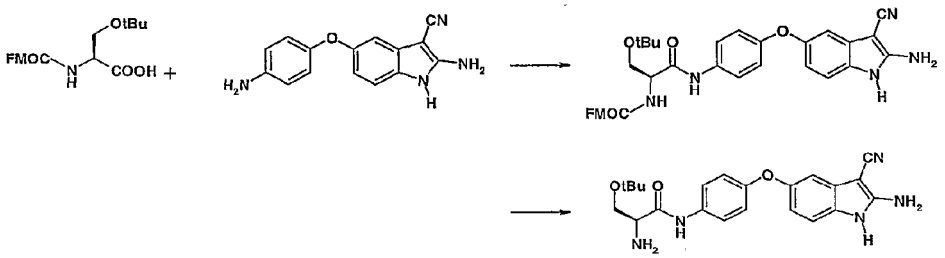
Yield : 73%

^{1}H NMR spectrum (DMSO d_6) : 3.87 (m, 2H) ; 4.03 (m, 1H) ; 5.58 (m, 1H) ; 6.61 (dd, 1H) ; 6.68 (d, 1H) ; 6.85 (s, 2H) ; 6.96 (d, 2H) ; 7.13 (d, 1H) ; 7.62 (d, 2H) ; 8.31 (br s, 3H) ; 10.69 (s, 1H) ; 10.82 (s, 1H).

LCMS-ESI : 352 $[M+H]^+$

Elemental analysis :	Found	C 53.55	H 4.87	N 17.27
$C_{18}H_{17}N_5O_3$; 1.4 HCl	Requires	C 53.73	H 4.61	N 17.40

5-[4-(O-*t*-Butyloxycarbonylserylamino)phenoxy]-2-amino-1*H*-indole-3-carbonitrile was prepared as follows:-



5-[4-({N-FMOC-O-*t*-butoxycarbonylseryl}amino)phenoxy]-2-amino-1*H*-indole-3-carbonitrile

Using the general coupling method described in Example 16, 5-[4-({N-FMOC-O-*t*-butoxycarbonylseryl}amino)phenoxy]-2-amino-1*H*-indole-3-carbonitrile (1.68 g) was obtained as a white solid from 5-(4-aminophenoxy)-2-amino-1*H*-indole-3-carbonitrile (Example 15)(800 mg ; 3.00 mmol) after purification by flash chromatography eluting with increasingly polar mixtures of EtOAc/hexanes (70 to 100% EtOAc).

Yield : 89%

^{1}H NMR spectrum (DMSO d_6) : 1.12 (s, 9H) ; 3.55 (m, 2H) ; 4.15 - 4.35 (m, 4H) ; 6.59 (dd, 1H) ; 6.69 (d, 1H) ; 6.80 (s, 2H) ; 6.92 (d, 2H) ; 7.11 (d, 1H) ; 7.33 (m, 2H) ; 7.42 (m, 2H) ; 7.5 - 7.7 (m, 3H) ; 7.75 (m, 2H) ; 7.89 (d, 2H) ; 9.98 (s, 1H) ; 10.70 (s, 1H).

MS-ESI : 630 $[M+H]^+$

5-[4-(O-*t*-Butyloxycarbonylserylamino)phenoxy]-2-amino-1*H*-indole-3-carbonitrile

A solution of 5-[4-({N-FMOC-O-*t*-butoxycarbonylseryl}amino)phenoxy]-2-amino-1*H*-indole-3-carbonitrile (1.45 g ; 2.3 mmol) in $CHCl_3$ (30 ml) was treated dropwise with piperidine (3.25 ml). The mixture was stirred for 3 hours at room temperature and was directly purified by flash chromatography eluting with increasingly polar mixtures of MeOH/CH_2Cl_2 (2.5 to 10% MeOH). The appropriate fractions were evaporated to give 5-[4-(O-*t*-butoxycarbonylserylamino)phenoxy]-2-amino-1*H*-indole-3-carbonitrile as a white solid (878 mg).

Yield : 94%

^{1}H NMR spectrum (DMSO d_6) : 1.34 (s, 9H) ; 1.93 (br s, 2H) ; 3.4 - 3.55 (m, 3H) ; 6.61 (dd, 1H) ; 6.69 (d, 1H) ; 6.82 (s, 2H) ; 6.92 (d, 2H) ; 7.12 (d, 1H) ; 7.62 (d, 2H) ; 9.83 (br s, 1H) ; 10.73 (br s, 1H).

MS-ESI : 408 $[M+H]^+$

Example 20

6-Benzyloxy-2-amino-1*H*-indole-3-carbonitrile



Example 20

Using the general method described in Example 2, except that two equivalents of aqueous NaOH were employed and the reaction was warmed to 100°C in the first step, 6-benzyloxy-2-amino-1*H*-indole-3-carbonitrile (110 mg) was obtained as a beige solid from 5-benzyloxy-2-chloro-nitrobenzene (1.31 g ; 5.0 mmol) after purification by flash chromatography eluting with increasingly polar mixtures of EtOAc/hexanes (30 to 70% EtOAc).

Yield : 8%

^{1}H NMR spectrum (DMSO d_6) : 5.05 (s, 2H) ; 6.59 (s, 2H) ; 6.70 (dd, 1H) ; 6.82 (d, 1H) ; 7.01 (d, 1H) ; 7.31 (t, 1H) ; 7.38 (t, 2H) ; 7.44 (d, 2H) ; 10.56 (s, 1H).

LCMS-ESI : 264 $[M+H]^+$

Elemental analysis :	Found	C 72.11	H 4.91	N 15.84
$C_{16}H_{13}N_3O$; 0.2 H_2O	Requires	C 72.0	H 5.06	N 15.74

5-Benzyloxy-2-chloro-nitrobenzene

This was prepared as follows:- A solution of benzyl bromide (10.26 g ; 60 mmol) and 4-chloro-3-nitrophenol (8.7 g ; 50 mmol) in DMF (250 ml) was treated with Na_2CO_3 (10.6 g ; 0.1 mol) and the mixture was stirred at room temperature for 3 days. The mixture was poured into water and extracted with Et_2O. The organic phase was washed with brine and dried over $MgSO_4$. The residue was purified by flash chromatography eluting with CH_2Cl_2 to give 5-benzyloxy-2-chloro-nitrobenzene as a pale yellow solid (12.32 g).

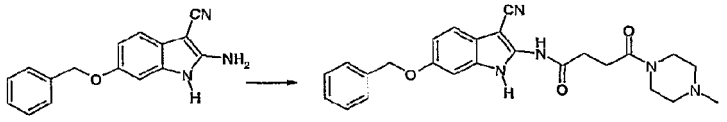
Yield : 94%

^{1}H NMR spectrum ($CDCl_3$) : 5.10 (s, 2H) ; 7.12 (dd, 1H) ; 7.36 - 7.43 (m, 6H) ; 7.47 (d, 1H).

Elemental analysis :	Found	C 58.72	H 3.81	N 5.26
$C_{13}H_{10}ClNO_3$; 0.1 H_2O	Requires	C 58.82	H 3.87	N 5.28

Example 21

6-Benzyloxy-2-(4-methylpiperazin-1-yl-4-oxobutanoylamino)-1*H*-indole-3-carbonitrile



Example 21

Using the general method described for Examples 12 and 13 , 6-benzyloxy-2-(4-methylpiperazin-1-yl-4-oxobutanoylamino)-1*H*-indole-3-carbonitrile (128 mg) was obtained as a white solid from 6-benzyloxy-2-amino-1*H*-indole-3-carbonitrile (Example 20)(120 mg ; 0.46 mmol) after purification by flash chromatography eluting with increasingly polar mixtures of MeOH/CH_2Cl_2 (3 to 10% MeOH).

Yield : 63%

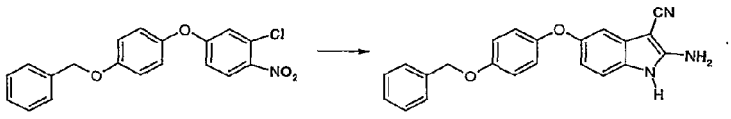
^{1}H NMR spectrum (DMSO d_6) : 2.18 (s, 3H) ; 2.22 (m, 2H) ; 2.31 (m, 2H) ; 2.6 - 2.75 (m, 4H) ; 3.4 - 3.5 (m, 4H) ; 5.08 (s, 2H) ; 6.68 (dd, 1H) ; 7.18 (d, 1H) ; 7.31 (d, 1H) ; 7.33 (t, 1H) ; 7.39 (t, 2H) ; 7.46 (d, 2H) ; 11.4 (br s, 1H) ; 11.9 (br s, 1H).

LCMS-ESI : 446 $[M+H]^+$

Elemental analysis :	Found	C 61.52	H 6.11	N 14.03
$C_{25}H_{27}N_5O_3$; 1.1 HCl 0.2 H_2O	Requires	C 61.38	H 5.87	N 14.31

Example 22

5-(4-Benzyloxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile



Example 22

Using the general method described in Example 2, except that two equivalents of aqueous NaOH were employed, 5-(4-benzyloxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile (1.28 g) was obtained as a white solid from 4-(4-benzyloxyphenoxy)-2-chloro-nitrobenzene (2.1 g ; 5.9

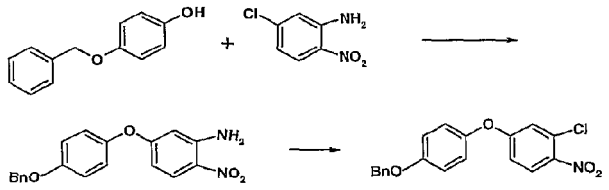
mmol) after purification by flash chromatography eluting with increasingly polar mixtures of CH_2Cl_2/EtOAc (0 to 80% EtOAc).

Yield : *61%*

^{1}H NMR spectrum (DMSO d_6) : 5.07 (s, 2H) ; 6.58 (dd, 1H) ; 6.63 (d, 1H) ; 6.78 (s, 2H) ; 6.92 (m, 2H) ; 7.00 (m, 2H) ; 7.09 (d, 1H) ; 7.34 (t, 1H) ; 7.40 (t, 2H) ; 7.46 (d, 2H) ; 10.69 (br s, 1H).

LCMS-ESI : 356 $[M+1]^+$

4-(4-Benzyloxyphenoxy)-2-chloro-nitrobenzene was prepared as follows:-



4-(4-Hydroxyphenylsulphanyl)-2-amino-nitrobenzene

Using the general method described for 4-(4-hydroxyphenylsulphanyl)-2-amino-nitrobenzene in Example 2, except that one equivalent of NaH was used, 4-(4-benzyloxyphenoxy)-2-amino-nitrobenzene (5.88 g) was prepared from 4-chloro-2-amino-nitrobenzene (4.30 g ; 25 mmol).

Yield : 70%

^{1}H NMR spectrum ($CDCl_3$) : 5.11 (s, 2H) ; 6.25 - 6.30 (m, 2H) ; 7.11 (s, 4H) ; 7.3 - 7.5 (m, 7H) ; 7.98 (d, 1H).

MS-ESI : 337 $[M+H]^+$

4-(4-Benzyloxyphenoxy)-2-chloro-nitrobenzene

Similarly, using the general method described for 4-(4-hydroxyphenylsulphanyl)-2-chloro-nitrobenzene in Example 2, 4-(4-benzyloxyphenoxy)-2-chloro-nitrobenzene (2.09 g) was prepared from 4-(4-benzyloxyphenoxy)-2-amino-nitrobenzene (9.6 g ; 28.5 mmol) after purification by flash chromatography eluting with increasingly polar mixtures of EtOAc/CH_2Cl_2 (10 to 20% EtOAc).

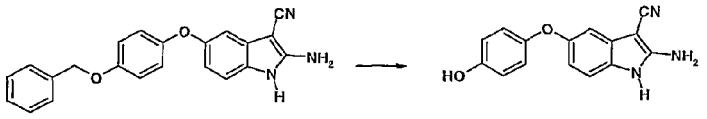
Yield : 20%

^{1}H NMR spectrum (DMSO d_6) : 5.13 (s, 2H) ; 7.01 (dd, 1H) ; 7.15 (m, 4H) ; 7.22 (d, 1H) ; 7.35 (t, 1H) ; 7.41 (t, 2H) ; 7.48 (d, 2H) ; 8.13 (d, 1H).

MS-ESI : 357 $[M+H]^+$

Example 23

5-(4-Hydroxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile



Example 23

Using the general method of hydrogenolysis described in Example 16, 5-(4-hydroxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile (570 mg) was obtained as a white solid from 5-(4-benzyloxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile (Example 22) (1.0 g ; 2.81 mmol) after purification by flash chromatography eluting with EtOAc.

Yield : 76%

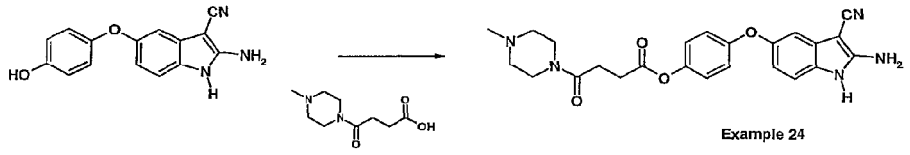
^1H NMR spectrum (DMSO d_6) : 6.54 (dd, 1H) ; 6.58 (d, 1H) ; 6.73 (d, 2H) ; 6.75 (s, 2H) ; 6.81 (d, 2H) ; 7.06 (d, 1H) 9.21 (br s , 1H) ; 10.64 (br s, 1H).

LCMS-ESI : 266 $[M+H]^+$

Elemental analysis :	Found	C 67.96	H 4.37	N 15.73
$C_{15}H_{11}N_3O_2$	Requires	C 67.92	H 4.18	N 15.84

Example 24

5-[4-(4-Methylpiperazin-1-yl-4-oxobutanoyloxy)phenoxy]-2-amino-1*H*-indole-3-carbonitrile



Example 24

A solution of 5-(4-hydroxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile (Example 23)(130 mg; 0.5 mmol) and 3-(N-methylpiperazin-1-ylcarbonyl)propanoic acid(128 mg 0.63 mmol) in a mixture of CH_2Cl_2 (5 ml) and CH_3CN (0.5 ml) was treated at room temperature with EDCI (122 mg ; 0.63 mmol) and a catalytic quantity of DMAP (20 mg). The mixture was stirred for overnight and was directly purified by flash chromatography eluting with increasingly polar mixtures of MeOH/CH_2Cl_2 (5 to 10% MeOH). The appropriate

fractions were evaporated to give 5-[4-(4-methylpiperazin-1-yl-4-oxobutanoyloxy)phenoxy]-2-amino-1*H*-indole-3-carbonitrile as a white solid (150 mg).

Yield : 67%

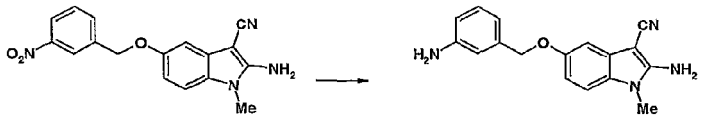
^{1}H NMR spectrum (DMSO d_6) : 2.17 (s, 3H) ; 2.23 (m, 2H) ; 2.30 (m, 2H) ; 2.72 (m, 4H) ; 3.45 (m, 4H) ; 6.61 (dd, 1H) ; 6.73 (d, 1H) ; 6.82 (s, 2H) ; 6.94 (d, 2H); 7.04 (d, 2H) ; 7.13 (d, 1H) ; 10.73 (s, 1H).

MS-ESI : 448 $[M+H]^+$

Elemental analysis :	Found	C 62.56	H 5.98	N 15.02
$C_{24}H_{25}N_5O_4$; 0.8 H_2O	Requires	C 62.41	H 5.80	N 15.16

Example 25

5-(3-Aminobenzyloxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile



Example 25

A solution of 5-(3-nitrobenzyloxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (40 mg ; 0.12 mmol) in MeOH (6 ml) was treated with K_2CO_3 (6 mg) and hydrogenated over PtO_2 (6 mg) at 45 psi at room temperature for 1 hour. After filtration and evaporation to dryness, the residue was purified by flash chromatography eluting with MeOH/CH_2Cl_2 (5 % MeOH) and the appropriate fractions were evaporated to give 5-(3-aminobenzyloxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (32 mg).

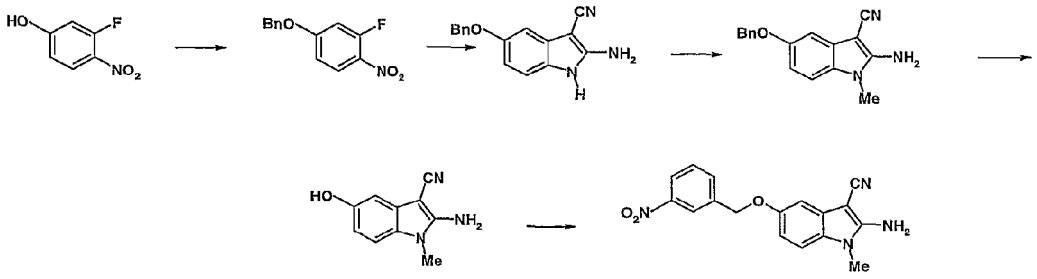
Yield : 91%

^{1}H NMR spectrum (DMSO d_6) : 3.48 (s, 3H) ; 4.94 (s, 2H) ; 5.09 (s, 2H) ; 6.50 (dd, 1H) ; 6.57 (d, 1H) ; 6.63 (m, 2H) ; 6.77 (d, 1H) ; 6.90 (s, 2H) ; 7.01 (t, 1H) ; 7.11 (d, 1H).

LCMS-ESI : 293 $[M+H]^+$

Elemental analysis :	Found	C 64.10	H 5.64	N 16.57
$C_{17}H_{16}N_4O$; 0.75 H_2O 0.1 MeOH 0.2 CH_2Cl_2	Requires	C 63.73	H 5.66	N 17.18

5-(3-Nitrobenzyloxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile was prepared as follows:-



4-Benzyloxy-2-fluoronitrobenzene

A solution of 3-fluoro-4-nitrophenol (25 g ; 0.159 mol) in DMF (600 ml) under argon was treated at room temperature with Na_2CO_3 (33.7 g ; 0.318 mol). The mixture was stirred and treated dropwise with benzyl bromide (32.6 g ; 0.19 mol). Stirring was continued for 3 days when the mixture was poured into water and extracted with Et_2O. The organic phase was washed with water, brine and dried over $MgSO_4$. Evaporation gave 4-benzyloxy-2-fluoronitrobenzene as a yellow solid (38 g).

Yield : 97%

^{1}H NMR spectrum ($CDCl_3$) : 5.15 (s, 2H) ; 6.8 - 6.9 (m, 2H) ; 7.35 - 7.5 (m, 5H) ; 8.1 (t, 1H).

Elemental analysis :	Found	C 63.25	H 4.24	N 5.73
$C_{13}H_{10}FNO_3$	Requires	C 63.16	H 4.08	N 5.67

5-Benzyloxy-2-amino-1*H*-indole-3-carbonitrile

Using the general method described in Example 2, except that two equivalents of aqueous NaOH were employed, 5-benzyloxy-2-amino-1*H*-indole-3-carbonitrile (31.5 g) was obtained as a pale rose solid from 4-benzyloxy-2-fluoronitrobenzene (38 g ; 0.15 mol) after purification by trituration with CH_2Cl_2.

Yield : 80%

^{1}H NMR spectrum (DMSO d_6) : 5.07 (s, 2H) ; 6.58 (dd, 1H) ; 6.66 (s, 2H) ; 6.75 (d, 1H) ; 7.01 (d, 1H) ; 7.25 - 7.5 (m, 5H) ; 10.51 (s, 1H).

LCMS-ESI : 264 $[M+H]^+$

Elemental analysis :	Found	C 70.91	H 4.95	N 15.77
$C_{16}H_{13}N_3O$; 0.4 H_2O	Requires	C 71.04	H 5.14	N 15.53

5-Benzyloxy-2-amino-1-methyl-1*H*-indole-3-carbonitrile

A solution of 5-benzyloxy-2-amino-1*H*-indole-3-carbonitrile (2.96 g ; 11.25 mmol) in DMF (20 ml) under argon was treated at room temperature with Cs_2CO_3 (4.4 g ; 13.5 mmol). The mixture was stirred and treated dropwise with methyl iodide (0.84 ml ; 13.48 mmol). Stirring was continued for 1 hour when the mixture was poured into water. The precipitate was filtered, washed with water and dried to give 5-benzyloxy-2-amino-1-methyl-1*H*-indole-3-carbonitrile as a beige solid (2.77 g).

Yield : 90%

^{1}H NMR (DMSO d_6) : 3.49 (s, 3H) ; 5.11 (s, 3H) ; 6.68 (dd, 1H) ; 6.82 (d, 1H) ; 6.91 (s, 2H) ; 7.13 (d, 1H) ; 7.35 (t, 1H) ; 7.41 (m, 2H) ; 7.47 (d, 2H).

Elemental analysis :	Found	C 72.95	H 5.39	N 15.16
$C_{17}H_{15}N_3O$; 0.2 H_2O	Requires	C 72.68	H 5.53	N 14.96

5-Hydroxy-2-amino-1-methyl-1*H*-indole-3-carbonitrile

Using the general method of hydrogenolysis described in Example16, 5-hydroxy-2-amino-1-methyl-1*H*-indole-3-carbonitrile (1.81 g) was obtained as a white solid from 5-benzyloxy-2-amino-1-methyl-1*H*-indole-3-carbonitrile (2.6 g ; 9.38 mmol) after purification by flash chromatography eluting with EtOAc.

Yield : 100%

^{1}H NMR spectrum (DMSO d_6) : 3.45 (s, 3H) ; 6.43 (dd, 1H) ; 6.56 (d, 1H) ; 6.81 (s, 2H) ; 6.99 (d, 1H) ; 8.83 (s, 1H).

LCMS-ESI : 188 $[M+H]^+$

Elemental analysis :	Found	C 64.05	H 4.92	N 22.20
$C_{10}H_9N_3O$	Requires	C 64.16	H 4.85	N 22.45

5-(3-Nitrobenzyloxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile

A solution of 5-hydroxy-2-amino-1-methyl-1*H*-indole-3-carbonitrile (100 mg ; 0.53 mmol) and 3-nitrobenzyl bromide (130 mg ; 0.6 mmol) in DMF (5 ml) was treated with Cs_2CO_3 (520 mg ; 1.6 mmol) and the mixture was stirred for 2 hours. Water (80 ml) was added and the precipitate was filtered, taken up in methanol, filtered, washed and dried to give 5-(3-nitrobenzyloxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (96 mg).

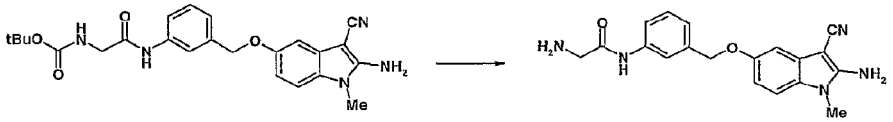
Yield : 56%

^{1}H NMR spectrum (DMSO d_6) : 3.49 (s, 3H) ; 5.28 (s, 2H) ; 6.71 (dd, 1H) ; 6.85 (d, 1H) ; 6.94 (s, 2H) ; 7.15 (d, 1H) ; 7.72 (t, 1H) ; 7.94 (d, 1H) ; 8.20 (d, 1H) ; 8.33 (s, 1H).

LCMS-ESI : 321 $[M-H]^-$

Example 26

5-(3-Glycylbenzyloxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile



Example 26

A solution of 5-[3-(N-*t*-butoxycarbonylglycyl)benzyloxy]-2-amino-1-methyl-1*H*-indole-3-carbonitrile (188 mg ; 0.42 mmol) in EtOAc (10 ml) that was saturated with gaseous HCl was stirred at room temperature for 24 hours. An excess of Et_2O was added and the precipitate was filtered and dried to give the hydrochloride of 5-(3-glycylbenzyloxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (170 mg).

Yield : 100%

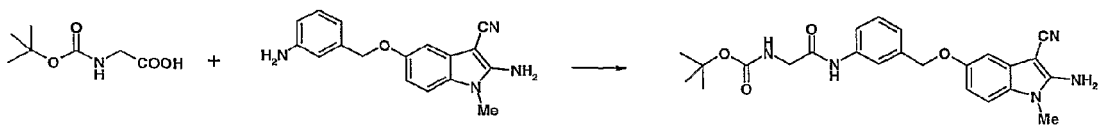
^{1}H NMR spectrum (DMSO d_6) : 3.47 (s, 3H) ; 3.80 (s, 2H) ; 5.10 (s, 2H) ; 6.65 (dd, 1H) ; 6.78 (d, 1H) ; 6.95 (br s, 2H) ; 7.10 (d, 1H) ; 7.18 (d, 1H) ; 7.36 (t, 1H) ; 7.58 (d, 1H) ; 7.68 (s, 1H) ; 8.14 (br s, 3H) ; 10.54 (s, 1H).

LCMS-ESI : 350 $[M+H]^+$

Elemental analysis:	Found	C 52.53	H 5.58	N 15.53
$C_{19}H_{19}N_5O_2$; 0.8 H_2O 0.3 EtOAc 1.9 HCl	Requires	C 52.80	H 5.46	N 15.24

5-[3-(N-*t*-Butyloxycarbonylglycyl)benzyloxy]-2-amino-1-methyl-1*H*-indole-3-carbonitrile

5-[3-(N-*t*-Butoxycarbonylglycyl)benzyloxy]-2-amino-1-methyl-1*H*-indole-3-carbonitrile (188 mg)was prepared using the general coupling method described in Example 16



as a white solid from 5-(3-aminobenzyloxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile

(150 mg ; 0.51 mmol) after purification by flash chromatography eluting with EtOAc/CH_2Cl_2 (50/50).

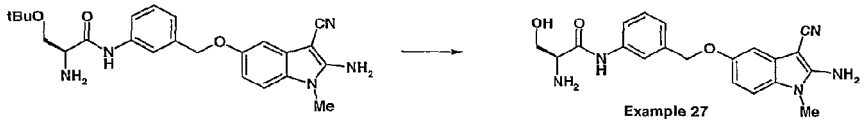
Yield : 80%

^{1}H NMR spectrum (DMSO d_6) : 1.42 (s, 9H) ; 3.50 (s, 3H) ; 3.74 (d, 2H) ; 5.09 (s, 2H) ; 6.67 (dd, 1H) ; 6.82 (d, 1H) ; 6.92 (s, 2H) ; 7.05 (t, 1H) ; 7.14 (m, 2H) ; 7.33 (t, 1H) ; 7.58 (d, 1H) ; 7.69 (s, 1H) ; 9.97 (s, 1H).

LCMS-ESI : 450 $[M+H]^+$

Example 27

5-(3-Serylbenzyloxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile



Using the general method of deprotection described in Example 18, 5-(3-serylbenzyloxy)-2-amino-1*H*-indole-3-carbonitrile (177 mg) was obtained as its hydrochloride as a glassy solid from 5-[3-(O-t-butylseryl)benzyloxy]-2-amino-1-methyl-1*H*-indole-3-carbonitrile (280 mg ; 0.64 mmol) after purification by reverse phase C18 preparative HPLC eluting with MeOH/H_2O/HCl (80/19/1)

Yield : 70%

^{1}H NMR spectrum (DMSO d_6) : 3.49 (s, 3H) ; 3.7 (s, 1H) ; 3.87 (d, 2H) ; 4.04 (m, 1H) ; 5.11 (s, 2H) ; 6.66 (dd, 1H) ; 6.80 (d, 1H) ; 6.95 (br s, 2H) ; 7.12 (d, 1H) ; 7.20 (d, 1H) ; 7.38 (t, 1H) ; 7.62 (d, 1H) ; 7.75 (s, 1H) ; 8.30 (m, 3H) ; 10.67 (s, 1H).

LCMS-ESI : 380 $[M+H]^+$

Elemental analysis :	Found	C 51.84	H 5.51	N 14.72
$C_{20}H_{21}N_5O_3$; 1.4 HCl 1.8 H_2O	Requires	C 51.69	H 5.65	N 15.07

5-[3-(O-*t*-Butyl-N-FMOC-seryl)benzyloxy]-2-amino-1-methyl-1*H*-indole-3-carbonitrile

This was prepared using the general coupling method described in Example 16, 5-[3-(O-*t*-butyl-N-FMOC-seryl)benzyloxy]-2-amino-1-methyl-1*H*-indole-3-carbonitrile (730 mg) was obtained as a white solid from 5-(3-aminobenzyloxy)-2-amino-1-methyl-1*H*-

indole-3-carbonitrile (Example 25)(700 mg ; 2.38 mmol) after purification by flash chromatography eluting with EtOAc/CH_2Cl_2 (20% EtOAc).

Yield : 64%

^{1}H NMR spectrum (DMSO d_6) : 1.13 (s, 9H) ; 3.48 (s, 3H) ; 3.55 (m, 2H) ; 4.2 - 4.35 (m, 4H) ; 5.09 (s, 2H) ; 6.66 (dd, 1H) ; 6.81 (d, 1H) ; 6.92 (s, 2H) ; 7.13 (m, 2H) ; 7.33 (m, 3H) ; 7.43 (m, 2H) ; 7.56 (m, 2H) ; 7.76 (m, 3H) ; 7.90 (d, 2H) ; 10.06 (s, 1H).

5-[3-(O-*t*-Butylseryl)benzyloxy]-2-amino-1-methyl-1*H*-indole-3-carbonitrile

Using the general FMOC deprotection method described in Example 16, 5-[3-(O-*t*-butylseryl)benzyloxy]-2-amino-1-methyl-1*H*-indole-3-carbonitrile (280 mg) was obtained as a white solid from 5-[3-(O-*t*-butyl-N-FMOC-seryl)benzyloxy]-2-amino-1-methyl-1*H*-indole-3-carbonitrile (730 mg ; 1.52 mmol) after purification by flash chromatography eluting with increasingly polar mixtures of MeOH/CH_2Cl_2 (2 to 5% MeOH).

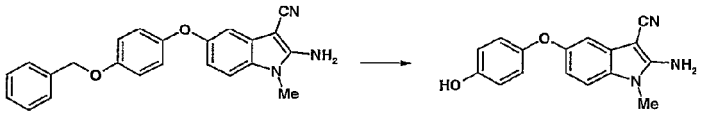
Yield : 42%

^{1}H NMR spectrum (DMSO d_6) : 1.13 (s, 9H) ; 1.93 (br s, 2H) ; 3.4 - 3.5 (m, 3H) ; 3.48 (s, 3H) ; 5.08 (s, 2H) ; 6.66 (dd, 1H) ; 6.80 (d, 1H) ; 6.91 (s, 2H) ; 7.13 (m, 2H) ; 7.32 (t, 1H) ; 7.61 (d, 1H) ; 7.76 (s, 1H) 9.88 (br s, 1H).

MS-ESI : 436 $[M+H]^+$

Example 28

5-(4-Hydroxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile



Example 28

Using the general method of hydrogenolysis described in Example 16 at 18 psi for 15 minutes, 5-(4-hydroxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (1.45 g) was obtained as a white solid from 5-(4-benzyloxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (2.02 g; 5.47 mmol) after purification by flash chromatography eluting with MeOH/CH_2Cl_2 (10 % MeOH).

Yield : 95%

^{1}H NMR spectrum (DMSO d_6) : 3.49 (s, 3H) ; 6.60 (dd, 1H) ; 6.61 (d, 1H) ; 6.74 (d, 2H) ; 6.82 (d, 2H) ; 6.99 (s, 2H) ; 7.16 (d, 1H) ; 9.22 (s, 1H).

LCMS-ESI : 280 $[M+H]^+$

5-(4-benzyloxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile

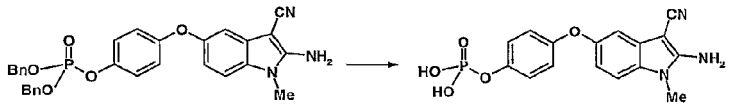
This was prepared using the general method of selective N-alkylation described for 5-benzyloxy-1*H*-indole-3-carbonitrile in Example 25, 5-(4-benzyloxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (1.77 g) was obtained as a white solid from 5-(4-benzyloxyphenloxy)-2-amino-1*H*-indole-3-carbonitrile (2.0 g ; 5.63 mmol) after purification by flash chromatography eluting with increasingly polar mixtures of CH_2Cl_2/EtOAc (10 to 20% EtOAc).

Yield : 85%

^{1}H NMR spectrum (DMSO d_6) : 3.50 (s, 3H) ; 5.07 (s, 2H) ; 6.65 (dd, 1H) ; 6.66 (d, 1H) ; 6.93 (d, 2H) ; 7.00 (d, 2H) ; 7.01 (s, 2H) ; 7.19 (d, 1H) ; 7.33 (t, 1H) ; 7.40 (t, 2H) ; 7.45 (d, 2H).

Example 29

5-(4-Phosponooxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile



Example 29

Using the general method of hydrogenolysis described in Example 16 at 10 psi for 1hour, 5-(4-phosponooxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (920 mg) was obtained as a white solid from 5-[4-(O,O-dibenzylphosponooxy)phenoxy]-2-amino-1-methyl-1*H*-indole-3-carbonitrile (1.71 g ; 3.16 mmol) after purification by HP20SS chromatography eluting with MeOH/H_2O (0 to 100 % MeOH).

Yield : 80%

A small sample (300 mg) was repurified by reverse phase C18 preparative HPLC eluting with MeOH/H_2O/AcOH (80/19/1) to give pure 5-(4-phosponooxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (150 mg).

^{1}H NMR spectrum (DMSO d_6 + TFA) : 3.49 (s, 3H) ; 6.69 (dd, 1H) ; 6.76 (d, 1H) ; 6.95 (d, 2H) ; 7.15 (d, 2H) ; 7.23 (d, 1H) ; 12.37 (br s, 2H) ; 12.64 (br s, 2H).

LCMS-ESI : 360 $[M+H]^+$

Elemental analysis :	Found	C 51.45	H 3.96	N 11.12
$C_{16}H_{14}N_3O_5P$; 0.77 H_2O	Requires	C 51.50	H 4.20	N 11.26

5-[4-(O,O-Dibenzylphosponooxy)phenoxy]-2-amino-1-methyl-1*H*-indole-3-carbonitrile

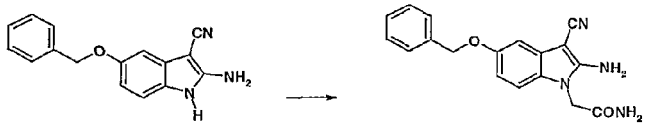
This was prepared as follows:- A solution of 5-(4-hydroxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (1.4 g ; 5 mmol) in acetonitrile (80 ml), CCl_4 (2.44 ml ; 25 mmol) and diisopropylethylamine (1.82 ml ; 10.5 mmol) was treated with a catalytic amount of DMAP (50 mg) and then cooled to 0°C. Dibenzyl phosphite (1.7 ml ; 7.51 mmol) was added and the temperature allowed to rise to room temperature over 30 minutes. The mixture was treated with aqueous KH_2PO_4 (0.5M), the acetonitrile evaporated and the mixture extracted with Et_2O. The organic phase was washed with water, brine and dried over $MgSO_4$. The residue was purified by flash chromatography eluting with increasingly polar mixtures of CH_2Cl_2/EtOAc (10 to 20% EtOAc) to give 5-[4-(O,O-dibenzylphosponooxy)phenoxy]-2-amino-1-methyl-1*H*-indole-3-carbonitrile (1.76 g).

Yield : 65%

^{1}H NMR spectrum ($CDCl_3$) : 3.52 (s, 3H) ; 5.15 (d, 4H) ; 6.68 (dd, 1H) ; 6.76 (d, 1H) ; 6.91 (d, 2H) ; 7.06 (s, 2H) ; 7.13 (d, 2H) ; 7.23 (d, 1H) ; 7.36 (m, 10H).

Example 30

5-Benzyloxy-1-carbamoylmethyl-2-amino-1*H*-indole-3-carbonitrile



Example 30

Using the general method of selective N-alkylation described for 5-benzyloxy-2-amino-1-methyl-1*H*-indole-3-carbonitrile in Example 25, using iodoacetamide (222 mg ; 1.2 mmol), 5-benzyloxy-1-carbamoylmethyl-2-amino-1*H*-indole-3-carbonitrile (163 mg) was obtained as a white solid from 5-benzyloxy-2-amino-1*H*-indole-3-carbonitrile (263 mg ; 1.0 mmol) after trituration with Et_2O/CH_2Cl_2.

Yield : 51%

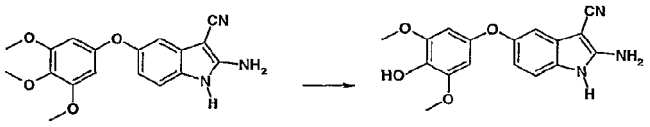
^{1}H NMR spectrum (DMSO d_6) : 4.61 (s, 2H) ; 5.11 (s, 2H) ; 6.65 (dd, 1H) ; 6.81 (d, 1H) ; 6.91 (s, 2H) ; 6.94 (d, 1H) ; 7.30 (s, 1H) ; 7.34 (d, 1H) ; 7.40 (m, 2H) ; 7.47 (m, 2H) ; 7.57 (s, 1H).

LCMS-ESI : 319 $[M-H]^-$

Elemental analysis :	Found	C 65.65	H 5.01	N 17.41
$C_{18}H_{16}N_4O_2$; 0.45 H_2O	Requires	C 65.82	H 5.19	N 17.06

Example 31

5-(3,5-Dimethoxy-4-hydroxy-phenoxy)-2-amino-1*H*-indole-3-carbonitrile



Example 31

A solution of 5-(3,4,5-trimethoxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile (Example 11) (50 mg ; 0.147 mmol) in CH_2Cl_2 (1.0 ml) was treated with trimethyl silyl iodide (30 ul ; 0.2 mmol) and the mixture was stirred at room temperature overnight. The mixture was treated with aqueous $Na_2S_2O_3$ (0.5M), the CH_2Cl_2 evaporated and the mixture extracted with EtOAc. The organic phase was washed with water, brine and dried over $MgSO_4$. Evaporation gave 5-(3,5-dimethoxy-4-hydroxy-phenoxy)-2-amino-1*H*-indole-3-carbonitrile (50 mg).

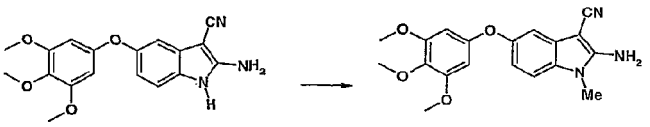
Yield : 100%

^{1}H NMR spectrum (DMSO d_6) : 3.66 (s, 6H) ; 6.26 (s, 2H) ; 6.55 (dd, 1H) ; 6.62 (d, 1H) ; 6.74 (s, 2H) ; 7.05 (d, 1H) ; 8.04 (s, 1H) ; 10.62 (s, 1H).

LCMS-ESI : 326 $[M+H]^+$

Example 32

5-(3,4,5-Trimethoxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile



Example 32

Using the general method of selective N-alkylation described in Example 25, 5-(3,4,5-trimethoxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (77 mg) was obtained as a white solid from 5-(3,4,5-trimethoxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile

(Example 11) (100 mg ; 0.29 mmol) after purification by flash chromatography eluting with CH_2Cl_2/EtOAc (10% EtOAc).

Yield : 75%

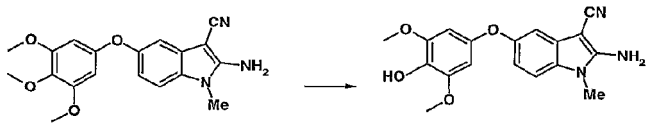
^{1}H NMR spectrum (DMSO d_6) : 3.52 (s, 3H) ; 3.64 (s, 3H) ; 3.69 (s, 6H) ; 6.28 (s, 2H) ; 6.70 (dd, 1H) ; 6.78 (d, 1H) ; 7.05 (s, 2H) ; 7.22 (d, 1H).

LCMS-ESI : 354 $[M+H]^+$

Elemental analysis :	Found	C 64.69	H 5.60	N 11.72
$C_{19}H_{19}N_3O_4$	Requires	C 64.58	H 5.42	N 11.89

Example 33

5-(3,5-Dimethoxy-4-hydroxy-phenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile



Example 33

Using the general method of selective demethylation described for Example 31, 5-(3,5-dimethoxy-4-hydroxy-phenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (50 mg) was obtained as a white solid from 5-(3,4,5-trimethoxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (Example 32) (160 mg ; 0.45 mmol) after purification by flash chromatography eluting with CH_2Cl_2/EtOAc (20% EtOAc).

Yield : 33%

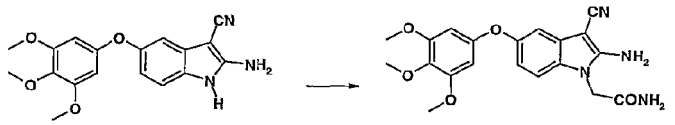
^{1}H NMR spectrum (DMSO d_6) : 3.51 (s, 3H) ; 3.69 (s, 6H) ; 6.30 (s, 2H) ; 6.66 (dd, 1H) ; 6.71 (d, 1H) ; 7.01 (s, 2H) ; 7.19 (d, 1H) ; 8.10 (s, 1H).

LCMS-ESI : 340 $[M+H]^+$

Elemental analysis :	Found	C 63.60	H 5.27	N 12.00
$C_{18}H_{17}N_3O_4$	Requires	C 63.71	H 5.05	N 12.38

Example 34

5-(3,4,5-Trimethoxyphenoxy)-2-amino-1-carbamoylmethyl-1*H*-indole-3-carbonitrile



Example 34

Using the general method of selective N-alkylation described for 5-benzyoxy-2-amino-1-methyl-1*H*-indole-3-carbonitrile in Example 25 using iodoacetamide (350 mg ; 1.88 mmol), 5-(3,4,5-trimethoxyphenoxy)-2-amino-1-carbamoylmethyl-1*H*-indole-3-carbonitrile (192 mg) was obtained as a white solid from 5-(3,4,5-trimethoxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile (Example 11)(320 mg ; 0.94 mmol) after trituration with Et_2O/CH_2Cl_2.

Yield : 52%

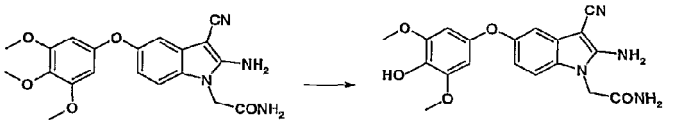
^1H NMR spectrum (DMSO d_6) : 3.64 (s, 3H) ; 3.70 (s, 6H) ; 4.66 (s, 2H) ; 6.31 (s, 2H) ; 6.68 (dd, 1H) ; 6.77 (d, 1H) ; 7.03 (d, 1H) ; 7.04 (s, 2H) ; 7.33 (s, 1H) ; 7.62 (s, 1H).

LCMS-ESI : 397 $[M+H]^+$

Elemental analysis :	Found	C 54.81	H 4.68	N 12.63
$C_{20}H_{20}N_4O_5$; 0.8 H_2O 0.45 CH_2Cl_2	Requires	C 54.70	H 5.05	N 12.48

Example 35

5-(3,5-Dimethoxy-4-hydroxyphenoxy)-2-amino-1-carbamoylmethyl-1*H*-indole-3-carbonitrile



Example 35

Using the general method of selective demethylation described for Example 31 5-(3,5-dimethoxy-4-hydroxyphenoxy)-2-amino-1-carbamoylmethyl-1*H*-indole-3-carbonitrile (95 mg) was obtained as a beige solid from 5-(3,4,5-trimethoxyphenoxy)-2-amino-1-carbamoylmethyl-1*H*-indole-3-carbonitrile (150 mg ; 0.37 mmol) after trituration with Et_2O.

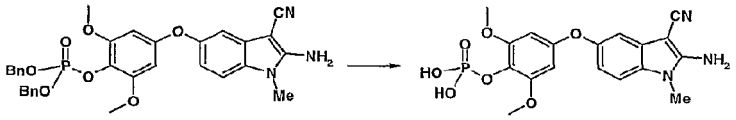
Yield : 67%

^{1}H NMR spectrum (DMSO d_6) : 3.70 (s, 6H) ; 4.64 (s, 2H) ; 6.32 (s, 2H) ; 6.63 (dd, 1H) ; 6.69 (d, 1H) ; 7.00 (d, 1H) ; 7.01 (s, 2H) ; 7.32 (s, 1H) ; 7.61 (s, 1H) ; 8.11 (s, 1H).

LCMS-ESI : 383 $[M+H]^+$

Example 36

5-(3,5-Dimethoxy-4-phosphonooxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile



Example 36

Using the general method of hydrogenolysis described in Example 16 at 2 psi for 2 hours, 5-(3,5-dimethoxy-4-phosphonooxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (37 mg) was obtained as a white solid from 5-[3,5-dimethoxy-4-(O,O-dibenzylphosphonooxy)phenoxy]-2-amino-1-methyl-1*H*-indole-3-carbonitrile (88 mg ; 0.14 mmol) after purification by HP20SS chromatography eluting with increasingly polar mixtures of MeOH/H_2O (0 to 50 % MeOH).

Yield : 60%

^{1}H NMR spectrum (DMSO d_6) : 3.49 (s, 3H) ; 3.62 (s, 6H) ; 6.20 (s, 2H) ; 6.68 (dd, 1H) ; 6.78 (d, 1H) ; 7.01 (s, 2H) ; 7.23 (d, 1H).

Elemental analysis :	Found	C 41.45	H 4.11	N 7.69
$C_{18}H_{16}N_3O_7P$; 2.7 H_2O 2.3 Na	Requires	C 41.67	H 4.16	N 8.10

5-[3,5-Dimethoxy-4-(O,O-dibenzylphosphonooxy)phenoxy]-2-amino-1-methyl-1*H*-indole-3-carbonitrile

Using the general method of phosphorylation described in Example 29, 5-[3,5-dimethoxy-4-(O,O-dibenzylphosphonooxy)phenoxy]-2-amino-1-methyl-1*H*-indole-3-carbonitrile (88 mg) was obtained as a white solid from 5-(3,5-dimethoxy-4-hydroxy-phenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (114 mg ; 0.33 mmol) after purification by flash chromatography eluting with CH_2Cl_2/EtOAc (20 % EtOAc).

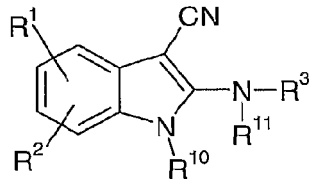
Yield : 44%

^{1}H NMR spectrum (DMSO d_6) : 3.54 (s, 3H) ; 3.69 (s, 6H) ; 5.22 (d, 4H) ; 6.36 (s, 2H) ; 6.74 (dd, 1H) ; 6.83 (d, 1H) ; 7.07 (s, 2H) ; 7.26 (d, 1H) ; 7.3 - 7.5 (m, 10H).

LCMS-ESI : 599 $[M-H]^-$

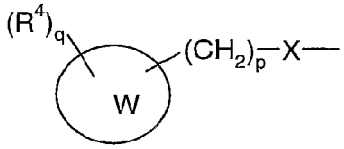
Claims

1. A compound of Formula (I).



Formula (I)

wherein R^1 and R^2 are independently selected from hydrogen, halogen, -CN, a hydrocarbyl group or a group of Formula (II);



Formula (II)

wherein W is aryl or a heterocyclic group,

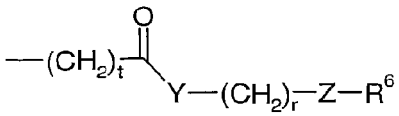
R^4 is independently selected from hydrogen, halogen, -OH, amino, alkanoylamino, —OPO_3H_2, or a hydrocarbyl group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified or two R^4 groups together form an optionally substituted cyclic or heterocyclic group;

X is selected from —S—, —O—, —S(O)—, —$S(O_2)$— and —NH—;

p is an integer from 0 to 4; and

q is an integer from 1 to 4;

$R^3$and R^{10} are independently selected from hydrogen, lower alkyl or a group of Formula (IV):



Formula (IV)

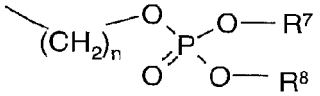
wherein Y is selected from —NH—, —O— or a bond;

Z is selected from —NH—, —O—, —C(O)— or a bond;

r is an integer from 0 to 4;

t is an integer from 0 to 1;

R^6 is hydrogen, a hydrocarbyl group or a group of Formula (V):



Formula (V)

wherein n is an integer of from 1 to 6, and;

R^7 and R^8 are independently selected from hydrogen or a hydrocarbyl group; and

R^{11} is hydrogen or lower alkyl;

or a salt or solvate thereof.

provided that:

iii) when R^1 is an unsubstituted phenylthio group (Ph-S-), R^2 is H, R^{10} is H and R^{11} is H then R^3 is neither H nor $-C(O)-O-CH_2CH_3$; and

iv) R^1, R^2 and R^3 are not all hydrogen.

2. A compound according to claim 1 wherein R^1 is a group of Formula (II).

3. A compound according to claim 2 wherein R^1 is a group of Formula (II) and R^2 is selected from hydrogen, halogen or lower alkyl.

4. A compound according to any one of claims 2 to 3 wherein X is selected from —S—, —O—, —$S(O_2)$— and —NH—.

5. A compound according to any one of claims 2 to 4 wherein R^4 is selected from hydrogen, fluoro, a lower alkanoylamino group, -OH, amino, —OPO_3H_2, or a lower alkoxy group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified.

6. A compound according to any one of claims 2 to 5 wherein q is 1 and R^4 is selected from hydrogen, -OH, amino, —OPO_3H_2, methoxy, acetylamino, serylamino, glutamylamino, glycylamino, alanylamino and 4-methylpiperazin-1-ylcarbonylpropanoyloxy.

7. A compound according to any one of claims 2 to 5 wherein q is 2 or 3 and R^4 is independently selected from hydrogen, fluoro, -OH, —OPO_3H_2, or a lower alkoxy group.

8. A compound according to any one of the preceding claims wherein R^3 is hydrogen and R^{10} is independently selected from hydrogen or lower alkyl.

9. A compound selected from

2-phenoxycarbonylamino-5-phenylsulphanyl-1*H*-indole-3-carbonitrile;

5-(4-hydroxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile;

5-(3,6-dimethoxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile;

5-phenylsulphanyl-2-(4-methylpiperazin-1-ylpropoxycarbonylamino)-1*H*-indole-3-carbonitrile;

5-phenylsulphanyl-1-(4-methylpiperazin-1-ylpropoxycarbonyl)-2-amino-1*H*-indole-3-carbonitrile;

6-benzyloxy-2-amino-1*H*-indole-3-carbonitrile;

5-[4-(4-methylpiperazin-1-yl-4-oxobutanoyloxy)phenoxy]-2-amino-1*H*-indole-3-carbonitrile;

5-(4-glycylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(4-alanylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(4-glutamylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(3-glycylbenzyloxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(4-hydroxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(4-phosponooxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(3-serylbenzyloxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(4-acetylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile; and

5-(4-serylaminophenoxy)-2-amino-1*H*-indole-3-carbonitrile;

or salt or solvate thereof.

10. A compound selected from:

5-(4-aminophenoxy)-2-amino-1*H*-indole-3-carbonitrile;

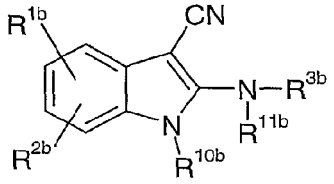
5-(3,4,5-trimethoxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(3,4-dimethoxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile; and

5-(3,4,5-trimethoxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile;

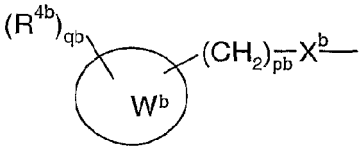
or salt or solvate thereof.

11. The use of a compound of Formula (Ib) as a medicament:



Formula (Ib)

wherein R^{1b} and R^{2b} are independently selected from hydrogen, halogen, -CN, a hydrocarbyl group or a group of Formula (IIb);



Formula (IIb)

wherein W^b is aryl or a heterocyclic group,

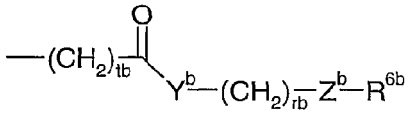
R^{4b} is independently selected from hydrogen, halogen, -OH, amino, alkanoylamino, —OPO_3H_2, or a hydrocarbyl group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified or two R^{4b} groups together form an optionally substituted cyclic or heterocyclic group;

X^b is selected from —S—, —O—, —S(O)—, —$S(O_2)$— and —NH—;

pb is an integer from 0 to 4; and

qb is an integer from 1 to 4;

$R^{3b}$and R^{10b} are independently selected from hydrogen, lower alkyl or a group of Formula (IVb):



Formula (IVb)

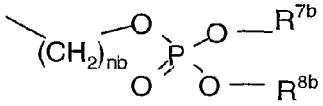
wherein Y^b is selected from —NH—, —O— or a bond;

Z^b is selected from —NH—, —O—, —C(O)— or a bond;

rb is an integer from 0 to 4;

tb is an integer from 0 to 1;

R^{6b} is hydrogen, a hydrocarbyl group or a group of Formula (Vb):



Formula (Vb)

wherein nb is an integer of from 1 to 6, and;

R^{7b} and R^{8b} are independently selected from hydrogen or a hydrocarbyl group; and

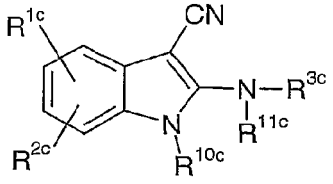
R^{11b} is hydrogen or lower alkyl;

or a salt or solvate thereof;

provided that:

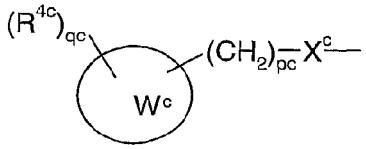
ii) when R^1 is an unsubstituted phenylthio group (Ph-S-), R^2 is H, R^{10} is H and R^{11} is H then R^3 is neither H nor –C(O)-O-CH_2CH_3.

12. The use of a compound of Formula (Ic) or a pharmaceutically-acceptable salt or solvate thereof, for the manufacture of a medicament to inhibit and/or reverse and/or alleviate symptoms of angiogenesis and/or any disease state associated with angiogenesis:



Formula (Ic)

wherein R^{1c} and R^{2c} are independently selected from hydrogen, halogen, -CN, a hydrocarbyl group or a group of Formula (IIc);



Formula (IIc)

wherein W^c is aryl or a heterocyclic group,

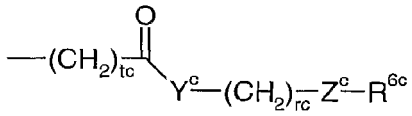
R^{4c} is independently selected from hydrogen, halogen, -OH, amino, alkanoylamino, —OPO_3H_2, or a hydrocarbyl group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified or two R^{4c} groups together form an optionally substituted cyclic or heterocyclic group;

X^c is selected from —S—, —O—, —S(O)—, —$S(O_2)$— and —NH—;

pc is an integer from 0 to 4; and

qc is an integer from 1 to 4;

R^{3c} and R^{10c} are independently selected from hydrogen, lower alkyl or a group of Formula (IVc):



Formula (IVc)

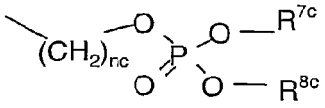
wherein Y^c is selected from —NH—, —O— or a bond;

Z^c is selected from —NH—, —O—, —C(O)— or a bond;

rc is an integer from 0 to 4;

tc is an integer from 0 to 1;

R^{6c} is hydrogen, a hydrocarbyl group or a group of Formula (Vc):



Formula (Vc)

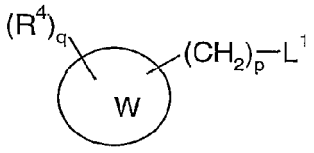
wherein nc is an integer of from 1 to 6, and;

R^{7c} and R^{8c} are independently selected from hydrogen or a hydrocarbyl group; and

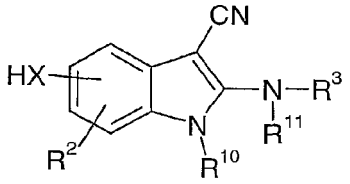
R^{11c} is hydrogen or lower alkyl;

13. A process for preparing a compound of Formula (I), or salt, or solvate thereof, which process (wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, m, n, p, q, r, t, W, X, Y and Z are unless otherwise specified as defined in Formula (I)) comprises:

a) for compounds of Formula (I) wherein R^1 is of the Formula (II) and X is —O—, —S— or —NH—, reacting a compound of Formula (A) with a compound of Formula (B),



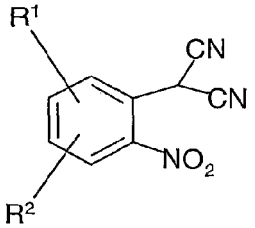
Formula (A)



Formula (B)

wherein L^1 is a leaving group;

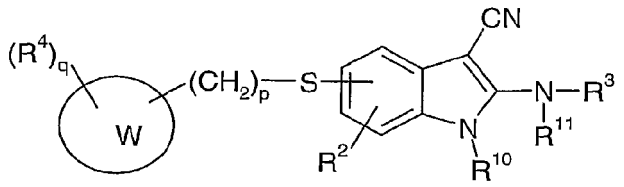
b) for compounds of Formula (I) in which R^3 is hydrogen and R^{11} is hydrogen may be prepared by reduction followed by ring closure of compounds of Formula (C):



Formula (C)

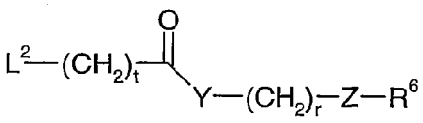
c) for compounds of Formula (I) wherein R^{10} is lower alkyl, reacting a compound of Formula (I) wherein R^{10} is hydrogen with a suitable alkylhalide,

d) for compounds of Formula (I) wherein R^1 is of the Formula (II) and X is —S(O)—, —$S(O_2)$—, oxidising a compound of Formula (D),



Formula (D)

e) for compounds of Formula (I) in which R^3 is a group of Formula (IV) may be prepared by the reaction of compounds of Formula (I) in which R^3 is hydrogen with compounds of Formula (F) below, in which L^2 is a leaving group.:



Formula (F)

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 salt, or solvate.

INTERNATIONAL SEARCH REPORT

ational Application No
PCT/GB 01/02335

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D209/42 A61K31/40 A61P43/00

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 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)
WPI Data, CHEM ABS Data, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 533 672 A (EAKIN MURDOCH A ET AL) 6 August 1985 (1985-08-06) cited in the application claim 1; examples 1,2	1-8,13
A	WO 95 17181 A (PHARMACIA SPA) 29 June 1995 (1995-06-29) page 11, line 2 - line 18; claim 1	1-12
A	WO 99 02166 A (ANGIOGENE PHARM LTD ;DOUGHERTY GRAEME (GB)) 21 January 1999 (1999-01-21) page 12, line 12 - line 23; claim 1	1-12
P,X	WO 00 41669 A (ANGIOGENE PHARM LTD ;DAVIS PETER DAVID (GB)) 20 July 2000 (2000-07-20) page 11, line 9 - line 17; claim 1	1-12

☐ 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
30 July 2001	09/08/2001

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	Usuelli, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

tional Application No
PCT/GB 01/02335

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4533672 A	06-08-1985	AT 26261 T	15-04-1987
		AU 563413 B	09-07-1987
		AU 2054883 A	03-05-1984
		CA 1205078 A	27-05-1986
		DE 3370619 D	07-05-1987
		EP 0107963 A	09-05-1984
		ES 526876 D	01-05-1985
		ES 8504707 A	16-07-1985
		FI 833958 A,B,	29-04-1984
		GR 79404 A	22-10-1984
		IE 56125 B	24-04-1991
		IL 70111 A	30-11-1987
		JP 59095257 A	01-06-1984
		NO 833918 A,B,	30-04-1984
		NZ 206080 A	23-01-1987
		PT 77574 A,B	01-11-1983
		ZA 8307829 A	29-08-1984
WO 9517181 A	29-06-1995	AU 676958 B	27-03-1997
		AU 8061294 A	10-07-1995
		CA 2155098 A	29-06-1995
		EP 0684820 A	06-12-1995
		HU 73176 A	28-06-1996
		IL 112010 A	30-10-1998
		JP 8507089 T	30-07-1996
		NO 953146 A	10-08-1995
		PL 310379 A	11-12-1995
		US 5576330 A	19-11-1996
		ZA 9410204 A	10-11-1995
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 A	24-05-2000
		NO 20000077 A	07-01-2000
		PL 337926 A	11-09-2000
		TR 9903149 T	21-09-2000
WO 0041669 A	20-07-2000	AU 1995700 A	01-08-2000

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [6] :
C07D 239/94, 239/88, 401/12, 403/12, 409/12, 413/12, 417/12, A61K 31/505

A1

(11) International Publication Number: **WO 97/22596**

(43) International Publication Date: 26 June 1997 (26.06.97)

(21) International Application Number: PCT/GB96/03075

(22) International Filing Date: 13 December 1996 (13.12.96)

(30) Priority Data:
95402846.0 18 December 1995 (18.12.95) EP
(34) Countries for which the regional or international application was filed: AT et al.
96402190.1 15 October 1996 (15.10.96) EP
(34) Countries for which the regional or international application was filed: AT 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]; Zone Industrielle Sud-est, Boîte postale 401, F-51064 Reims Cédex (FR).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: LOHMANN, Jean-Jacques, Marcel [FR/FR]; Zeneca-Pharma S.A., Zone Industrielle Sud-est, Boîte postale 401, F-51064 Reims Cédex (FR). HENNEQUIN, Laurent, François, André [FR/FR]; Zeneca-Pharma S.A., Zone Industrielle Sud-est, Boîte postale 401, F-51064 Reims Cédex (FR). THOMAS, Andrew, Peter [GB/GB]; Zeneca Pharmaceuticals, 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).

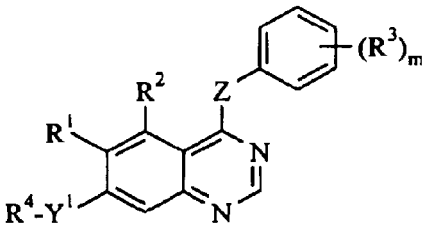
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: QUINAZOLINE DERIVATIVES

(57) Abstract

The invention relates to quinazoline derivatives of formula (I) [wherein: Y^1 represents -O-, -S-, -CH_2-, -SO-, -SO_2-, -NR^5CO-, -$CONR^6$-, -SO_2NR^7-, -NR^8SO_2- or -NR^9- (wherein R^5, R^6, R^7, R^8 and R^9 each independently represents hydrogen, alkyl or alkoxyalkyl); R^1 represents hydrogen, hydroxy, halogeno, nitro, trifluoromethyl, cyano, alkyl, alkoxy, alkylthio, amino or alkylamino. R^2 represents hydrogen, hydroxy, halogeno, alkyl, alkoxy, trifluoromethyl, cyano, amino or nitro; m is an integer from 1 to 5; R^3 represents hydroxy, halogeno, alkyl, alkoxy, alkanoyloxy, trifluoromethyl, cyano, amino or nitro; R^4 represents a group which is or which contains an optionally substituted pyridone, phenyl or aromatic heterocyclic group] 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.



(I)

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

QUINAZOLINE DERIVATIVES.

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 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.

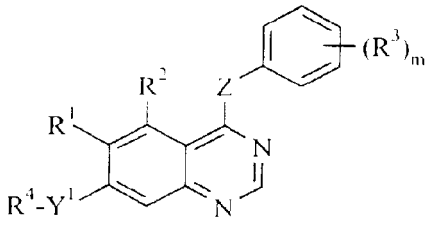
Normal angiogenesis plays an important role in a variety of processes including embryonic development, wound healing and several components of female reproductive function. Undesirable or pathological angiogenesis has been associated with disease states including diabetic retinopathy, psoriasis, cancer, 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 No. 0566226. 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 than 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:

Y^1 represents -O-, -S-, $-CH_2-$, -SO-, $-SO_2-$, $-NR^5CO-$, $-CONR^6-$, $-SO_2NR^7-$, $-NR^8SO_2-$ or $-NR^9-$ (wherein R^5, R^6, R^7, R^8 and R^9 each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

R^1 represents hydrogen, hydroxy, halogeno, nitro, trifluoromethyl, cyano, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkylthio, or $NR^{10}R^{11}$ (wherein R^{10} and R^{11}, which may be the same or different, each represents hydrogen or C_{1-3}alkyl);

R^2 represents hydrogen, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3} alkoxy, trifluoromethyl, cyano, amino or nitro;

m is an integer from 1 to 5;

R^3 represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkanoyloxy, trifluoromethyl, cyano, amino or nitro;

R^4 is selected from one of the following eight groups:

1) X^1 (wherein X^1 represents a pyridone group, a phenyl group or a 5 or 6-membered aromatic heterocyclic group with 1 to 3 heteroatoms selected from O, N and S, which pyridone, phenyl or heterocyclic group may carry up to 5 substituents selected from halogeno, amino, C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}aminoalkyl, C_{1-4}alkylamino, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{12}R^{13}$ and $-NR^{14}COR^{15}$ (wherein R^{12}, R^{13}, R^{14} and R^{15}, which may be the same or different, each represents hydrogen, C_{1-4}alkyl or C_{1-3}alkoxyC_{2-3}alkyl));

2) C_{1-5}alkylX^1 (wherein X^1 is as defined hereinbefore);

3) C_{2-5}alkenylX^1 (wherein X^1 is as defined hereinbefore);

4) C_{2-5}alkynylX^1 (wherein X^1 is as defined hereinbefore);

5) C_{1-5}alkylY^2X^1 (wherein Y^2 represents -O-, -S-, -SO-, $-SO_2$-, -OCO-, $-NR^{16}CO$-, $-CONR^{17}$-, $-SO_2NR^{18}$-, $-NR^{19}SO_2$- or $-NR^{20}$- (wherein R^{16}, R^{17}, R^{18}, R^{19} and R^{20} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and X^1 is as defined hereinbefore);

6) C_{2-5}alkenylY^3X^1 (wherein Y^3 represents -O-, -S-, -SO-, $-SO_2$-, -OCO-, $-NR^{21}CO$-, $-CONR^{22}$-, $-SO_2NR^{23}$-, $-NR^{24}SO_2$- or $-NR^{25}$- (wherein R^{21}, R^{22}, R^{23}, R^{24} and R^{25} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and X^1 is as defined hereinbefore);

7) C_{2-5}alkynylY^4X^1 (wherein Y^4 represents -O-, -S-, -SO-, $-SO_2$-, -OCO-, $-NR^{26}CO$-, $-CONR^{27}$-, $-SO_2NR^{28}$-, $-NR^{29}SO_2$- or $-NR^{30}$- (wherein R^{26}, R^{27}, R^{28}, R^{29} and R^{30} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and X^1 is as defined hereinbefore); and

8) C_{1-4}alkylY^5C_{1-4}alkylX^1 (wherein Y^5 represents -O-, -S-, -SO-, $-SO_2$-, $-NR^{31}CO$-, $-CONR^{32}$-, $-SO_2NR^{33}$-, $-NR^{34}SO_2$- or $-NR^{35}$- (wherein R^{31}, R^{32}, R^{33}, R^{34} and R^{35} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and X^1 is as defined hereinbefore);

Z represents -NH-, -O-, -S-, or $-CH_2$-; with the proviso that where R^4 is selected from one of the groups 1), 2), and 5) above and X^1 is unsubstituted phenyl or substituted phenyl with 1 to 2 substituents selected from halogeno, C_{1-4}alkyl and C_{1-4}alkoxy, then m is an integer from 3 to 5 and/or Z is -O-, -S-, or $-CH_2$-];

and salts thereof.

Advantageously Y^1 represents -O-, -S-, $-CH_2$-, $-NR^5CO$-, $-NR^8SO_2$- or $-NR^9$- (wherein R^5, R^8 and R^9 each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Preferably Y^1 represents -O-, -S-, $-CH_2$-, $-NR^5CO$-, $-NR^8SO_2$- or -NH- (wherein R^5 and R^8 each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

More preferably Y^1 represents -O-, -S-, $-CH_2$- or -NH-, especially -O-.

In another embodiment of the present invention Y^1 represents -O-, $-NR^5CO$- or $-NR^8SO_2$- (wherein R^5 and R^8 each independently represents hydrogen or C_{1-2}alkyl).

In a further embodiment of the present invention Y^1 is -NHCO-.

In one embodiment of the invention R^1 represents hydrogen, hydroxy, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkylthio, or $NR^{10}R^{11}$ (wherein R^{10} and R^{11} are as defined hereinbefore). Conveniently however R^1 is hydrogen, hydroxy, cyano, nitro, trifluoromethyl, C_{1-3}alkyl, C_{1-3}alkoxy or amino.

R^1 is advantageously hydrogen, hydroxy, C_{1-3}alkyl, C_{1-3}alkoxy or amino.

R^1 is preferably hydrogen, hydroxy, methyl, ethyl, methoxy or ethoxy, more preferably hydrogen, hydroxy, methyl or methoxy, particularly hydrogen, methyl or methoxy but especially methoxy.

In another embodiment of the present invention R^1 represents hydrogen, hydroxy, cyano, nitro, trifluoromethyl, methyl, ethyl, methoxy or ethoxy.

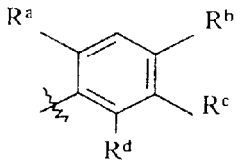
R^2 is advantageously hydrogen, halogeno, amino or nitro.

R^2 is preferably hydrogen, chloro 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.

Advantageously in another embodiment of the present invention one R^3 substituent is meta-hydroxy and the other one or more are each selected from halogeno and methyl.

In another embodiment of the invention the phenyl group bearing $(R^3)_m$ is of the formula IIa:



(IIa)

(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, especially hydrogen or fluoro).

In a further embodiment of the invention the phenyl group bearing $(R^3)_m$ is preferably of the formula IIa wherein:

R^a represents hydrogen, fluoro or chloro;

R^b represents hydrogen, methyl, methoxy, bromo, fluoro or chloro, especially hydrogen, methyl or chloro;

R^c represents hydrogen or hydroxy; and

R^d represents hydrogen;

with the proviso that R^a, R^b and R^c do not each represent hydrogen.

Preferably the phenyl group bearing $(R^3)_m$ is the 3-hydroxy-4-methylphenyl group, the 2-fluoro-5-hydroxy-4-methylphenyl group, the 2-fluoro-4-bromophenyl, the 2-fluoro-4-chloro-5-hydroxyphenyl or the 4-chloro-2-fluorophenyl group.

In a particular aspect of the present invention, the phenyl group bearing $(R^3)_m$ is the 3-hydroxy-4-methylphenyl group, but especially the 2-fluoro-5-hydroxy-4-methylphenyl group.

In a further embodiment of the present invention the phenyl group bearing $(R^3)_m$ is the 4-chloro-2-fluorophenyl group.

Advantageously Y^2 represents -O-, -S-, -SO-, $-SO_2$-, $-NR^{19}CO$-, $-NR^{19}SO_2$- or $-NR^{20}$- (wherein R^{19}, R^{19} and R^{20} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Preferably Y^2 represents, -O-, -S-, -SO-, $-SO_2$- or $-NR^{20}$- (wherein R^{20} represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

More preferably Y^2 represents -S-, -O- or $-NR^{20}$- (wherein R^{20} represents hydrogen or C_{1-2}alkyl), but most preferably is -O-, or $-NR^{20}$ (wherein R^{20} is as hereinbefore defined).

Advantageously Y^3 represents -O-, -S-, -SO-, $-SO_2$-, $-NR^{24}CO$-, $-NR^{24}SO_2$- or $-NR^{25}$- (wherein R^{21}, R^{24} and R^{25} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Preferably Y^3 represents -O-, -S-, -SO-, $-SO_2$- or $-NR^{25}$- (wherein R^{25} represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

More preferably Y^3 represents -O- or $-NR^{25}$- (wherein R^{25} represents hydrogen or C_{1-2}alkyl).

Advantageously Y^4 represents -O-, -S-, -SO-, $-SO_2$-, $-NR^{26}CO$-, $-NR^{29}SO_2$- or $-NR^{30}$- (wherein R^{26}, R^{29} and R^{30} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Preferably Y^4 represents -O-, -S-, -SO-, $-SO_2$- or $-NR^{30}$- (wherein R^{30} represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

More preferably Y^4 represents -O- or $-NR^{30}$- (wherein R^{30} represents hydrogen or C_{1-2}alkyl).

Advantageously Y^5 represents -O-, -S-, -SO-, $-SO_2$- or $-NR^{35}$- (wherein R^{35} represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

Preferably Y^5 represents -O-, -S- or -NR^{35}- (wherein R^{35} represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

m is preferably 2 or 3.

Z may for example represent -NH- or -O- but Z is preferably -NH-.

X^1 preferably represents a pyridone group or a 5 or 6-membered aromatic heterocyclic group with 1 to 3 heteroatoms selected from O, N and S, which pyridone group or heterocyclic group may be substituted as hereinbefore defined.

Where X^1 is a 5 or 6-membered aromatic heterocyclic group, it preferably has 1 or 2 heteroatoms, selected from O, N and S, of which more preferably one is N, and may be substituted as hereinbefore defined.

X^1 is particularly a pyridone, pyridyl, imidazolyl, thiazolyl, thienyl, triazolyl or pyridazinyl group which group may be substituted as hereinbefore defined, more particularly a pyridone, pyridyl, imidazolyl, thiazolyl or triazolyl group, especially a pyridone, pyridyl, imidazolyl or triazolyl group which group may be substituted as hereinbefore defined.

Where R^4 is C_{1-5}alkylX^1, C_{2-5}alkenylX^1, C_{2-5}alkynylX^1 or C_{1-3}alkylY^5C_{1-3}alkylX^1, and X^1 is a nitrogen-containing 6-membered aromatic heterocyclic group, said group is advantageously linked to the alkyl, alkenyl or alkynyl moiety via a carbon atom of X^1, said group being such that a nitrogen atom is positioned para- to the carbon atom linked to the alkyl, alkenyl or alkynyl moiety. The C_{1-5}alkyl moiety may if desired be -$(CH_2)_n$-.

Where R^4-Y^1 is X^1-$(CH_2)_n$-Y^1- and n is an integer from 0 to 5, Y^1 is -O-, -NH-, -S- or -CH_2- and X^1 is a nitrogen-containing 6-membered aromatic heterocyclic group, said group is advantageously linked to -$(CH_2)_n$-Y^1- via a carbon atom of X^1, said group being such that a nitrogen atom is positioned para- to the carbon atom linked to -$(CH_2)_n$-Y^1-.

In another example of interest, X^1 is pyrimidine which may be substituted as hereinbefore defined.

In one embodiment of the invention X^1 represents a pyridone, phenyl or 5 or 6-membered aromatic heterocyclic group with 1 to 3 heteroatoms selected from O, N and S, which group may preferably carry up to 2 substituents, more preferably up to one substituent, selected from the group of substituents as hereinbefore defined.

In the definition of X^1, conveniently substituents are selected from halogeno, C_{1-4}alkyl, C_{1-4}alkoxy and cyano, more conveniently substituents are selected from chloro, fluoro, methyl and ethyl.

Conveniently R^4 is selected from one of the following eight groups:

1) X^1 (wherein X^1 is as defined hereinbefore);

2) C_{1-5}alkylX^1 (wherein X^1 is as defined hereinbefore);

3) C_{3-5}alkenylX^1 (wherein X^1 is as defined hereinbefore);

4) C_{3-5}alkynylX^1 (wherein X^1 is as defined hereinbefore);

5) C_{1-5}alkylY^2X^1 (wherein Y^2 and X^1 are as defined hereinbefore);

6) C_{3-5}alkenylY^3X^1 (wherein Y^3 and X^1 are as defined hereinbefore);

7) C_{3-5}alkynylY^4X^1 (wherein Y^4 and X^1 are as defined hereinbefore); and

8) C_{2-5}alkylY^5C_{1-5}alkylX^1 (wherein Y^5 and X^1 are as defined hereinbefore).

Advantageously R^4 is selected from one of the following eight groups:

1) X^1 (wherein X^1 is as defined hereinbefore);

2) C_{1-5}alkylX^1 (wherein X^1 is as defined hereinbefore);

3) 1-X^1prop-1-en-3-yl, 1-X^1but-2-en-4-yl, 1-X^1but-1-en-3-yl, 1-X^1pent-2-en-4-yl or 2-X^1pent-3-en-5-yl (wherein X^1 is as defined hereinbefore with the proviso that when R^4 is 1-X^1prop-1-en-3-yl, X^1 is linked to the alkenyl group via a carbon atom);

4) 1-X^1prop-1-yn-3-yl, 1-X^1but-2-yn-4-yl, 1-X^1but-1-yn-3-yl, 1-X^1pent-2-yn-4-yl or 2-X^1pent-3-yn-5-yl (wherein X^1 is as defined hereinbefore with the proviso that when R^4 is 1-X^1prop-1-yn-3-yl, X^1 is linked to the alkynyl group via a carbon atom);

5) C_{1-5}alkylY^2X^1 (wherein Y^2 and X^1 are as defined hereinbefore);

6) 1-(X^1Y^3)prop-1-en-3-yl, 1-(X^1Y^3)but-2-en-4-yl, 1-(X^1Y^3)but-1-en-3-yl, 1-(X^1Y^3)pent-2-en-4-yl or 2-(X^1Y^3)pent-3-en-5-yl (wherein Y^3 and X^1 are as defined hereinbefore);

7) 1-(X^1Y^4)prop-1-yn-3-yl, 1-(X^1Y^4)but-2-yn-4-yl, 1-(X^1Y^4)but-1-yn-3-yl, 1-(X^1Y^4)pent-2-yn-4-yl or 2-(X^1Y^4)pent-3-yn-5-yl (wherein Y^4 and X^1 are as defined hereinbefore); and

8) C_{2-5}alkylY^5C_{1-5}alkylX^1 (wherein Y^5 and X^1 are as defined hereinbefore).

Preferably R^4 is selected from one of the following eight groups:

1) X^1 (wherein X^1 is as defined hereinbefore);

2) C_{1-5}alkylX^1 (wherein X^1 is as defined hereinbefore);

3) 1-X^1but-2-en-4-yl (wherein X^1 is as defined hereinbefore);

4) 1-X^1but-2-yn-4-yl (wherein X^1 is as defined hereinbefore);

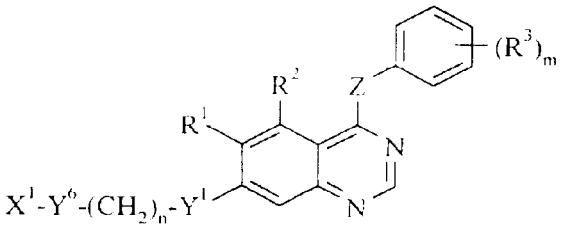
5) C_{1-3}alkylY^2X^1 (wherein Y^2 and X^1 are as defined hereinbefore);

6) 1-(X^1Y^3)but-2-en-4-yl (wherein Y^3 and X^1 are as defined hereinbefore);

7) 1-(X^1Y^4)but-2-yn-4-yl (wherein Y^4 and X^1 are as defined hereinbefore); and

8) ethylY^5methylX^1 (wherein Y^5 and X^1 are as defined hereinbefore).

More preferably the compounds of formula (I) are of the formula (Ia):



(Ia)

(wherein R^1, R^2, R^3, m, X^1, Y^1 and Z are as defined hereinbefore n is an integer from 0 to 5 and Y^6 represents a direct bond, -O-, -S-, -SO-, -SO_2-, -NR^{36}CO-, -$CONR^{37}$-, -SO_2NR^{38}-, -$NR^{39}SO_2$- or -NR^{40}- (wherein R^{36}, R^{37}, R^{38}, R^{39} and R^{40} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl)).

Advantageously Y^6 is a direct bond, -O-, -S-, -SO-, -SO_2- or -NR^{40}- (wherein R^{40} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyethyl).

Preferably Y^6 is a direct bond, -O-, -S- or -NH-.

More preferably Y^6 is a direct bond.

n is advantageously an integer from 0 to 3, preferably 1 to 3.

Therefore, for example, in a particular embodiment of the invention the compounds of formula I are of the formula Ia wherein:

[Y^1 represents -O-, -NH-, -S- or -CH_2-;

n is an integer from 0 to 5;

X^1 represents a phenyl group or a 5 or 6-membered aromatic heterocyclic group with 1 to 3 heteroatoms selected from O, N and S, which phenyl or heterocyclic group may carry up to 5

substituents selected from halogeno, C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}hydroxyalkyl, C_{1-4}hydroxyalkoxy, carboxy, cyano, $-CONR^{41}R^{42}$ and $-NR^{43}COR^{44}$ (wherein R^{41}, R^{42}, R^{43} and R^{44}, which may be the same or different, each represents hydrogen or C_{1-4}alkyl);

R^1 represents hydrogen, hydroxy, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkylthio, or $NR^{45}R^{46}$ (wherein R^{45} and R^{46}, which may be the same or different, each represents hydrogen or C_{1-3}alkyl);

R^2 represents hydrogen, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, trifluoromethyl, cyano, amino or nitro;

m is an integer from 1 to 5;

R^3 represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkanoyloxy, trifluoromethyl, cyano, amino or nitro;

Z represents -NH- or -O-; and

Y^6 is a direct bond;

with the proviso that where X^1 is unsubstituted phenyl or substituted phenyl with 1 to 2 substituents selected from halogeno, C_{1-4}alkyl and C_{1-4}alkoxy, m is an integer from 3 to 5 or Z is -O-];

and salts thereof.

Preferred compounds of the present invention are:-

4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(3-pyridylmethoxy)quinazoline

4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(3-thienylmethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-pyridyloxy)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-[N-methyl-N-(4-pyridyl)]aminoethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-[2-(2-oxo-1,2-dihydro-1-pyridyl)ethoxy]quinazoline

7-(4 cyanobenzyloxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(2-methylimidazol-1-yl)propoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-((2-methyl-4-pyridyl)methoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(2-oxo-1,2-dihydro-1-pyridyl)propoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(1-methylimidazol-2-ylthio)propoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(4-pyridyloxy)propoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-pyridylthio)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(3-pyridyloxy)ethoxy)quinazoline

7-benzyloxy-4-(2-fluoro-5-hydroxy-4-methylanilino)quinazoline

7-benzyloxy-4-(4-chloro-2-fluoro-5-hydroxyanilino)quinazoline

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-((2-methylthiazol-4-yl)methoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-((3-thienyl)methoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(N-methyl-N-(pyridazin-4-yl)amino)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(N-methyl-N-(6-methylpyrimidin-4-yl)amino)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-7-(2-(3,5-dimethyl-[1,2,4]-triazol-4-yl)ethoxy)-6-methoxyquinazoline

4-(4-chloro-2-fluoroanilino)-7-(2-(2,4-dimethylimidazol-1-yl)ethoxy)-6-methoxyquinazoline

4-(4-chloro-2-fluoroanilino)-7-(2-(2,5-dimethylimidazol-1-yl)ethoxy)-6-methoxyquinazoline

4-(3-hydroxyanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1,2,4-triazol-4-yl)ethoxy)quinazoline

4-(4-bromo-2-fluoroanilino)-7-(2-([1,2,4]-triazol-1-yl)ethoxy)-6-methoxyquinazoline

and salts thereof, especially hydrochloride salts thereof.

The following compounds of the present invention are especially preferred:-

4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(4-pyridylmethoxy)quinazoline

4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(2-pyridylmethoxy)quinazoline

4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(1-methylimidazol-2-ylmethoxy)quinazoline

4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(2-methylthiazol-4-ylmethoxy)quinazoline

7-(2-acetamidothiazol-4-ylmethoxy)-4-(3-hydroxy-4-methylanilino)-6-methoxyquinazoline

4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(4-pyridylpropoxy)quinazoline

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(4-pyridylpropoxy)quinazoline

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(4-pyridylmethoxy)quinazoline

7-benzyloxy-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-pyridyloxy)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-[2-(4-oxo-1,4-dihydro-1-pyridyl)ethoxy]quinazoline

7-benzyloxy-4-(2-fluoro-5-hydroxy-4-methylphenoxy)-6-methoxyquinazoline

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-((2-methylthiazol-4-yl)methoxy)quinazoline

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(4-pyridylmethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-((1-methylimidazol-2-yl)methoxy)quinazoline

7-((2-acetamidothiazol-4-yl)methoxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline

7-benzyloxy-4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxyquinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(4-pyridyl)propoxy)quinazoline

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(3-(4-pyridyl)propoxy)quinazoline

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline

4-(4-chloro-2-fluoro-5-hydroxyanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-(4-pyridyl)ethoxy)quinazoline

4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-thienylmethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-pyridyl)ethoxy)quinazoline

4-(3-hydroxy-4-methylanilino)-6-methoxy-7-((1-methylbenzimidazol-2-yl)methoxy)quinazoline

7-((2-chloro-6-methyl-4-pyridyl)methoxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline

4-(4-chloro-2-fluorophenoxy)-6-methoxy-7-((4 pyridyl)methoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-((4 pyridyl)methoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-methylimidazol-1-yl)ethoxy)quinazoline

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-((4-pyridyl)methoxy)quinazoline

7-((2-chloro-4-pyridyl)methoxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1-methylimidazol-2-ylthio)ethoxy)quinazoline

7-(3,4-difluorobenzyloxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-((1-methylimidazol-2-yl)methoxy)quinazoline

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-((1-methylimidazol-2-yl)methoxy)quinazoline

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(2-(1,2,4-triazol-1-yl)ethoxy)quinazoline

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-((3-thienyl)methoxy)quinazoline

4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(2-(4-pyridyl)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1,2,4-triazol-1-yl)ethoxy)quinazoline

4-(2-fluoro-5-hydroxy-4-methylanilino)-7-((4-pyridyl)carboxamido)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(N-(4-pyridyl)amino)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1-methylimidazol-2-yl)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-7-((2-cyano-4-pyridyl)methoxy)-6-methoxyquinazoline

and salts thereof, especially hydrochloride salts thereof,

of which the following are particularly preferred:-

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-pyridyloxy)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-[2-(4-oxo-1,4-dihydro-1-pyridyl)ethoxy]quinazoline

4-(4-chloro-2-fluoroanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(4-pyridyl)propoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-pyridyl)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-((4-pyridyl)methoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-methylimidazol-1-yl)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1-methylimidazol-2-ylthio)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1,2,4-triazol-1-yl)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(N-(4-pyridyl)amino)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1-methylimidazol-2-yl)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-7-((2-cyano-4-pyridyl)methoxy)-6-methoxyquinazoline

and salts thereof, especially hydrochloride salts thereof.

Another compound of interest is 4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(4-pyrimidinylmethoxy)quinazoline and salts thereof especially hydrochloride salts thereof.

For the avoidance of doubt it is to be understood that where in this specification a group is qualified by '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. 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 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 2-6 carbon atoms, preferably 4-5 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 2-6 carbon atoms, preferably 4-5 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 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 Y^1 is, for example, a group of formula $-NR^5CO-$, it is the nitrogen atom bearing the R^5 group which is attached to the quinazoline ring and the carbonyl (CO) group is attached to R^4, whereas when Y^1 is, for example, a group of formula $-CONR^6-$, it is the carbonyl group which is attached to the quinazoline ring and the nitrogen atom bearing the R^6 group is attached to R^4. A similar convention applies to the other two atom Y^1 linking groups such as $-NR^8SO_2-$ and $-SO_2NR^7-$. When Y^1 is $-NR^9-$ it is the nitrogen atom bearing the R^9 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 Y^1 represents $-NR^9-$ and R^9 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 in a compound of the formula I when R^4 is, for example, a group of formula $C_{1-5}alkylY^5C_{1-5}alkylX^1$, it is the terminal $C_{1-5}alkyl$ moiety which is bound to Y^1, similarly when R^4 is, for example, a group of formula $C_{2-5}alkenylX^1$ it is the $C_{2-5}alkenyl$ moiety which is bound to Y^1 and an analgous convention applies to other groups. When R^4 is a group 1-X^1prop-1-en-3-yl it is the first carbon to which the group X^1 is attached and it is the third carbon which is linked to Y^1, similarly when R^4 is a group 2-X^1pent-3-en-5-yl it is the second carbon to which the group X^1 is attached and it is the fifth carbon which is linked to Y^1, and an analogous convention applies to other groups.

For the avoidance of any doubt, it is to be understood that when X^1 carries a C_{1-4}aminoalkyl substituent it is the $C_{1-4}alkyl$ moiety which is attached to X^1 whereas when X^1

carries a C_{1-4}alkylamino substituent it is the amino moiety which is attached to X^1 and an analogous convention applies to other groups.

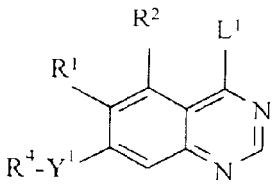
The present invention relates to the compounds of formula I as hereinbefore defined as well as to the salts thereof. Salts for use in pharmaceutical compositions will be pharmaceutically acceptable salts, but other salts may be useful in the production of the compounds of formula I and their pharmaceutically 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 acids such as with hydrogen halides (especially hydrochloric or hydrobromic acid of which hydrochloric acid is particularly preferred) or with sulphuric or phosphoric acid as well as salts with organic acids affording pharmaceutically acceptable anions, such as for example 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 substance or organic base which affords a pharmaceutically acceptable cation. Such salts 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 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.

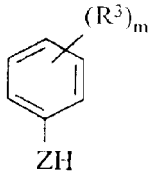
Synthesis of Compounds of Formula I

(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, R^4 and Y^1 are as defined hereinbefore and L^1 is a displaceable group), with a compound of the formula IV:



(IV)

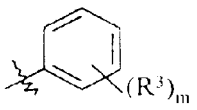
(wherein Z, R^3 and m are as defined hereinbefore) whereby to obtain compounds of the formula I and salts thereof. A convenient displaceable group L^1 is, for example, a halogeno, alkoxy (preferably C_{1-4}alkoxy), aryloxy or sulphonyloxy group, for example a chloro, bromo, methoxy, phenoxy, methanesulphonyloxy 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 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.

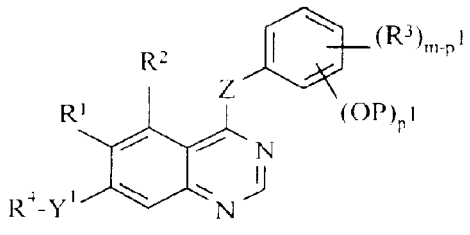
The compound of the invention may be obtained from this process in the form of the free base or alternatively it may be obtained in the form of a salt with the acid of the formula H-L^1 wherein L^1 has the meaning defined hereinbefore. When it is desired to obtain the free base from the salt, the salt may be treated with a base as defined hereinbefore using a conventional procedure.

(b) Where the group of formula IIb:



(IIb)

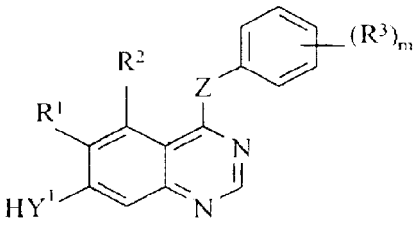
(wherein R^3 and m are as hereinbefore defined) represents a phenyl group carrying one or more hydroxy groups, a compound of the formula I and salts thereof can be prepared by the deprotection of a compound of formula V:



(V)

(wherein Y^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 or near 20°C.

(c) Production of those compounds of formula I and salts thereof wherein the substituent Y^1 is -O-, -S- or -NR^9- can be achieved by the reaction, conveniently in the presence of a base as defined hereinbefore, of a compound of the formula VI:



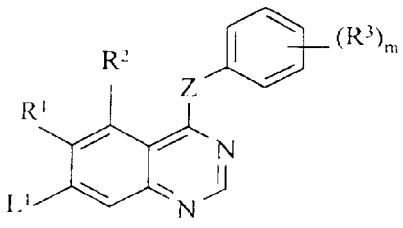
(VI)

(wherein m, Y^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 group 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 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 or near 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:

$$R^4\text{-}Y^1\text{-}H \qquad (IX)$$

(wherein L^1, R^1, R^2, R^3, R^4, Z, m and Y^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 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 or near 100°C.

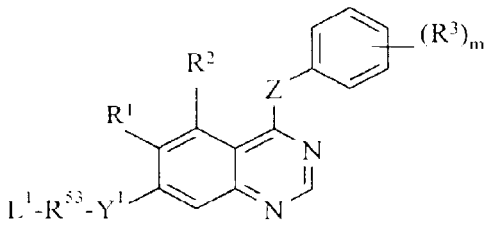
(e) Compounds of the formula I and salts thereof wherein R^4 is C_{1-5}alkylX^2, [wherein X^2 is selected from one of the following three groups:

1) X^1 (wherein X^1 is as defined hereinbefore);

2) Y^7X^1 (wherein Y^7 represents -O-, -S-, $-SO_2-$, $-NR^{47}CO-$, $-NR^{48}SO_2-$ or $-NR^{49}-$ (wherein R^{47}, R^{48} and R^{49} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and X^1 is as defined hereinbefore); and

3) Y^8C_{1-5}alkylY^5X^1 (wherein Y^8 represents -O-, -S-, $-SO_2-$, $-NR^{50}CO-$, $-NR^{51}SO_2-$ or $-NR^{52}-$ (wherein R^{50}, R^{51} and R^{52} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and Y^5 and X^1 are as defined hereinbefore);]

may be prepared by reacting a compound of the formula X:



(X)

(wherein L^1, Y^1, R^1, R^2, R^3, Z and m are as hereinbefore defined and R^{53} is C_{1-3}alkyl) with a compound of the formula XI:

$$X^2\text{-}H \qquad \text{(XI)}$$

(wherein X^2 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.

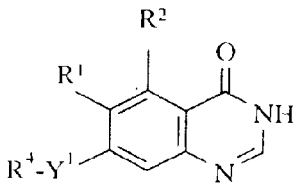
(f) The production of those compounds of the formula I and salts thereof wherein the substituent R^1 is represented by $NR^{10}R^{11}$, where one or both of R^{10} and R^{11} are C_{1-3}alkyl, may be effected by the reaction of compounds of formula I wherein the substituent R^1 is an amino group and 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 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:

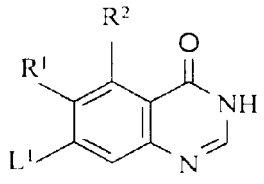


(XII)

(wherein R^1, R^2, R^4 and Y^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.

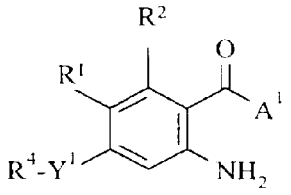
The compounds of formula XII and salts thereof which constitute a further feature of the present invention may for example be prepared by reacting a compound of the formula XIII:



(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 or near 100°C.

The compounds of formula XII and salts thereof may also be prepared by cyclising a compound of the formula XIV:

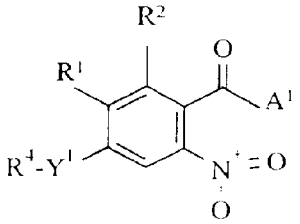


(XIV)

(wherein R^1, R^2, R^4 and Y^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 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:

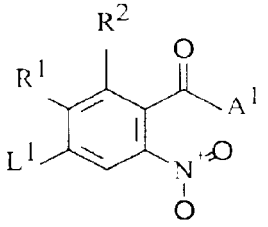


(XV)

(wherein R^1, R^2, R^4, Y^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 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 catalyst 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 a mixture of 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 or near 70°C.

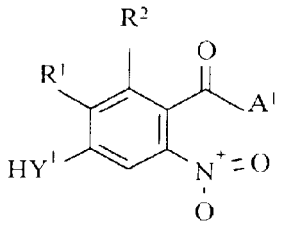
Compounds of the formula XV and salts thereof which constitute a further feature of the present invention, may for example be prepared by the reaction of a compound of the formula XVI:



(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.

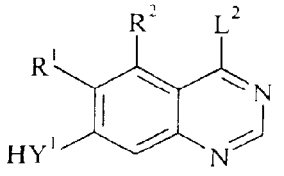
Compounds of formula XV and salts thereof, may for example also be prepared by the reaction of a compound of the formula XVII:



(XVII)

(wherein R^1, R^2, Y^1 and A^1 are as hereinbefore defined with the proviso that Y^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:



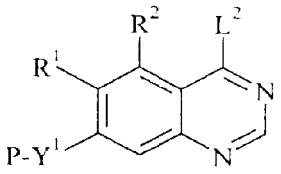
(XVIII)

(wherein R^1, R^2 and Y^1 are as hereinbefore defined with the proviso that Y^1 is not $-CH_2-$ and L^2 represents a displaceable protecting group) 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:

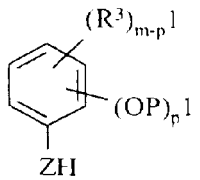


(XIX)

(wherein R^1, R^2, P, Y^1 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.

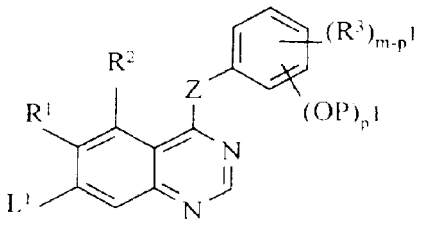
(ii) The compounds of formula V and salts thereof, constitute a further feature of the present invention, and may for example be prepared by the reaction of a compound of formula III as hereinbefore defined with a compound of the formula XX:



(XX)

(wherein R^3, m, p^1, P and Z are as hereinbefore defined). The reaction may for example be effected as described for process (a) hereinbefore.

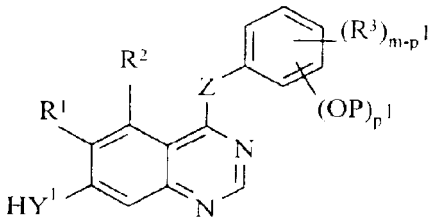
The compounds of formula V and salts thereof may also be prepared by reacting a compound of formula XXI:



(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.

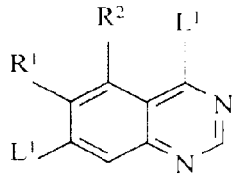
The compounds of formula V and salts thereof may also be prepared by reacting a compound of formula XXII:



(XXII)

(wherein R^1, R^2, R^3, Y^1, Z, P, p^1 and m are as hereinbefore defined with the proviso that Y^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.

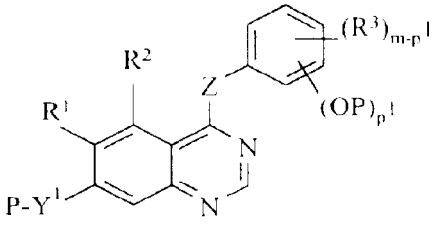
The compounds of formula XXI and salts thereof may for example be prepared by reaction of a compound of formula XXIII:



(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.

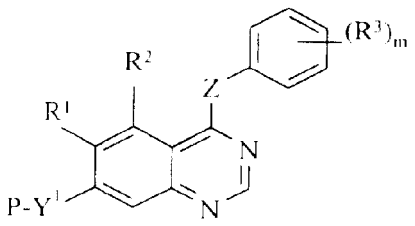
Compounds of the formula XXII and salts thereof may be made by reacting compounds of the formulae XIX and XX as hereinbefore defined, under conditions described in (a) hereinbefore, to give a compound of formula XXIV:



(XXIV)

(wherein R^1, R^2, R^3, P, Z, Y^1, p^1 and m are as hereinbefore defined with the proviso that Y^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 and salts thereof, as hereinbefore defined, may be made by deprotecting the compound of formula XXV:



(XXV)

(wherein R^1, R^2, R^3, P, Z, Y^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 may be effected by a process as described in (a) above.

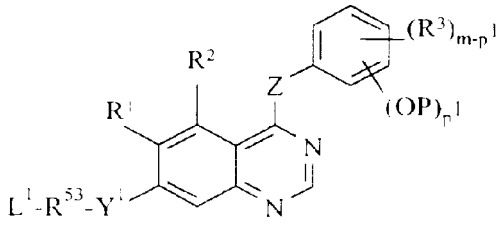
(v) Compounds of the formula X as defined hereinbefore and salts thereof may for example be made by the reaction of a compound of formula VI as defined hereinbefore with a compound of the formula XXVI:

$$L^1\text{-}R^{53}\text{-}L^1$$

(XXVI)

(wherein L^1 and R^{53} 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^{53}, Y^1, R^1, R^2, R^3, 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.

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 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, or a pharmaceutically acceptable salt thereof, as defined hereinbefore 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 intraveous, subcutaneous, intramuscular, intravascular or infusion) as a sterile solution, suspension or emulsion, for topical administration as an ointment or cream or for rectal administration 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 5-5000 mg per square metre body area of the animal, i.e. approximately 0.1-100 mg/kg. A unit dose in the range, for example, 1-100 mg/kg, preferably 1-50 mg/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 1-250 mg 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 now 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.

Thus according to this 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 1-50 mg/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 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

NMP 1-methyl-2-pyrrolidinone

THF tetrahydrofuran

TFA trifluoroacetic acid.]

Example 1

To a solution of 4-(3-acetoxy-4-methylanilino)-6-methoxy-7-(4-pyridylmethoxy)quinazoline (93mg, 0.2mmol) in a mixture of methanol (6ml) and methylene chloride (3ml) was added at ambient temperature a 2M aqueous sodium hydroxide solution (0.3ml, 0.6mmol). The mixture was stirred for 10 minutes at ambient temperature, the solvents were partially evaporated, water was added to the residue and the solution was acidified with 0.1M hydrochloric acid to pH6. The precipitate was filtered off, washed with water and dried under vacuum to give **4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(4-pyridylmethoxy)quinazoline** hydrochloride (67mg, 87%).

m.p. 249-251°C

^{1}H NMR Spectrum: (DMSOd_6) 2.13(s, 3H); 4.01(s, 3H); 5.40(s, 2H); 7.05(br s, 2H); 7.24(s, 1H); 7.34(s, 1H); 7.51(d, 2H); 7.92(s, 1H); 8.44(s, 1H); 8.63(d, 2H); 9.34(s, 1H); 9.47(br s, 1H)

MS - ESI: 389 [MH]$^+$

Elemental analysis:	Found	C 61.4	H 5.3	N 12.8
$C_{23}H_{20}N_4O_3$ 1.8H_2O, 0.2HCl	Requires	C 61.7	H 5.6	N 13.1%

The starting material was prepared as follows:

A mixture of 2-amino-4-benzyloxy-5-methoxybenzamide (10g, 0.04mol), (J. Med. Chem. 1977, vol 20, 146-149), 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 at 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 hydrochloride (3.45g).

A mixture of 7-benzyloxy-4-chloro-6-methoxyquinazoline hydrochloride (2.18g, 6.47mmol), 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 filtered off, washed with isopropanol and ether to give 4-(3-acetoxy-4-methylanilino)-7-benzyloxy-6-methoxyquinazoline hydrochloride (2.69g, 89%).

A mixture of 4-(3-acetoxy-4-methylanilino)-7-benzyloxy-6-methoxyquinazoline hydrochloride (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 an atmosphere of hydrogen (1.5 atmospheres) for 30 minutes. The catalyst was filtered off and the filtrate evaporated. The residual solid was triturated in ether, filtered off and dried under vacuum at 50°C to give 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (2.1g, 100%).

To a solution of 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (375mg, 1mmol) in DMF (16ml) were added at ambient temperature potassium carbonate (415mg, 3mmol) and 4-(bromomethyl)pyridine hydrobromide (J.Org.Chem. 1958, 23, 575, 278mg, 1.1mmol). The reaction mixture was heated at 60°C for 2 hours. The mixture was evaporated and the residue was partitioned between ethyl acetate and water. The organic layer was washed with a saturated aqueous sodium chloride solution, dried ($MgSO_4$) and evaporated. The residue was purified by column flash chromatography, eluting with methylene chloride/methanol (95/5) to give 4-(3-acetoxy-4-methylanilino)-6-methoxy-7-(4-pyridylmethoxy)quinazoline (93mg, 22%).

m.p. 201-202°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.12(s, 3H); 2.34(s, 3H); 4.02(s, 3H); 5.40(s, 2H); 7.27(s, 1H); 7.30(d, 1H); 7.51(d, 2H); 7.62(s, 1H); 7.65(d, 1H); 7.91(s, 1H); 8.47(s, 1H); 8.63(d, 2H); 9.53(s, 1H)

MS - ESI: 453 $[MNa]^+$, 431 $[MH]^+$

Elemental analysis:	Found	C 65.4	H 5.5	N 12.7
$C_{24}H_{22}N_4O_4$ $0.6H_2O$	Requires	C 65.3	H 5.3	N 12.7%

3-Acetoxy-4-methylaniline used as a starting material was prepared as follows:

To a mixture of 2-methyl-5-nitrophenol (2.5g, 16.3mmol) and 1M aqueous sodium hydroxide (24.5ml) was added at ambient temperature acetic anhydride (1.9ml, 20.3mmol). The mixture was stirred for 40 minutes, the solid was filtered off 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 filtered off and the filtrate evaporated to give 3-acetoxy-4-methylaniline (2.45g, 94%).

Example 2

Using an analogous procedure to that described for the starting material in Example 1, 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (750mg) was reacted with 3-(bromomethyl)pyridine hydrobromide (Can. J. Chem. 1978, 56, 3068) (378mg) to give

4-(3-acetoxy-4-methylanilino)-6-methoxy-7-(3-pyridylmethoxy)quinazoline (293mg, 34%).

m.p. 113-115°C

^1H NMR Spectrum: ($DMSOd_6$) 2.09(s, 3H); 2.30(s, 3H); 3.94(s, 3H); 5.32(s, 2H); 7.27(d, 1H); 7.32(s, 1H); 7.43-7.46(m, 1H); 7.58(s, 1H); 7.59-7.63(m, 1H); 7.85(s, 1H); 7.89-7.92(m, 1H); 8.45(s, 1H); 8.57(dd, 1H); 8.71(d, 1H); 9.48(s, 1H)

MS - ESI: 453 $[MNa]^+$, 431 $[MH]^+$

Elemental analysis:	Found	C 64.4	H 5.7	N 11.7
$C_{24}H_{22}N_4O_4$ $0.85H_2O$	Requires	C 64.7	H 5.4	N 12.6%

Example 3

The compound synthesised in Example 2 was further subjected to basic cleavage of the acetoxy protecting group using an analogous procedure to that described in Example 1 to give **4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(3-pyridylmethoxy)quinazoline** (215mg, 83%).

m.p. 258-259°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.12(s, 3H); 3.94(s, 3H); 5.34(s, 2H); 7.05(s, 2H); 7.32(s, 1H); 7.35(s, 1H); 7.46-7.49(m, 1H); 7.88(s, 1H); 7.93-7.95(m, 1H); 8.43(s, 1H); 8.60(dd, 1H); 8.74(d, 1H); 9.33(s, 1H); 9.35(s, 1H)

MS - ESI: 411 $[MNa]^+$, 389 $[MH]^+$

Elemental analysis:	Found	C 59.2	H 5.5	N 12.6
$C_{22}H_{20}N_4O_3$ $3H_2O$ 0.07HCl	Requires	C 59.4	H 5.9	N 12.6%

Example 4

4-(3-Acetoxy-4-methylanilino)-6-methoxy-7-(2-pyridylmethoxy)quinazoline (170mg, 0.39mmol) was subjected to basic cleavage of the acetoxy protecting group using an analogous procedure to that described in Example 1 to give **4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(2-pyridylmethoxy)quinazoline** hydrochloride (58mg, 38%).

m.p. 236-238°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.30(s, 3H); 3.97(s, 3H); 5.34(s, 2H); 7.02(s, 2H); 7.23(s, 1H); 7.33(s, 1H); 7.36-7.39(m, 1H); 7.56(d, 1H); 7.84-7.88(m, 1H); 7.87(s, 1H); 8.39(s, 1H); 8.91(d, 1H); 9.32(s, 2H)

MS - ESI: 389 $[MH]^+$

Elemental analysis:	Found	C 55.8	H 5.5	N 11.8
$C_{22}H_{20}N_4O_3$ $3H_2O$ 0.75HCl	Requires	C 56.2	H 5.7	N 11.9%

The starting material was prepared as follows:

Using an analogous procedure to that described for the starting material in Example 1, 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (376mg)

was reacted with 2-(chloromethyl)pyridine hydrochloride (328mg) to give 4-(3-acetoxy-4-methylanilino)-6-methoxy-7-(2-pyridylmethoxy)quinazoline (170mg, 40%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.12(s, 3H); 2.34(s, 3H); 4.00(s, 3H); 5.37(s, 2H); 7.29(s, 1H); 7.31(s, 1H); 7.39 - 7.42(m, 1H); 7.58 - 7.66(m, 3H); 7.87 - 7.90(m, 1H); 7.91(s, 1H); 8.47(s, 1H); 8.64(d, 1H); 9.52(s, 1H)

Example 5

4-(3-Acetoxy-4-methylanilino)-6-methoxy-7-(pyrimidin-4-ylmethoxy)quinazoline (496mg, 1.15mmol) was subjected to basic cleavage of the acetoxy protecting group using an analogous procedure to that described in Example 1 to give

4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(pyrimidin-4-ylmethoxy)quinazoline (278mg, 62%).

m.p. 290-291°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.13(s, 3H); 4.02(s, 3H); 5.43(s, 2H); 7.05(s, 2H); 7.24(s, 1H); 7.35(s, 1H); 7.67(d, 1H); 7.92(s, 1H); 8.41(s, 1H); 8.89(d, 1H); 9.24(s, 1H); 9.36(s, 1H); 9.38(s, 1H)

MS - ESI: 390 $[MH]^+$

Elemental analysis:	Found	C 58.8	H 5.4	N 16.3
$C_{21}H_{19}N_5O_3.2.2H_2O$	Requires	C 58.8	H 5.5	N 16.3%

The starting material was prepared as follows:

Using an analogous procedure to that described for the starting material in Example 1, 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (560mg) was reacted in the presence of catalytic potassium iodide with 4-(chloromethyl)pyrimidine (375mg) to give 4-(3-acetoxy-4-methylanilino)-6-methoxy-7-(pyrimidin-4-ylmethoxy)quinazoline (496mg, 74%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.13(s, 3H); 2.35(s, 3H); 4.03(s, 3H); 5.44(s, 2H); 7.27(s, 1H); 7.31(d, 1H); 7.62 - 7.68(m, 3H); 7.93(s, 1H); 8.47(s, 1H); 8.89(d, 1H); 9.24(d, 1H); 9.54(s, 1H)

4-(Chloromethyl)pyrimidine was synthesised as follows:

A mixture of 4-methylpyrimidine (2g, 21.2mmol), N-chlorosuccinimide (4.26g, 31.9mmol) and dibenzoylperoxide (500mg) in carbon tetrachloride (100ml) was heated at 80°C for 2 hours. After cooling, the mixture was filtered and the filtrate was evaporated. The resulting oil was purified by flash chromatography using methylene chloride as eluant to give 4-(chloromethyl)pyrimidine as an orange oil (1g, 37%).

Example 6

A solution of 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (400mg, 1.06mmol), (prepared as described for the starting material in Example 1), 2-chloromethyl-1-methylimidazole hydrochloride (354mg, 2.12 mmol), and potassium carbonate (585mg) in DMF (15ml) was heated at 60°C for 15 hours. After cooling to ambient temperature the reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried ($MgSO_4$) and evaporated. The residue was diluted with methanol (20ml) and 2M sodium hydroxide (1ml) was added. After stirring for 1 hour, the reaction mixture was diluted with water (20ml) and 2M hydrochloric acid (3 ml) was added. The resulting solid was filtered off, washed with water and dried under vacuum to give **4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(1-methylimidazol-2-ylmethoxy)quinazoline** hydrochloride (150mg, 29%).

m.p. 257-260°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.17(s, 3H); 3.95(s, 3H); 4.03(s, 3H); 5.68(s, 2H); 7.02(dd, 1H); 7.16(s, 2H); 7.64(s, 1H); 7.72(s, 1H); 7.80(s, 1H); 8.42(s, 1H); 8.8(s, 1H); 9.7(s, 1H); 11.38(s, 1H)

MS - ESI: 392 $[MH]^+$

Elemental analysis:	Found	C 51.7	H 5.5	N 14.2
$C_{21}H_{21}N_5O_3$ 1.65H_2O 1.9HCl	Requires	C 51.4	H 5.4	N 14.3%

Example 7

A solution of 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (400mg, 1.06mmol), (prepared as described for the starting material in Example 1), 4-chloromethyl-2-methylthiazole hydrochloride (390mg, 2.12mmol), potassium carbonate (438mg) and potassium iodide (40mg) in DMF (15ml) was heated at 60°C for 15

hours. After cooling to ambient temperature the reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried ($MgSO_4$) and evaporated. The residue was diluted with methanol (10ml) and 2M sodium hydroxide (2ml) was added. After stirring for 1 hour, the reaction mixture was diluted with water (20ml) and 2M hydrochloric acid (3ml) was added. The resulting solid was filtered off, washed with water and dried under vacuum to give

4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(2-methylthiazol-4-ylmethoxy) quinazoline hydrochloride (300mg, 59%).

m.p. 243-245°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.17(s, 3H); 2.7(s, 3H); 4.0(s, 3H); 5.35(s, 2H); 7.0(dd, 1H); 7.12(d, 1H); 7.16(d, 1H); 7.58(s, 1H); 7.75(s, 1H); 8.3(s, 1H); 8.8(s, 1H); 9.5-9.8(br s, 1H); 11.3(s, 1H)

MS - ESI: 409 $[MH]^+$

Elemental analysis:	Found	C 51.9	H 5.0	N 11.6	S 6.8	
$C_{21}H_{20}N_4O_3S\ 1H_2O\ 1.7HCl$	Requires	C 51.6	H 4.9	N 11.5	S 6.6%	

Example 8

To a solution of 4-(3-acetoxy-4-methylanilino)-6-methoxy-7-(3-thienylmethoxy)quinazoline (200mg, 0.45mmol) in methylene chloride/methanol (1/1) (20ml) was added, a 2M aqueous sodium hydroxide solution (0.67ml, 1.35mmol). The mixture was stirred for 35 minutes at ambient temperature, the solvents were evaporated, water was added to the residue and the solution was extracted with ethyl acetate. The organic layer was washed with water, brine, then dried ($MgSO_4$) and evaporated to give a white solid. This solid was then dissolved into a saturated solution of hydrochloric acid in methanol (10ml) and stirred for 10 minutes. The solid product was filtered and dried under a vacuum, to give **4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(3-thienylmethoxy)quinazoline** hydrochloride (127mg, 66%).

m.p. 246-248°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.15(s, 3H); 3.98(s, 3H); 5.32(s, 2H); 6.98(dd, 1H); 7.10(s, 1H); 7.14(d, 1H); 7.25(d, 1H); 7.40(s, 1H); 7.61(dd, 1H); 7.70(d, 1H); 8.12(s, 1H); 8.74(s, 1H); 9.60(s, 1H)

MS - ESI: 394 $[MH]^+$

Elemental analysis: Found C 58.3 H 4.8 N 9.4 S 7.3 Cl 7.5

$C_{21}H_{19}N_3O_3S$ 0.2H_2O 0.95HCl Requires C 58.4 H 4.8 N 9.7 S 7.4 Cl 7.8%

The starting material was prepared as follows:

Using an analogous procedure to that described for the starting material in Example 1, 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (400mg) was reacted in the presence of catalytic potassium iodide with 3-chloromethylthiophene (Journal of the Chemical Society 1958, 4202) (168mg) to give 4-(3-acetoxy-4-methylanilino)-6-methoxy-7-(3-thienylmethoxy)quinazoline (210mg, 46%).

m.p. 201-203°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.11(s, 3H); 2.32(s, 3H); 3.95(s, 3H); 5.27(s, 2H); 7.23(dd, 1H); 7.28(d, 1H); 7.32(s, 1H); 7.58-7.66(m, 4H); 7.85(s, 1H); 8.46(s, 1H); 9.49(s, 1H)

MS - ESI: 436 $[MH]^+$

Elemental analysis: Found C 63.0 H 5.2 N 9.1 S 7.3

$C_{23}H_{21}N_3O_4S$ 0.3H_2O Requires C 62.7 H 5.0 N 9.5 S 7.3%

Example 9

7-(2-Acetamidothiazol-4-ylmethoxy)-4-(3-acetoxy-4-methylanilino)-6-methoxyquinazoline (220mg, 0.44mmol) was subjected to basic cleavage of the acetoxy protecting group using an analogous procedure to that described in Example 8 to give, **7-(2-acetamidothiazol-4-ylmethoxy)-4-(3-hydroxy-4-methylanilino)-6-methoxyquinazoline** hydrochloride (41mg, 19%).

m.p. 202-204°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.16(s, 3H); 2.17(s, 3H); 4.01(s, 3H); 5.31(s, 2H); 6.98(dd, 1H); 7.10(d, 1H); 7.17(d, 1H); 7.34(s, 1H); 7.47(s, 1H); 8.22(s, 1H); 8.80(s, 1H); 9.68(br s, 1H)

MS - ESI: 452 [MH]$^+$

Elemental analysis:	Found	C	47.1	H	4.7	N	12.5	S	5.8	Cl	12.2
$C_{22}H_{21}N_5O_5S\ 2H_2O\ 2HCl$	Requires	C	47.2	H	4.9	N	12.5	S	5.7	Cl	12.7%

The starting material was prepared as follows:

Using an analogous procedure to that described for the starting material in Example 1, 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (400mg) was reacted in the presence of catalytic potassium iodide with 2-acetamido-4-chloromethylthiazole (252mg) to give 7-(2-acetamidothiazol-4-ylmethoxy)-4-(3-acetoxy-4-methylanilino)-6-methoxyquinazoline (220mg, 42%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.13(s, 3H); 2.15(s, 3H); 2.35(s, 3H); 3.97(s, 3H); 5.24(s, 2H); 7.24-7.31(m, 2H); 7.37(s, 1H); 7.63-7.66(m, 2H); 7.87(s, 1H); 8.48(s, 1H); 9.50(s, 1H)

MS - ESI: 494 [MH]$^+$

Example 10

4-(3-Acetoxy-4-methylanilino)-7-(3,5-dimethylisoxazol-4-ylmethoxy)-6-methoxyquinazoline (342mg, 0.76mmol) was subjected to basic cleavage of the acetoxy protecting group using an analogous procedure to that described in Example 8 to give **4-(3-hydroxy-4-methylanilino)-7-(3,5-dimethylisoxazol-4-ylmethoxy)-6-methoxyquinazoline** hydrochloride (209mg, 62%).

m.p. 252-254°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.20(s, 3H); 2.29(s, 3H); 2.52(s, 3H); 4.03(s, 3H); 5.23(s, 2H); 7.03(dd, 1H); 7.15(d, 1H); 7.19(d, 1H); 7.44(s, 1H); 8.22(s, 1H); 8.82(s, 1H); 9.67(s, 1H)

MS - ESI: 407 [MH]$^+$

Elemental analysis:	Found	C	59.1	H	5.4	N	12.6	Cl	8.0
$C_{22}H_{22}N_4O_4\ 0.25H_2O\ 1HCl$	Requires	C	59.1	H	5.3	N	12.5	Cl	7.9%

The starting material was prepared as follows:

Using an analogous procedure to that described for the starting material in Example 1, 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (400mg)

was reacted in the presence of potassium iodide (16mg) with 4-chloromethyl-3,5-dimethylisoxazole (177mg) to give 4-(3-acetoxy-4-methylanilino)-7-(3,5-dimethylisoxazol-4-ylmethoxy)-6-methoxyquinazoline (342mg, 72%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.18(s, 3H); 2.33(s, 3H); 2.35(s, 3H); 2.46(s, 3H); 3.98(s, 3H); 4.98(s, 2H); 7.00(s, 1H); 7.15(s, 1H); 7.22-7.25(m, 1H); 7.32(s, 1H); 7.43(dd, 1H); 7.51(s, 1H); 8.66(s, 1H)

Example 11

A solution of 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (400mg, 1.06mmol), (prepared as described for the starting material in Example 1), 4-(3-chloropropyl)pyridyl hydrochloride 410mg, 2.1mmol), potassium carbonate (438mg) and potassium iodide (40mg) in DMF (15ml) was heated at 60°C for 15 hours. After cooling to ambient temperature the reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried ($MgSO_4$) and evaporated. The residue was diluted with methanol (20ml) and 2M sodium hydroxide (2ml) was added. After stirring for 1 hour, the reaction mixture was diluted with water (20ml) and concentrated hydrochloric acid (1ml) was added. The resulting solid was filtered off and was purified by preparative C18 HPLC using a gradient of methanol/water (0% to 80%) as eluant. After evaporation of the methanol, concentrated hydrochloric acid (0.3ml) was added and the solution was evaporated to dryness. After trituration with acetone, the solid was filtered off and dried under vacuum to give **4-(3-hydroxy-4-methylanilino)-6-methoxy-7-(4-pyridylpropoxy)quinazoline** hydrochloride (305mg, 59%).

m.p. 278-282°C

^{1}H NMR Spectrum: (DMSO-d_6) 2.15(s, 3H); 2.3(m, 2H); 3.1(m, 2H); 3.96(s, 3H); 4.24(t, 2H); 6.98(dd, 1H); 7.15(m,2H); 7.44(s, 1H); 7.96(d, 2H); 8.31(s, 1H); 8.77(s, 1H); 8.81(d, 2H); 9.7(br s, 1H); 11.34(s, 1H)

MS - ESI: 417 $[MH]^+$

Elemental analysis:	Found	C 57.3	H 5.4	N 11.0
$C_{24}H_{24}N_4O_3$ 0.7H_2O 1.95HCl	Requires	C 57.6	H 5.5	N 11.2%

The starting material was prepared as follows:

Thionyl chloride (1.6ml) was added to a solution of 4-pyridine propanol (2g, 14.5mmol) in trichloromethane (20ml) cooled at 0°C. After stirring for 1 hour at ambient temperature followed by 1 hour at 60°C, the solvent was evaporated and the residue was triturated with ether to give 4-(3-chloropropyl)pyridyl hydrochloride as a white solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 2.15(m, 2H); 3.02(t, 2H); 3.69(t, 2H); 7.96(d, 2H); 8.84(d, 2H)

Example 12

A solution of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (410mg, 1.00mmol), 4-(3-chloropropyl)pyridyl hydrochloride (480mg, 2.5mmol), potassium carbonate (480mg) and potassium iodide (40mg) in DMF (15ml) was heated at 60°C for 15 hours. After cooling to ambient temperature the reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried ($MgSO_4$) and evaporated. The residue was diluted with methanol (10ml) and 2M sodium hydroxide (2ml) was added. After stirring for 1 hour, the reaction mixture was diluted with water (20ml) and concentrated hydrochloric acid (0.5 ml) was added. The resulting solid was filtered off and was purified by preparative C18 HPLC using a gradient of methanol/water (0% to 80%) as eluant. After evaporation of the methanol, concentrated hydrochloric acid (0.3ml) was added and the solution was evaporated to dryness. After trituration with acetone, the solid was filtered off and dried under vacuum to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(4-pyridylpropoxy)quinazoline** hydrochloride (243mg, 48%).

m.p. 246-248°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.16(s, 3H); 2.30(m, 2H); 3.09(t, 2H); 3.95(s, 3H); 4.26(t, 2H); 6.90(d, 1H); 7.11(d, 1H); 7.41(s, 1H); 7.94(d, 2H); 8.3(s, 1H); 8.77(s, 1H); 8.80(d, 2H); 9.7(br s, 1H); 11.46(s, 1H)

MS - ESI: 435 $[MH]^+$

Elemental analysis: Found C 55.3 H 5.3 N 10.2 Cl 13.0

$C_{24}H_{23}N_4O_3F$ $0.9H_2O$ 1.95HCl Requires C 55.3 H 5.2 N 10.7 Cl 13.3%

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 hydrochloride (800mg, 2.4mmol), (prepared as described for the starting material in Example 1), and 2-fluoro-5-methoxycarbonyloxy-4-methylaniline (570mg, 2.89mmol) 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 hydrochloride (1.0g, 87%)

^{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 hydrochloride (700mg, 1.45mmol) in DMF (10ml), methanol (10ml) and trichloromethane (10ml) containing 10% palladium-on-charcoal (100mg) was stirred under an 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 hydrochloride (570 mg, 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 13

A stirred solution of 4-chloro-6-methoxy-7-(4-pyridylmethoxy)quinazoline (35mg, 0.1mmol) and 2-fluoro-5-hydroxy-4-methylaniline (15mg, 0.1mmol) in a mixture of ethereal

hydrogen chloride (2ml) and isopropanol (5ml) was heated at reflux for 4 hours. The precipitated product was collected by filtration, washed with acetone and dried under vacuum to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(4-pyridylmethoxy)quinazoline** hydrochloride (23mg, 47%).

m.p. 257-260°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.15(s, 3H); 4.08(s, 3H); 5.60(s, 2H); 6.90(d, 1H); 7.07(d, 1H); 7.47(s, 1 H); 7.93(br d, 2H); 8.74(s, 1H); 8.89(br d, 2 H); 9.62(br s, 1H); 11.46(s, 1H)

MS - ESI: 407 $[MH]^+$

Elemental analysis:	Found	C 52.8	H 4.6	N 10.9
$C_{22}H_{19}N_4O_3F$ $1H_2O$ 2HCl	Requires	C 53.1	H 4.6	N 11.3%

The starting chloroquinazoline 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 N-methylpyrrolidone (20ml) and the mixture stirred for 10 minutes.

7-Benzyloxy-4-chloro-6-methoxyquinazoline hydrochloride (1.6g, 4.7mmol), (prepared as described for the starting material in Example 1), 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 (3x100ml). 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, 95%) 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: 359 $[MH]^+$

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]$^+$

A mixture of 7-hydroxy-6-methoxy-4-phenoxyquinazoline (95mg, 0.35mmol), 4-chloromethyl pyridine hydrochloride (120mg, 0.74mmol) and potassium carbonate (200mg, 1.4mmol) in DMF (5ml) were heated at 80°C for 2 hours. The reaction mixture was allowed to cool, water was added and the mixture extracted with ethyl acetate (3x50ml). The combined extracts were then washed with water and dried ($MgSO_4$). The solvent was removed by evaporation and the residue trituated with an ethyl acetate/hexane mixture to give 6-methoxy-4-phenoxy-7-(4-pyridylmethoxy)quinazoline (44mg, 35%) as a white solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 4.02(s, 3H); 5.47(s, 2H); 7.25-7.35(m, 3H); 7.45(s, 1H); 7.4-7.55(m, 4H); 7.62(s, 1H); 8.52(s, 1 H); 8.63(dd, 2H)

MS - ESI: 360 [MH]$^+$

A solution of 6-methoxy-4-phenoxy-7-(4-pyridylmethoxy)quinazoline (200mg, 0.56mmol) in 2M hydrochloric acid (15ml) was heated at reflux for 90 minutes. The reaction mixture was allowed to cool and neutralised to pH6-7 with aqueous ammonia. The precipitated product was extracted with methanol/methylene chloride (1:9) and the extract solution dried ($MgSO_4$). Removal of the solvent by evaporation gave 6-methoxy-7-(4-pyridylmethoxy)-3,4-dihydroquinazolin-4-one (90mg, 57%) as a grey solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.93(s, 3H); 5.35(s, 2H); 7.18(s, 1H); 7.48(s, 1H); 7.50(m, 2H); 8.04(s, 1 H); 8.62(m, 2H)

MS - ESI: 284 [MH]$^+$

Phosphorus oxytrichloride (0.1ml) was added to a mixture of 6-methoxy-7-(4-pyridylmethoxy)-3,4-dihydroquinazolin-4-one (81mg, 0.29mmol) and N,N-dimethylaniline (0.1ml) in toluene (5ml), and the mixture heated at reflux for 1 hour. The volatiles were removed by evaporation and the residue partitioned between methylene chloride and aqueous ammonia. The organic extract was separated, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by chromatography on silica eluting with ethyl acetate progressing through increasingly polar mixtures to methanol/methylene chloride (1/9) to give 4-chloro-6-methoxy-7-(4-pyridylmethoxy)quinazoline (40mg, 41%).

1H NMR Spectrum: ($DMSOd_6$) 4.04(s, 3H); 5.47(s, 2H); 7.46(s, 1H); 7.50(d, 2H); 7.53(s, 1H); 8.60(d, 2H); 8.85(s, 1 H)

MS - ESI: 302 $[MH]^+$

The starting aniline was prepared as described below:

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.

^1H NMR Spectrum: ($DMSOd_6$) 2.14(s, 3H); 3.81(s, 3H); 7.05(m, 1H); 7.1-7.25(m, 2 H)

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.

^1H NMR Spectrum: ($DMSOd_6$; CD_3COOD) 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.

^1H 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 14

A solution of 7-benzyloxy-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxyquinazoline hydrochloride (259mg, 0.54 mmol), (prepared as described for the starting material in Example 12), in methanol (15ml) containing 1M sodium hydroxide (1.6ml) was stirred at ambient temperature for 1 hour. After addition of water (15ml), concentrated hydrochloric acid (1ml) was added and the mixture was stirred at ambient temperature for 15 minutes. After evaporation of methanol, the precipitate was filtered, washed with water and dried under vacuum to give **7-benzyloxy-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline** hydrochloride (192mg, 80%).

m.p. 294-298°C

^{1}H NMR Spectrum: (DMSOd_6) 2.2(s, 3H); 4.05(s, 3H); 5.35(s, 2H); 6.9(d, 1H); 7.12(d, 1H); 7.35-7.5(m, 4H); 7.55-7.6(m, 2H); 8.25(s, 1H); 8.8(s, 1H); 9.7(s, 1H); 11.35(s, 1H)

MS - ESI: 406 [MH]$^+$

Elemental analysis:	Found	C 62.3	H 4.9	N 9.3
$C_{23}H_{20}N_3O_3F$ 0.16H_2O 1HCl	Requires	C 62.1	H 4.8	N 9.5%

Example 15

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (200mg, 0.63mmol), 2-(2-chloroethoxy)pyridine hydrochloride (120mg, 0.61mmol) and potassium carbonate (260mg, 1.9mmol) in DMF (25ml) was heated at 90°C for 16 hours. The mixture was diluted with water and extracted with ethyl acetate. The extract was dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with ethyl acetate/methanol mixtures (100/0 increasing to 90/10) to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-pyridyloxy)ethoxy)quinazoline** (20mg, 7%) as an off-white solid.

^{1}H NMR Spectrum: (DMSOd_6) 3.99(s, 3H); 4.35(t, 2H); 4.42(t, 2H); 6.22(t, 1H); 6.40(d, 1H); 7.42(s, 1H); 7.55(d, 2H); 7.71(d, 1H); 7.85(t, 1H); 8.55(d, 1H); 9.62(s, 1H)

MS - ESI: 441 [MH]$^+$

The starting material was prepared as follows:

A solution of 7-benzyloxy-4-chloro-6-methoxyquinazoline hydrochloride (1.34g, 4mmol), (prepared as described for the starting material in Example 1), 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, 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
$C_{22}H_{17}N_3ClFO_2$ 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 $[MH]^+$

Thionyl chloride (0.55ml, 7.55mmol) was added to a solution of 2-(2-hydroxyethoxy)pyridine (700mg, 5.04mmol), (J. Org. Chem. 1977, **42**, 1500), in trichloromethane (20ml) at 5°C. The mixture was stirred for 1 hour at 5°C, allowed to warm to ambient temperature and stirred for a further 1 hour. The volatiles were removed by evaporation and by azeotroping with toluene to give 2-(2-chloroethoxy)pyridine hydrochloride (970mg, 99%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.90(t, 2H); 4.20(t, 2H); 6.22(d, 1H); 6.40(d, 1H); 7.44(dd, 1H); 7.64(d, 1H)

MS - ESI: 158 $[MH]^+$

Example 16

Triphenylphosphine (5.5g, 21mmol) followed by 2-[N-methyl-N-(4-pyridyl)]aminoethanol (1.49g, 9.8mmol), (prepared as described in EP 0359389 A1), were added to a stirred solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (2.23g, 7mmol), (prepared as described for the starting material in Example 15), in methylene chloride (60ml) under nitrogen. Diethyl azodicarboxylate (3.65g, 21mmol) was then added dropwise and the mixture was stirred at ambient temperature for 2 hours. Ethyl acetate (200ml) was added and the mixture was stirred for a further 2 hours. The solid product was collected by filtration, washed with ethyl acetate, dried under vacuum and finally purified by column chromatography eluting with methylene chloride/methanol (75/25 followed by 60/40 and 50/50) to give a white solid. The purified product was dissolved in methylene chloride/methanol and the insolubles removed by filtration. Ethereal hydrogen chloride (10ml of 3M solution) was added to the filtrate and the volatiles were removed by evaporation. The residue was triturated with ether and the solid product collected by filtration and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-[N-methyl-N-(4-pyridyl)]aminoethoxy)quinazoline** hydrochloride (2.75g, 75%) as a white solid.

m.p. 222-227°C

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.29(s, 3H); 3.95(s, 3H); 4.16(t, 2H); 4.48(t, 2H); 7.05(br s, 1H); 7.37(s, 2H); 7.42(d, 1H); 7.58(t, 1H); 7.65(dd, 1H); 8.18(s, 1H); 8.28(br s, 2H); 8.86(s, 1H)

MS - ESI: 454 $[MH]^+$

Elemental Analysis: Found C 51.2 H 4.8 N 12.9

$C_{23}H_{21}N_5O_2ClF$ $0.9H_2O$ 2HCl Requires C 50.9 H 4.6 N 12.9%

Example 17

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (300mg, 0.94mmol), (prepared as described for the starting material in Example 15), 4-(2-

chloroethoxy)pyridine hydrochloride (155mg, 0.79mmol) and potassium carbonate (260mg, 1.9mmol) in NMP (20ml) was heated at 90°C for 2 hours, allowed to cool to ambient temperature and stirred for a further 18 hours. The mixture was diluted with water and extracted with ethyl acetate. The extract was washed with water, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol mixtures (100/0 increasing to 95/5) to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-pyridyloxy)ethoxy)quinazoline** (20mg, 7%).

m.p. 200-202°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.90(s, 3H); 4.50(s, 4H); 7.04(d, 2H); 7.26(s, 1H); 7.33(dd, 1H); 7.5-7.6(m, 2H); 7.80(s, 1H); 8.35(s, 1H); 8.39(d, 2H); 9.52(s, 1H)

MS - ESI: 441 $[MH]^+$

The starting material was prepared as follows:

Thionyl chloride (0.75ml, 10mmol) was added to a solution of 4-(2-hydroxyethoxy)pyridine (0.9g, 6.5mmol), (J. Chem. Soc. Perkin II, 1987, 1867), in trichloromethane (20ml) at 5°C. The mixture was stirred for 1 hour at 5°C, allowed to warm to ambient temperature and stirred for a further 2 hours. The volatiles were removed by evaporation and by azeotroping with toluene to give 4-(2-chloroethoxy)pyridine hydrochloride (1.3g, 100%).

^{1}H NMR Spectrum: ($DMSOd_6$) 4.03(t, 2H); 4.62(t, 2H); 7.58(d, 2H); 8.77(d, 2H)

MS - ESI: 158 $[MH]^+$

Example 18

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (300mg, 0.94mmol), (prepared as described for the starting material in Example 15), 1-(2-chloroethyl)-1,2-dihydro-2-pyridone (175mg, 1.11mmol), (J. Am. Chem. Soc. 1951, **73**, 3635), and potassium carbonate (260mg, 1.9mmol) in DMF (30ml) was heated at 80°C for 3 hours, allowed to cool to ambient temperature and stirred for a further 18 hours. The mixture was diluted with water and extracted with ethyl acetate. The extract was washed with water, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol/triethylamine mixtures (100/0/0

increasing to 70/30/0.5) to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-[2-(2-oxo-1,2-dihydro-1-pyridyl)ethoxy]quinazoline** (50mg, 12%).

m.p. 209-211°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.94(s, 3H); 4.35(t, 2H); 4.41(t, 2H); 6.22(dd, 1H); 6.40(d, 1H); 7.14(s, 1H); 7.35(dd, 1H); 7.42(ddd, 1H); 7.5-7.6(m, 2H); 7.70(d, 1H); 7.80(s, 1H); 8.35(s, 1H); 9.53(s, 1H)

MS - ESI: 441 $[MH]^+$

Example 19

1-(3-Hydroxypropyl)-1,4-dihydro-4-pyridone (220mg, 1.44mmol) in methylene chloride (4ml) followed by 1,1'-(Azodicarbonyl)dipiperidine (720mg, 2.86mmol) were added to a stirred solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (300mg, 0.94mmol), (prepared as described for the starting material in Example 15), and tributylphosphine (0.69ml, 2.8mmol) in methylene chloride (20ml) under nitrogen at 5°C. The mixture was stirred at 5°C for 3 hours, allowed to warm to ambient temperature and stirred for a further 18 hours. The solvent was removed by evaporation and the residue partitioned between ethyl acetate and water. The organic phase was separated, dried ($MgSO_4$), and solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol/triethylamine mixtures (100/0/0 increasing to 70/30/0.5) to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-[3-(4-oxo-1,4-dihydro-1-pyridyl)propoxy]quinazoline** (48mg, 11%).

m.p. >250°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.56(m, 2H); 4.00(s, 3H); 3.54(t, 2H); 4.38(t, 2H); 7.42(d, 1H); 7.5-7.65(m, 5H); 8.43(s, 1H); 8.65-8.75(m, 4H)

MS - ESI: 455 $[MH]^+$

The starting material was prepared as follows:

Sodium hydride (946mg of a 50% suspension in mineral oil, 19.7mmol) was added to a solution of 4-hydroxypyridine (1.88g, 19.7mmol) in DMF (50ml) and the mixture stirred for 30 minutes. 2-(3-Bromopropoxy)tetrahydropyran (4.0g, 17.9mmol), (J. Chem. Soc. 1963, 3440), was added and the mixture heated at 100°C for 3 hours. The reaction mixture was

allowed to cool, diluted with water and extracted with ethyl acetate. The extract was washed with water, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol mixtures (100/0 increasing to 95/5) to give 1-[3-(tetrahydropyran-2-yloxy)propyl]-1,4-dihydro-4-pyridone (1.5g, 35%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.35-1.75(m, 6H); 1.95(t, 2H); 3.35-3.5(m, 2H); 3.65-3.8(m, 2H); 4.12(t, 2H); 4.57(s, 1H); 6.95(s, 2H); 7.94(s, 2H).

A solution of 1-[3-(tetrahydropyran-2-yloxy)propyl]-1,4-dihydro-4-pyridone (0.75g, 3.16mmol) in acetic acid (8ml), THF (4ml) and water (4ml) was heated at 50°C for 4 hours. The volatiles were removed by evaporation to give 1-(3-hydroxypropyl)-1,4-dihydro-4-pyridone (480mg, 99%) as an off-white solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 1.9-1.95(m, 2H); 1.97-2.05(m, 2H); 4.0-4.1(m, 2H); 6.91(m, 2H); 8.36(m, 2H)

MS - ESI: 154 $[M-H]^-$

Example 20

1-(2-Hydroxyethyl)-1,4-dihydro-4-pyridone (221mg, 1.6mmol) was added to a stirred solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (230mg, 0.7mmol), (prepared as described for the starting material in Example 15), and tributylphosphine (0.53ml, 2.1mmol) in methylene chloride (20ml) under nitrogen at 5°C. 1,1'-(Azodicarbonyl)dipiperidine (552mg, 2.2mmol) was added in portions over 10 minutes and the mixture was stirred at 5°C for 2 hours, allowed to warm to ambient temperature and stirred for a further 18 hours. The mixture was diluted with ether, the insolubles removed by filtration and the solvent was removed from the filtrate by evaporation. The residue was partitioned between ethyl acetate and water, the organic phase was separated and dried ($MgSO_4$), and solvent was removed by evaporation. The residue was dissolved in acetone and ethereal hydrogen chloride (1.2ml of a 3M soultion) was added. The mixture was left to stand for 15 minutes and the precipitated product was collected by filtration, washed with ether and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-[2-(4-oxo-1,4-dihydro-1-pyridyl)ethoxy]quinazoline** hydrochloride (54mg, 16%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.98(s, 3H); 4.63(dd, 2H); 4.83(dd, 2H); 7.42(d, 1H); 7.50(s, 1H); 7.56(d, 1H); 7.6-7.65(m, 3H); 8.39(s, 1H); 8.77(s, 1H); 8.80(s, 2H)
MS - ESI: 441 $[MH]^+$

The starting material was prepared as follows:

Sodium hydride (1.27g of a 50% suspension in mineral oil, 26.4mmol) was added to a solution of 4-hydroxypyridine (2.5g, 26mmol) in DMF (50ml) and the mixture stirred for 30 minutes. 2-(2-Bromoethoxy)tetrahydropyran (5.0g, 23.9mmol), (J. Am. Chem. Soc. 1948, **70**, 4187), in DMF (5ml) was added and the mixture heated at 80°C for 3 hours. The reaction mixture was allowed to cool, diluted with water and extracted with ethyl acetate. The extract was washed with water, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol mixtures (100/0 increasing to 97/3) to give 1-[2-(tetrahydropyran-2-yloxy)ethyl]-1,4-dihydro-4-pyridone (1.5g, 28%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.39-1.68(m, 6H); 3.39-3.44(m, 1H); 3.64-3.78(m, 2H); 3.86-3.92(m, 1H); 4.20(t, 2H); 4.64(s, 1H); 6.95(d, 2H); 8.36(d, 2H)
MS - ESI: 224 $[MH]^+$

A solution of 1-[2-(tetrahydropyran-2-yloxy)ethyl]-1,4-dihydro-4-pyridone (500mg, 2.23mmol) in acetic acid (4ml), THF (2ml) and water (1ml) was heated at 45°C for 4 hours. The volatiles were removed by evaporation to give 1-(2-hydroxyethyl)-1,4-dihydro-4-pyridone (221mg, 71%) as an off-white solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.70(t, 2H); 4.06(t, 2H); 6.95(d, 2H); 8.37(d, 2H)

Example 21

A solution of 7-benzyloxy-4-chloro-6-methoxyquinazoline hydrochloride (132mg, 0.4mmol), (prepared as described for the starting material in Example 1), and 2-fluoro-5-methoxycarbonyloxy-4-methylphenol (96mg, 0.48mmol) in pyridine (2ml) was heated at reflux for 3 hours. The mixture was allowed to cool, the solvent was removed by evaporation and the residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with water, brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/ether

(70/30). The resulting solid was crystallised from methylene chloride and methanol to give 7-**benzyloxy-4-(2-fluoro-5-hydroxy-4-methylphenoxy)-6-methoxyquinazoline** (120mg, 64%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.15(s, 3H); 3.98(s, 3H); 5.35(s, 2H); 6.75(d, 1H); 7.13(d, 1H); 7.37(d, 1H); 7.45(t, 2H); 7.48-7.56(m, 3H); 7.58(s, 1H); 8.54 (s, 1H); 9.65(br s, 1H)

MS - ESI: 454 $[MH]^+$

Elemental Analysis:	Found	C 67.8	H 4.9	N 6.9
$C_{23}H_{19}N_2O_4F$ $0.1H_2O$	Requires	C 67.7	H 4.7	N 6.9%

The starting material was prepared as follows:

A mixture of (4-fluoro-2-methyl-5-nitrophenyl) methyl carbonate (8g, 35mmol), (EP 0307777 A2), and platinum(IV)oxide (174mg) in ethanol (100ml) and ethyl acetate (70ml) was stirred under hydrogen at 1.3 atmospheres pressure for 1.5 hours. The catalyst was removed by filtration through diatomaceous earth and the solvent removed by evaporation. The residue was purified by column chromatography eluting with petroleum ether/ethyl acetate (7/3) to give 2-fluoro-5-methoxycarbonyloxy-4-methylaniline (6.56g, 94%) as an oil which crystallised.

^{1}H NMR Spectrum ($CDCl_3$): 2.09(s, 3H); 3.66(br s, 2H); 3.90(s, 3H); 6.54(d, 1H); 6.83(d, 1H)

A solution of sodium nitrite (1.63g, 23mmol) in water (19ml) and ice (48g) was added dropwsie to a solution of 2-fluoro-5-methoxycarbonyloxy-4-methylaniline (3.93g, 20mmol) in 35% sulphuric acid (48ml) at 0°C. The reaction mixture was stirred at 0°C for 30 minutes and a solution of copper(II)nitrate trihydrate (467g, 1.93mol) in water (780ml) followed by copper(II)oxide (2.65g, 18mmol) were added. The solution was extracted with ethyl acetate, the organic layer was washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with petroleum ether/ethyl acetate (8/2) to give 2-fluoro-5-methoxycarbonyloxy-4-methylphenol (2.13g, 53%) as a yellow solid.

^{1}H NMR Spectrum ($CDCl_3$): 2.13(s, 3H); 3.91(s, 3H); 5.11(br s, 1H); 6.78(d, 1H); 6.93(d, 1H)

Example 22

A mixture of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (470mg, 1mmol), 4-chloromethyl-2-methylthiazole hydrochloride (368mg, 2mmol), potassium carbonate (414mg, 3mmol) and potassium iodide (40mg) in DMF (15ml) was heated at 60°C for 24 hours. The mixture was allowed to cool and partitioned between ethyl acetate and water. The organic layer was washed with water and brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was dissolved in methanol (15ml) and 1M sodium hydroxide (2ml) was added and the mixture stirred for 30 minutes. Concentrated hydrochloric acid (0.5ml) was added. The solvent was removed by evaporation. The residue was purified by reverse phase HPLC eluting with a gradient (0-70%) of methanol in water. Concentrated hydrochloric acid (0.3ml) was added to the combined fractions of pure product and the solvent was removed by evaporation. The residue was triturated with acetone, collected by filtration, washed with acetone and dried under vacuum at 55°C to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-((2-methylthiazol-4-yl)methoxy)quinazoline** hydrochloride (225mg, 48%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.17(s, 3H); 2.69(s, 3H); 4.00(s, 3H); 4.7(br s, 1H); 5.34(s, 2H); 6.91(d, 1H); 7.1(d, 1H); 7.60(s, 1H); 7.74(s, 1H); 8.33(s, 1H); 8.79(s, 1H); 11.5(s, 1H)
MS - ESI: 427 $[MH]^+$

The starting material was prepared as follows:

A mixture of (4-fluoro-2-methyl-5-nitrophenyl) methyl carbonate (3g, 13mmol), (EP 0307777 A2), and platinum(IV)oxide (300mg) in ethanol (60ml) was stirred under hydrogen at 0.3 atmosphere for 1 hour. The catalyst was removed by filtration through diatomaceous earth and the solvent removed by evaporation to give 2-fluoro-5-methoxycarbonyloxy-4-methylaniline (2.6g, 100%) as a solid.

^{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 hydrochloride (800mg, 2.4mmol), (prepared as described for the starting material in Example 1), and 2-fluoro-5-methoxycarbonyloxy-4-methylaniline (570mg, 2.89mmol) in isopropanol (20ml) 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 dried under

vacuum to give 7-benzyloxy-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxyquinazoline hydrochloride (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 mixture of 7-benzyloxy-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxyquinazoline hydrochloride (700mg, 1.4mmol) and 10% palladium-on-charcoal (100mg) in DMF (10ml), methanol (10ml) and trichloromethane (10ml) was stirred under hydrogen at 1 atmosphere pressure for 1 hour. The catalyst was removed by filtration through diatomaceous earth and the solvent was removed by evaporation. The residue was triturated with ether, collected by filtration and dried under vacuum to give 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (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 23

A mixture of 4-chloro-7-(4-pyridylmethoxy)quinazoline hydrochloride (350mg, 1mmol) and 2-fluoro-5-hydroxy-4-methylaniline (155mg, 1.1mmol), (prepared as described for the starting material in Example 13), in isopropanol (15ml) was heated at reflux for 1 hour. The resulting precipitate was collected by filtration and purified by reverse phase HPLC using a gradient (0-75%) of methanol in water. Concentrated hydrochloric acid (0.5ml) was added to the combined fractions of pure product and the solvent was removed by evaporation to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-7-(4-pyridylmethoxy)quinazoline** hydrochloride (140mg, 28%).

^{1}H NMR Spectrum: (DMSOd_6) 2.16(s, 3H); 5.69(s, 2H); 6.19(d, 1H); 7.1(d, 1H); 7.48(d, 1H); 7.66(dd, 1H); 8.06(d, 2H); 8.84(s, 1H); 8.86(d, 1H); 8.90(d, 2H); 9.7(br s, 1H); 11.71(s, 1H)

MS - ESI: 377 [MH]$^+$

Elemental Analysis: Found C 50.9 H 4.9 N 11.1

$C_{21}H_{17}N_4O_2F$ 2.4H_2O 2HCl Requires C 51.2 H 4.9 N 11.4%

The starting material was prepared as follows:-

Sodium hydride (0.72g of a 60% suspension in mineral oil, 18mmol) was added to a solution of 4-hydroxymethylpyridine (4g, 36mmol) in THF (30ml) and the mixture heated at reflux for 15 minutes. 7-Fluoro-3,4-dihydroquinazolin-4-one (1g, 6mmol), (J. Chem. Soc. section B 1967, 449), was added, the THF was removed by evaporation, and the mixture was heated at 120°C for 30 minutes. The mixture was allowed to cool, diluted with water (40ml) and was adjusted to pH8 with concentrated hydrochloric acid. The resulting precipitate was collected by filtration, washed with water, then ether and dried under vacuum to give 7-(4-pyridylmethoxy)-3,4-dihydroquinazolin-4-one (1.12g, 71%).

^{1}H NMR Spectrum (DMSOd_6) 5.35(s, 2H); 7.15-7.22(m, 2H); 7.5(d, 2H); 8.05(d, 1H); 8.07 (s, 1H); 8.6(d,2H).

A mixture of 7-(4-pyridylmethoxy)-3,4-dihydroquinazolin-4-one (320mg, 1.26mmol), DMF (1 drop) and thionyl chloride (10ml) was heated at 60°C for 1 hour. 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-chloro-7-(4-pyridylmethoxy)quinazoline hydrochloride (435mg, 98%).

^{1}H NMR Spectrum (DMSOd_6) 5.7(s, 2H); 7.32(s, 1H); 7.35(d, 1H); 8.1-8.2(m,3H); 8.62(s,1H); 9.0(d,2H).

MS - ESI: 272 $[MH]^+$

Example 24

A solution of 1,1'-(azodicarbonyl)dipiperidine (378mg, 1.5mmol) in methylene chloride (5ml) was added dropwise to a suspension of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (160mg, 0.5mmol), tributylphosphine (303mg, 1.5mmol) and 2-(imidazol-1-yl)ethanol (67mg, 0.6mmol), (J. Med. Chem. 1993, 25, 4052-4060), in methylene chloride (8ml) and the mixture was stirred for 3 hours at ambient temperature. Acetic acid (60mg, 1mmol) was added and the solvent was removed by evaporation. The solid residue was adsorbed on silica and purified by column chromatography eluting with methylene chloride/methanol (9/1 followed by 8/2). The resulting white solid was dissolved in methylene chloride/methanol and a solution of 5M hydrochloric acid in isopropanol was

added. The solvent was removed by evaporation and the solid was triturated with ether, filtered, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline** hydrochloride (180mg, 74%).

^{1}H NMR Spectrum: ($DMSOd_6$) 4.01(s, 3H); 4.62(t, 2H); 4.76(t, 2H); 7.44(dd, 1H); 7.48(s, 1H); 7.59(t, 1H); 7.66(dd, 1H); 7.72(s, 1H); 7.84(s, 1H); 8.41(s, 1H); 8.78(s, 1H); 9.22(s, 1H)

MS - ESI: 414 $[MH]^+$

Elemental Analysis:	Found	C 48.3	H 4.1	N 14.0
$C_{20}H_{17}N_5O_2ClF$ $0.4H_2O$ 2HCl Requires		C 48.6	H 4.0	N 14.2%

The starting material was prepared as follows:

A solution of 7-benzyloxy-4-chloro-6-methoxyquinazoline hydrochloride (1.2g, 3.6mmol), (prepared as described for the starting material in Example 1), and 4-chloro-2-fluoroaniline (444µl, 4mmol) in isopropanol (40ml) was heated at reflux for 1.5 hours. The mixture was allowed to cool, 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
$C_{22}H_{17}N_3O_2ClF$ HCl	Requires	C 59.2	H 4.1	N 9.4%

A solution of 7-benzyloxy-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (892mg, 2mmol) in TFA (10ml) was heated at reflux for 50 minutes. The mixture was allowed to cool and then poured on to ice. The precipitate was collected by filtration, dissolved in methanol (10ml) and basified to pH11 with aqueous ammonia. The mixture was concentrated by evaporation, the resulting 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 (460mg, 72%) as a yellow solid.

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 $[MH]^+$

Example 25

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (448mg, 1.4mmol), (prepared as described for the starting material in Example 24), and potassium carbonate (676mg, 4.9mmol) in DMF (10ml) was stirred at ambient temperature for 10 minutes. 4-Chloromethyl-2-methylthiazole hydrochloride (310mg, 1.68mmol) was added and the mixture was heated at 70°C for 3.5 hours. The reaction mixture was allowed to cool and was partitioned between ethyl acetate and water. The organic layer was separated, washed with water, brine, dried ($MgSO_4$) and the solvent removed by evaporation. The solid residue was purified by column chromatography eluting with a mixture of methylene chloride/acetonitrile/methanol (50/45/5 followed by 50/40/10). The resulting purified solid was dissolved in methylene chloride/methanol and a solution of 5M hydrogen chloride in isopropanol (1ml) was added. Partial evaporation led to the precipitation of a white solid. This solid was collected by filtration and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-((2-methylthiazol-4-yl)methoxy)quinazoline** hydrochloride (240mg, 35%).

m.p. 220-225°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.68(s, 3H); 4.0(s, 3H); 5.36(s, 2H); 7.46(dd, 1H); 7.54(s, 1H); 7.61(t, 1H); 7.7(d, 1H); 7.71(s, 1H); 8.26(s, 1H); 8.83(s, 1H)

MS - ESI: 431 $[MH]^+$

Elemental Analysis:	Found	C 49.3	H 4.0	N 11.3
$C_{20}H_{16}N_4O_2ClFS$ 0.3H_2O 1.5HCl	Requires	C 48.9	H 3.7	N 11.4%

Example 26

Using an analogous procedure to that described in Example 25, 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (224mg, 0.7mmol), (prepared as described for the starting material in Example 24), and 2-chloromethyl-1-methylimidazole

hydrochloride (140mg, 0.8mmol) were combined to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-((1-methylimidazol-2-yl)methoxy)quinazoline** hydrochloride (150mg, 44%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.94(s, 3H); 4.02(s, 3H); 5.69(s, 2H); 7.44(dd, 1H); 7.6(t, 1H); 7.64(s, 1H); 7.67(dd, 1H); 7.72(d, 1H); 7.81(d, 1H); 8.46(s, 1H); 8.81(s, 1H)

MS - ESI: 414 $[MH]^+$

Elemental Analysis: Found C 48.7 H 4.6 N 13.6

$C_{20}H_{17}N_5O_2ClF$ $0.5H_2O$ 2HCl Requires C 48.8 H 4.3 N 13.7%

0.25isopropanol

Example 27

A mixture of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (470mg, 1mmol), (prepared as described for the starting material in Example 22), 2-chloromethyl-1-methylimidazole hydrochloride (335mg, 2mmol), potassium carbonate (414mg, 3mmol) and potassium iodide (20mg) in DMF (15ml) was heated at 60°C for 2 hours. The mixture was allowed to cool and partitioned between ethyl acetate and water. The organic layer was separated, washed with water and brine, dried ($MgSO_4$) and the solvent removed by evaporation. The crude product was dissolved in methanol (20ml), 2M sodium hydroxide (1ml) was added and the mixture stirred for 15 minutes. Concentrated hydrochloric acid (0.5ml) was added and the solvent was removed by evaporation. The crude product was purified by reverse phase chromatography eluting with methanol/water (1/1). Concentrated hydrochloric acid (0.3ml) was added to the combined fractions containing the pure product and the solvent was removed by evaporation to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-((1-methylimidazol-2-yl)methoxy)quinazoline** hydrochloride (100mg, 21%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.17(s, 3H); 3.95(s, 3H); 4.01(s, 3H); 5.70(s, 2H); 6.92(d, 1H); 7.12(d, 1H); 7.63(s, 1H); 7.77(s, 1H); 7.83(s, 1H); 8.43(s, 1H); 8.82(s, 1H); 9.7(br s, 1H); 11.62(br s, 1H)

MS - ESI: 410 $[MH]^+$

Example 28

Using an analogous procedure to that described in Example 27, 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (470mg 1.14mmol), (prepared as described for the starting material in Example 22), and 2-acetamido-4-chloromethylthiazole (381mg, 1.68mmol) were combined to give **7-((2-acetamidothiazol-4-yl)methoxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline** (135mg, 25%).

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD) 2.16(s, 3H); 2.19(s, 3H); 4.00(s, 3H); 5.33(s, 2H); 6.91(d, 1H); 7.12(d, 1H); 7.33(s, 1H); 7.49(s, 1H); 8.16(s, 1H); 8.82(s, 1H)

MS - ESI: 470 [MH]$^+$

Elemental Analysis:	Found	C 51.5	H 4.5	N 13.8
$C_{22}H_{20}N_5O_4FS$ 0.4H_2O 0.95HCl	Requires	C 51.7	H 4.3	N 13.7%

Example 29

A suspension of 7-benzyloxy-4-chloro-6-methoxyquinazoline hydrochloride (169mg, 0.5mmol), (prepared as described for the starting material in Example 1), and 4-chloro-2-fluoro-5-hydroxyaniline (97mg, 0.6mmol), (EP 061741 A2), in isopropanol (5ml) was heated at reflux for 2 hours. The resulting precipitate was collected by filtration, washed with isopropanol and ether and dried under vacuum to give **7-benzyloxy-4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxyquinazoline** hydrochloride (197mg, 85%).

^{1}H NMR Spectrum: (DMSOd_6) 4.0(s, 3H); 5.36(s, 2H); 7.15(d, 1H); 7.4-7.5(m, 4H); 7.52(s, 1H); 7.54(d, 2H); 8.23(s, 1H); 8.8(s, 1H); 10.6(s, 1H); 11.39(br s, 1H)

MS - ESI: 426 [MH]$^+$

Elemental Analysis:	Found	C 57.1	H 4.2	N 8.9
$C_{22}H_{17}N_3O_3ClF$ 0.15H_2O 1HCl	Requires	C 56.8	H 4.0	N 9.0%

0.4isopropanol

Example 30

1,1'-(Azodicarbonyl)dipiperidine (1.06g, 4.2mmol) in methylene chloride (15ml) was added dropwise to a solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (448mg, 1.4mmol), (prepared as described for the starting material in Example 24), tributylphosphine (848mg, 4.2mmol) and 4-(3-hydroxypropyl)pyridine (322mg,

2.4mmol) in methylene chloride (15ml) and the mixture stirred for 3 hours at ambient remperature. Acetic acid (126mg, 2.1mmol) was added and the solvent was removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (95/5). The purified product was triturated with ether, the resulting solid collected and dissolved in methylene chloride (20ml). 5M Hydrogen chloride in isopropanol solution (0.7ml) was added, the solution was diluted with isopropanol (5ml) and concentrated by evaporation to a total volume of 4ml. Ether was added and the resulting soild was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(4-pyridyl)propoxy)quinazoline** hydrochloride (520mg, 73%).

^{1}H NMR Spectrum: (DMSOd_6) 2.30(m, 2H); 3.09(t, 2H); 3.97(s, 3H); 4.27(t, 2H); 7.42(s, 1H); 7.44(d, 1H); 7.59(t, 1H); 7.67(dd, 1H); 7.95(d, 2H); 8.34(s, 1H); 8.8(s, 1H); 8.82(d, 2H)
MS - ESI: 439 [MH]$^+$

Elemental Analysis: Found C 53.6 H 4.8 N 10.7

$C_{23}H_{20}N_4O_2ClF$ 0.5H_2O 2HCl Requires C 53.1 H 4.6 N 10.6%

0.1isopropanol

Example 31

2M Aqueous sodium hydroxide (1.5ml, 3mmol) was added to a solution of 4-(4-chloro-2-fluoro-5-methoxycarbonyloxyanilino)-6-methoxy-7-(3-(4-pyridyl)propoxy)quinazoline (1.28g, 2.5mmol) in methanol (13ml) and the mixture stirred for 2 hours at ambient temperature. Water was added and the mixture was adjusted to pH7 with 2M hydrochloric acid. The resulting precipitate was collected by filtration, washed with water and dried under vacuum. This solid was dissolved in methylene chloride (30ml) and methanol (5ml) and a solution of 5M hydrogen chloride in isopropanol (2.5ml) was added. The solution was diluted with isopropanol and concentrated under vacuum to a total volume of 10ml. The resulting solid was collected by filtration, washed with isopropanol and then ether and dried under vacuum to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-(3-(4-pyridyl)propoxy)quinazoline** hydrochloride (924mg, 70%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.3(t, 2H); 3.12(t, 2H); 4.0(s, 3H); 4.28(t, 2H); 7.18(d, 1H); 7.4(s, 1H); 7.52(d, 1H); 7.95(d, 2H); 8.32(s, 1H); 8.82(s, 1H); 8.84(d, 2H); 10.65(s, 1H); 11.65(br s, 1H)

MS - ESI: 455 $[MH]^+$

Elemental Analysis:	Found	C 51.9	H 4.5	N 10.7
$C_{23}H_{20}N_4O_5ClF\ 0.55H_2O\ 1.9HCl$	Requires	C 51.5	H 4.7	N 10.5%

The starting material was prepared as follows:

1,1'-(Azodicarbonyl)dipiperidine (2.52g, 10mmol) in methylene chloride (10ml) was added dropwise to a solution of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (1.38g, 3.5mmol), (prepared as described for the starting material in Example 22), tributylphosphine (2g, 10.5mmol) and 4-(3-hydroxypropyl)pyridine (720mg, 5.25mmol) in methylene chloride (25ml) and the mixture stirred for 2.5 hours at ambient temperature. The solvent was removed by evaporation and the residue was triturated with petroleum ether. The solid product was collected by filtration and purified by column chromatography eluting with methylene chloride/methanol (95/5) to give 4-(4-chloro-2-fluoro-5-methoxycarbonyloxyanilino)-6-methoxy-7-(3-(4-pyridyl)propoxy)quinazoline (1.2g, 67%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.18(m, 2H); 2.84(t, 2H); 3.90(s, 3H); 3.97(s, 3H); 4.2(t, 2H); 7.21(s, 1H); 7.3(d, 2H); 7.72-7.82(m, 3H); 8.41(s, 1H); 8.47(d, 2H); 9.67(s, 1H)

MS - ESI: 513 $[MH]^+$

Example 32

2M Aqueous sodium hydroxide (0.3ml, 6mmol) was added to a solution of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline (257mg, 5.5mmol) in methanol (5ml) and the mixture stirred for 1 hour at 40°C. Water and 1M hydrochloric acid (0.6ml) were added and the mixture concentrated to half volume by evaporation. The resulting solid was collected by filtration, dissolved in methylene chloride/methanol and a solution of 7M hydrogen chloride in isopropanol (0.4ml) was added. The volatiles were removed by evaporation, the solid residue was triturated with ether, collected by filtration and dried under vacuum to give **4-(2-fluoro-5-hydroxy-4-**

methylanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline hydrochloride (160mg, 60%).

m.p. 195-220°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.16(s, 3H); 4.0(s, 3H); 4.63(t, 2H); 4.76(t, 2H); 6.90(d, 1H); 7.1(d, 1H); 7.44(s, 1H); 7.72(s, 1H); 7.83(s, 1H); 8.31(s, 1H); 8.76(s, 1H); 9.20(s, 1H); 9.7(s, 1H); 11.4(br s, 1H)

MS - ESI: 410 $[MH]^+$

Elemental Analysis:	Found	C 52.3	H 5.1	N 13.7
$C_{21}H_{20}N_5O_3F$ $0.3H_2O$ 1.9HCl 0.22isopropanol	Requires	C 52.3	H 4.9	N 14.1%

The starting material was prepared as follows:

Diethyl azodicarboxylate (160mg, 1.4mmol) was added to a solution of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (261mg, 0.7mmol), (prepared as described for the starting material in Example 22), triphenylphosphine (367mg, 1.4mmol) and 2-(imidazol-1-yl)ethanol (94mg, 0.84mmol), (J. Med. Chem. 1993, 25, 4052-4060), in methylene chloride (5ml) and the mixture stirred for 1 hour at ambient temperature. Acetic acid (42mg, 0.7mmol) was added and the solvent was removed by evaporation. The residue was triturated with ether, the solid collected by filtration, dried under vacuum and purified by chromatography eluting with methylene chloride/methanol (9/1 followed by 8/2) to give 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline (250mg, 76%).

Example 33

A 1M solution of tetrabutylammonium fluoride in THF (560µl, 0.56mmol) was added to a suspension of 4-(4-chloro-5-diphenyl-t-butylsilyloxy-2-fluoroanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline (186mg, 0.28mmol) in THF (5ml) and the mixture stirred at 40°C for 1 hour. Water was added and the organic solvent was removed by evaporation. The resulting precipitate was collected by filtration, washed with water and dried by azeotroping with ethanol. The solid was dissolved in methylene chloride/methanol and a solution of 5M hydrogen chloride in isopropanol (0.5ml) was added. The volatiles were

removed by evaporation and the residue was dissolved in isopropanol (1ml) and ether was added. The resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline** hydrochloride (110mg, 78%).

^{1}H NMR Spectrum: (DMSOd_6) 4.01(s, 3H); 4.63(t, 2H); 4.75(t, 2H); 7.17(d, 1H); 7.46(s, 1H); 7.51(d, 1H); 7.72(s, 1H); 7.83(s, 1H); 8.36(s, 1H); 8.79(s, 1H); 9.21(s, 1H); 10.63(br s, 1H); 11.6(br s, 1H)

MS - ESI: 430 [MH]$^+$

Elemental Analysis:	Found	C 45.7	H 3.9	N 12.8
$C_{20}H_{17}N_5O_2ClF$ $1H_2O$ 2HCl	Requires	C 45.8	H 4.1	N 13.1%

0.09isopropanol 0.09methylene chloride

The starting material was prepared as follows:

A mixture of 7-benzyloxy-4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxyquinazoline (2.35g, 7mmol), (prepared as described for Example 29), imidazole (1.2g, 17.5mmol), t-butyldiphenylsilylchloride (2.1g, 7.7mmol) and 4-(dimethylamino)pyridine (20mg, 0.16mmol) in DMF (10ml) was stirred for 2 hours at ambient temperature. Water (100ml) and ethyl acetate (30ml) were added, the resulting precipitate was collected by filtration, washed with water and dried under vacuum to give 7-benzyloxy-4-(4-chloro-5-diphenyl-t-butylsilyloxy-2-fluoroanilino)-6-methoxyquinazoline (2g, 43%).

^{1}H NMR spectrum (DMSOd_6) 1.09(s, 9H); 3.86(s, 3H); 5.25(s, 2H); 7.04(d, 1H); 7.23(s, 1H); 7.32-7.5(m, 11H); 7.58(d, 1H); 7.65-7.72(m, 5H); 8.1(s, 1H); 9.25 (br s, 1H)

MS - ESI: 663 [MH]$^+$

A mixture of 7-benzyloxy-4-(4-chloro-5-diphenyl-t-butylsilyloxy-2-fluoroanilino)-6-methoxyquinazoline (2g, 3mmol) and 10% palladium-on-charcoal catalyst (400mg) in DMF (20ml), methanol (20ml) and ethyl acetate (20ml) was stirred under hydrogen at 1.7 atmospheres pressure for 2 hours. The catalyst was removed by filtration and the solvent 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 triturated

with ether, collected by filtration and dried under vacuum to give 4-(4-chloro-5-diphenyl-t-butylsilyloxy-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (1.65g, 95%).

^{1}H NMR spectrum ($DMSOd_6$) 1.09(s, 9H); 3.87(s, 3H); 7.00(s, 1H); 7.07(d, 1H); 7.4-7.5(m, 6H); 7.55(d, 1H); 7.62(s, 1H); 7.7(m, 4H); 8.04(s, 1H); 9.15(br s, 1H); 10.34(br s, 1H)

Diethyl azodicarboxylate (174mg, 1mmol) was added to a solution of 4-(4-chloro-5-diphenyl-t-butylsilyloxy-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (288mg, 0.5mmol), triphenylphosphine (262mg, 1mmol) and 2-(imidazol-1-yl)ethanol (62mg, 0.55mmol), (J. Med. Chem. 1993, 25, 4052-4060), in methylene chloride (5ml) and the mixture stirred for 1 hour at ambient temperature. Acetic acid (30mg, 0.5mmol) was added and the volatiles were removed by evaporation. The residue was triturated with ether, the solid collected by filtration and dried under vacuum to give 4-(4-chloro-5-diphenyl-t-butylsilyloxy-2-fluoroanilino)-7-(2-(imidazol-1-yl)ethoxy)-6-methoxyquinazoline (186mg, 55%).

MS - ESI: 668 $[MH]^+$

Example 34

A suspension of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxy-7-(2-(4-pyridyl)ethoxy)quinazoline (300mg, 0.63mmol) and 2M aqueous sodium hydroxide (0.38ml, 0.76mmol) in methanol (6ml) was stirred at ambient temperature for 2 hours. Water was added and the mixture adjusted to pH7 with 2M hydrochloric acid. The precipitate was collected by filtration, washed with water, and dried under vacuum. The solid was dissolved in methylene chloride/methanol and a 5M solution of hydrogen chloride in isopropanol (0.5ml) was added. The mixture was diluted with isopropanol, and the methylene chloride and methanol solvents were removed by evaporation. The resulting precipitate was collected by filtration, washed with methylene chloride and dried under vacuum to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(2-(4-pyridyl)ethoxy)quinazoline** hydrochloride (270mg, 94%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.16(s, 3H); 3.5(t, 2H); 3.99(s, 3H); 4.57(t, 2H); 6.89(d, 1H); 7.12(d, 1H); 7.44(s, 1H); 7.98(d, 2H); 8.24(s, 1H); 8.78(s, 1H); 8.81(d, 2H); 9.7(br s, 1H); 11.38(br s, 1H)

MS - ESI: 421 [MH]$^+$

Elemental Analysis:	Found	C 55.5	H 5.3
$C_{23}H_{21}N_4O_5F$ 0.3H_2O 1HCl 0.3isopropanol	Requires	C 55.6	H 5.1

The starting material was prepared as follow :

Diethyl azodicarboxylate (244mg, 1.4mmol) was added to a suspension of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (261mg, 0.7mmol), (prepared as described for the starting material in Example 22), triphenylphosphine (367mg, 1.4mmol) and 2-(4-pyridyl)ethanol (104mg, 0.84mmol), (Zhur. Obshchei. Khim. 1958, 28, 103-110), in methylene chloride and the mixture stirred for 30 minutes at ambient temperature. The solvent was removed by evaporation. The residue was suspended in ether and the ether then decanted. The resulting crude oil was purified by column chromatography eluting with methylene chloride/methanol (90/10) to give 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxy-7-(2-(4-pyridyl)ethoxy)quinazoline (300mg, 90%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.18(s, 3H); 3.16(t, 2H); 3.84(s, 3H); 3.92(s, 3H); 4.44(t, 2H); 7.24(s, 1H); 7.29(d, 1H); 7.40(d, 2H); 7.79(s, 1H); 8.35(s, 1H); 8.49(d, 2H); 9.51(s, 1H)

MS - ESI: 501 [MNa]$^+$

Example 35

Using an analogous procedure to that described in Example 34, 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxy-7-(3-thienylmethoxy)quinazoline (220mg, 0.47mmol) was treated with 2M aqueous sodium hydroxide (0.47ml) to give **4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxy-7-(3-thienylmethoxy)quinazoline** hydrochloride (180mg, 86%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.17(s, 3H); 3.98(s, 3H); 5.34(s, 2H); 6.89(d, 1H); 7.15(d, 1H); 7.27(d, 1H); 7.47(s, 1H); 7.65(dd, 1H); 7.75(s, 1H); 8.18(s, 1H); 8.77(s, 1H); 9.7(br s, 1H)

MS - ESI: 412 [MH]$^+$

Elemental Analysis:	Found	C 55.5	H 4.5	N 9.0
$C_{21}H_{18}N_3O_5FS$ 0.2H_2O 1HCl 0.09isopropanol	Requires	C 55.9	H 4.4	N 9.2%

The starting material was prepared as follows:

Using an analogous procedure to that described for the starting material in Example 34, 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (261mg, 0.7mmol), (prepared as described for the starting material in Example 22), was combined with 3-thiophenemethanol (96mg, 0.84mmol) to give, after purification by flash chromatography eluting with methylene chloride/methanol (98/2), 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxy-7-(3-thienylmethoxy)quinazoline (220mg, 67%).

^{1}H NMR Spectrum: (DMSOd_6) 2.18(s, 3H); 3.85(s, 3H); 3.93(s, 3H); 5.27(s, 2H); 7.23(d, 1H); 7.30(d, 1H); 7.32(s, 1H); 7.40(d, 1H); 7.59(dd, 1H); 7.66(s, 1H); 7.81(s, 1H); 8.35(s, 1H); 9.53(s, 1H)

MS - ESI: 492 [MNa]$^+$

Example 36

A mixture of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (187mg, 0.75mmol), (prepared as described for the starting material in Example 22), 4-bromomethylbenzonitrile (147mg, 0.75mmol) and potassium carbonate (173mg, 1.25mmol) in DMF (5ml) was heated at 50°C for 1 hour. Methanol (5ml) and potassium carbonate (138mg, 1mmol) were added and the mixture stirred at 65°C for 2 hours. The solvent was removed by evaporation, water was added to the residue and the mixture adjusted to pH7 with 2M hydrochloric acid. The resulting precipitate was collected by filtration, washed with water and dried under vacuum. The solid was purified by column chromatography eluting with methylene chloride/methanol (95/5). The purified product was triturated with ether, collected by filtration and dried. The solid was dissolved in methylene chloride/isopropanol and a 5M solution of hydrogen chloride in isopropanol (0.5ml) was added. The mixture was concentrated by evaporation and the resulting precipitate collected by filtration, washed with methylene chloride and dried under vacuum to give 7-

(4-cyanobenzyloxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline hydrochloride (60mg, 25%).

m.p. 265-270°C

^{1}H NMR Spectrum: (DMSOd_6) 2.17(s, 3H); 4.02(s, 3H); 5.47(s, 2H); 6.89(d, 1H); 7.11(d, 1H); 7.38(s, 1H); 7.71(d, 2H); 7.93(d, 2H); 8.23(s, 1H); 8.75(s, 1H); 9.67(s, 1H); 11.24(br s, 1H)

MS - ESI: 431 $[MH]^+$

Elemental Analysis:	Found	C 61.2	H 4.5	N 11.7
$C_{24}H_{19}N_4O_3F$ 0.1H_2O 1HCl	Requires	C 61.5	H 4.3	N 12.0%

Example 37

Diethyl azodicarboxylate (315µl, 2mmol) was added dropwise to a solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (319.5mg, 1mmol), (prepared as described for the starting material in Example 24), triphenylphosphine (524mg, 2mmol) and 2-(4-pyridyl)ethanol (160mg, 1.25mmol), (Zhur. Obshchei. Khim. 1958, 28, 103-110), in methylene chloride (7ml). The mixture was stirred for 1 hour at ambient temperature and the solvent was removed by evaporation. The residue was triturated with ether, the solid collected by filtration and purified by column chromatography eluting with methylene chloride/acetonitrile/methanol (85/10/5). The purified solid product was dissolved in a mixture of methylene chloride (50ml) and methanol (50ml) and 5M hydrochloric acid in isopropanol (0.5ml) was added. After diluting with isopropanol (20ml), the mixture was concentrated by evaporation. The precipitated solid was collected by filtration and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-pyridyl)ethoxy)quinazoline** hydrochloride (125mg, 25%).

m.p. 189-191°C

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD) 3.55(t, 2H); 3.99(s, 3H); 4.64(t, 2H); 7.46(s, 1H); 7.48(d, 1H); 7.62(t, 1H); 7.67(dd, 1H); 8.16(d, 2H); 8.17(s, 1H); 8.88(s, 1H); 8.94(d, 1H)

MS - ESI: 425 $[MH]^+$

Elemental Analysis:	Found	C 52.0	H 4.3	N 11.1
$C_{22}H_{18}N_3O_2ClF$ 0.5H_2O 1.95HCl	Requires	C 52.3	H 4.2	N 11.1%

Example 38

3-(Chloromethyl)pyridine hydrochloride (328mg, 2mmol) 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 24), potassium carbonate (442mg, 3.2mmol) and potassium iodide (33mg, 0.2mmol) in DMF (25ml) at ambient temperature and the reaction mixture then heated at 80°C for 2.5 hours. The mixture was allowed to cool and the volatiles were removed by evaporation. The residue was dissolved in a mixture of ethyl acetate (19ml) and methanol (1ml) and the insolubles 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 (50/45/5). The purified product was dissolved in hot methylene chloride and saturated ethereal hydrogen chloride was added. The mixture was concentrated to half volume by evaporation, the resulting precipitate was collected by filtration and dried under vacuum at 70°C to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-((3-pyridyl)methoxy)quinazoline** hydrochloride(103mg, 25%).

m.p. 216-221°C

^{1}H NMR Spectrum: ($DMSOd_6$) 4.03(s, 3H); 5.48(s, 2H); 7.47(d, 1H); 7.54(s, 1H); 7.65(t, 1H); 7.7-7.8(m, 2H); 8.25(d, 1H); 8.35(s, 1H); 8.75(d, 1H); 8.84(s, 1H); 8.90(s, 1H); 11.65(br s, 1H)

MS - ESI: 411 $[MH]^+$

Elemental Analysis:	Found	C 51.9	H 4.2	N 11.4
$C_{21}H_{16}N_4O_2ClF$ 0.8H_2O 1.6HCl	Requires	C 52.2	H 4.0	N 11.6%

Example 39

Using an analogous procedure to that described in Example 38, 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (319.5mg, 1mmol), (prepared as described for the starting material in Example 24), was reacted with 2-(chloromethyl)pyridine hydrochloride (310mg, 1.9mmol) to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-((2-pyridyl)methoxy)quinazoline** (146mg, 33%).

m.p. 215-218°C

^{1}H NMR Spectrum: (DMSOd_6) 3.98(s, 3H); 5.4(s, 2H); 7.3(s, 1H); 7.32-7.42(m, 2H); 7.52-7.62(m, 3H); 7.85(s, 1H); 7.90(t, 1H); 8.35(s, 1H); 8.65(d, 1H); 9.6(s, 1H)

MS - ESI: 411 [MH]

Elemental Analysis:	Found	C 59.7	H 3.9	N 13.1
$C_{21}H_{16}N_5O_2ClF$ $0.5H_2O$	Requires	C 60.1	H 4.1	N 13.3%

Example 40

Diethyl azodicarboxylate (128µl, 1.5mmol) was added dropwise to a solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (250mg, 0.78mmol), (prepared as described for the starting material in Example 24), triphenylphosphine (410mg, 1.5mmol) and 2-(1-methylimidazol-2-yl)ethanol (147mg, 1.15mmol), (EP 0675112 A1), in methylene chloride (4ml) and the mixture was stirred for 30 minutes at ambient temperature. Further triphenylphosphine (143mg, 0.52mmol) and diethyl azodicarboxylate (85µl, 1mmol) were added and the mixture stirred for 1 hour at ambient temperature. The solid product was collected by filtration and washed with methylene chloride. The solid was dissolved in a mixture of methylene chloride (25ml) and methanol (25ml), and a solution of 2.9M ethereal hydrogen chloride (2ml) 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-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1-methylimidazol-2-yl)ethoxy)quinazoline** hydrochloride (133mg, 34%).

m.p. 224-229°C

^{1}H NMR Spectrum: (DMSOd_6) 3.62(t, 2H); 3.94(s, 3H); 4.0(s, 3H); 4.59(t, 2H); 7.43(d, 1H); 7.46(s, 1H); 7.6(t, 1H); 7.6-7.7(m, 3H); 8.41(s, 1H); 8.78(s, 1H); 11.75(br s, 1H)

MS - ESI: 428 [MH]$^+$

Elemental Analysis:	Found	C 48.8	H 4.4	N 13.4
$C_{21}H_{19}N_5O_2ClF$ $1H_2O$ $2HCl$	Requires	C 48.6	H 4.5	N 13.5%

Example 41

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (319.5mg, 1mmol), (prepared as described for the starting material in Example 24), potassium

carbonate (414mg, 3mmol), potassium iodide (16mg, 0.1mmol) and 4-chloromethylpyrimidine (257mg, 2mmol) in DMF (20ml) was heated at 80°C for 2 hours. The solvent was removed by evaporation and the residue was triturated with water. The solid was collected by filtration and dried under vaccum. The solid was purified by column chromatography eluting with methylene chloride/methanol (95/5). The purified white solid was suspended in methanol (25ml) and a solution of 7.5M hydrogen chloride in methanol (20ml) was added. The resulting solid product was collected by filtration, washed with methanol and then pentane and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-((pyrimidin-4-yl)methoxy)quinazoline** hydrochloride (172mg, 42%).

m.p. 237-239°C

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 4.07(s, 3H); 5.53(s, 2H); 7.40(s, 1H); 7.46(dd, 1H); 7.65(t, 1H); 7.68-7.72(m, 2H); 8.26(s, 1H); 8.85(s, 1H); 8.91(d, 1H); 9.25(s, 1H)

MS - ESI: 412 $[MH]^+$

Elemental Analysis:	Found	C 49.5	H 3.6	N 14.1
$C_{20}H_{15}N_5O_2ClF$ 0.5H_2O 1.85HCl	Requires	C 49.2	H 3.7	N 14.3%

The starting material was prepared as follows:

A solution of 4-methylpyrimidine (2g, 21.2mmol), N-chlorosuccinimide (4.26g, 31.9mmol) and benzoyl peroxide (500mg, 2.1mmol) in carbon tetrachloride (100ml) was heated at 80°C for 2 hours. The mixture was allowed to cool, 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 to give 4-chloromethylpyrimidine (257mg, 10%).

^{1}H NMR Spectrum: ($DMSOd_6$) 4.81(s, 2H); 7.70(d, 1H); 8.88(d,1H); 9.21(s, 1H)

Example 42

2M Aqueous sodium hydroxide solution (900µl) was added to a solution of 4-(3-acetoxy-4-methylanilino)-6-methoxy-7-((1-methylbenzimidazol-2-yl)methoxy)quinazoline (290mg, 0.6mmol) in methanol (15ml) and methylene chloride (12ml) and the mixture stirred for 25 minutes at ambient temperature. The solvent was

removed by evaporation and the residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with water and brine, dried ($MgSO_4$) and the solvent removed by evaporation. The solid residue was purified by column chromatography eluting with methylene chloride/methanol (97/3 and 95/5). The purified white solid was suspended in methanol (20ml) and a solution of 7.5M hydrochloric acid in methanol (2 equivalents) was added. The solid was collected by filtration, washed with methanol and then pentane and dried under vacuum at 50°C to give **4-(3-hydroxy-4-methylanilino)-6-methoxy-7-((1-methylbenzimidazol-2-yl)methoxy)quinazoline** hydrochloride (106mg, 37%).

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD) 2.17(s, 3H); 4.04(s, 3H); 4.15(s, 3H); 6.01(s, 2H); 7.0(dd, 1H); 7.11(d, 1H); 7.18(d, 1H); 7.6-7.75(m, 3H); 7.89(d, 1H); 8.05(d, 1H); 8.27(s, 1H); 8.86(s, 1H)

MS - ESI: 469 $[MNa]^+$

The starting material was prepared as follows:

A solution of 1-methylbenzimidazole (2.5g, 19mmol), (J. Chem. Soc. 1929, 2820-2828), and paraformaldehyde (2g) was heated at 165°C for 30 minutes. Further paraformaldehyde (1g) was added and heating continued for 2 hours. The mixture was allowed to cool and was purified by column chromatography eluting with methylene chloride, followed by methylene chloride/methanol (95/5) to give 2-hydroxymethyl-1-methylbenzimidazole (1.34g, 45%).

^{1}H NMR Spectrum: (DMSOd_6) 3.84(s, 3H); 4.73(s, 2H); 5.57(br s, 1H); 7.19(t, 1H); 7.25(t, 1H); 7.54(d, 1H); 7.60(d, 1H)

MS - ESI: 185 $[MNa]^+$

A solution of 2-hydroxymethyl-1-methylbenzimidazole (1.1g, 6.7mmol) in thionyl chloride (10ml) was stirred at ambient temperature for 15 minutes and then heated at reflux for 15 minutes. The volatiles were removed by evaporation and the residue purified by column chromatography eluting with methylene chloride/methanol (95/5) to give 2-chloromethyl-1-methylbenzimidazole (506mg, 36%).

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD) 4.07(s, 3H); 5.38(s, 2H); 7.6-7.7(m, 2H); 7.9(d, 1H); 8.05(dd, 1H)

MS - ESI: 181 $[MH]^+$

A mixture of 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (240mg, 0.64mmol), (prepared as described for the starting material in Example 1), potassium carbonate (310mg, 2.25mmol), potassium iodide (10mg, 0.064mmol) and 2-chloromethyl-1-methylbenzimidazole (153mg, 0.7mmol) in DMF (12ml) was heated at 65°C for 3 hours. Further 2-chloromethyl-1-methylbenzimidazole (90mg, 0.41mmol) and potassium carbonate (165mg, 1.2mmol) were added and heating continued for 2 hours. The solvent was removed by evaporation and the residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with water and brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was triturated with water and the solid product collected by filtration, washed with ether and dried under vacuum to give 4-(3-acetoxy-4-methylanilino)-6-methoxy-7-((1-methylbenzoimidazol-2-yl)methoxy)quinazoline (292mg, 95%).

MS - ESI: 506 $[MNa]^+$

Example 43

2M Aqueous sodium hydroxide solution (700µl, 1.4mmol) was added to a suspension of 7-((2-chloro-6-methyl-4-pyridyl)methoxy)-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxyquinazoline (360mg, 0.7mmol) in methanol (10ml) cooled at 5°C and the mixture was then stirred for 30 minutes at ambient temperature. The solvent was removed by evaporation, the residue diluted with water (10ml) and the mixture adjusted to pH7 with 1M hydrochloric acid. The resulting solid was collected by filtration, washed with water and ether, and dried under vacuum. This solid was dissolved in methanol (5ml) and a 7M solution of hydrogen chloride in methanol (3ml) was added. The precipitate was collected by filtration, washed with methanol and dried under vacuum to give **7-((2-chloro-6-methyl-4-pyridyl)methoxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline** hydrochloride (273mg, 74%).

^1H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.18(s, 3H); 2.50(s, 3H); 4.04(s, 3H); 5.42(s, 2H); 6.9(d, 1H); 7.12(d, 1H); 7.35(s, 1H); 7.38(s, 1H); 7.42(s, 1H); 8.21(s, 1H); 8.81(s, 1H)

MS - ESI: 455 $[MH]^+$

Elemental Analysis:	Found	C 49.8	H 4.8	N 10.0
$C_{23}H_{20}N_4O_3ClF\ 1.5H_2O\ 1.9HCl$	Requires	C 50.1	H 4.6	N 10.2%

The starting material was prepared as follows:

A solution of 2-chloro-6-methyl-4-pyridinecarboxylic acid (2g, 12mmol) in ethanol (100ml) and concentrated sulphuric acid (10ml) was heated at reflux for 2 hours. The volatiles were removed by evaporation and the residue was dissolved in methylene chloride. The solution was washed with a saturated aqueous sodium hydrogen carbonate solution and brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with ethyl acetate/petroleum ether (1/9) to give ethyl 2-chloro-6-methyl-4-pyridinecarboxylate (2g, 86%).

^{1}H NMR Spectrum: ($CDCl_3$) 1.41(t, 3H); 2.6(s, 3H); 4.40(q, 2H); 7.63(s, 1H); 7.69(s, 1H)

MS - ESI: 200 $[MH]^+$

Elemental Analysis:	Found	C 54.4	H 5.3	N 7.0
$C_9H_{10}NO_2Cl$	Requires	C 54.1	H 5.0	N 7.0%

Lithium aluminium hydride (350mg, 9.26mmol) was added in portions to a solution of ethyl 2-chloro-6-methyl-4-pyridinecarboxylate (1.85g, 9.26mmol) in THF (40ml) cooled at 0°C. The mixture was stirred for 15 minutes at 0°C and acetic acid (2ml) was added. The mixture was partitioned between ethyl acetate and water and the aqueous layer was adjusted to pH7.5 with 5 % aqueous sodium hydrogen carbonate solution. 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 ethyl acetate/petroleum ether (35/65) to give 2-chloro-4-hydroxymethyl-6-methylpyridine (1.28g, 88%).

^{1}H NMR Spectrum: ($CDCl_3$) 1.92(t, 1H); 2.53(s, 3H); 4.70(d, 2H); 7.06(s, 1H); 7.16(s, 1H)

MS - ESI: 157 $[MH]^+$

Elemental Analysis:	Found	C 53.1	H 5.3	N 8.7
C_7H_8NOCl	Requires	C 53.3	H 5.1	N 8.9%

Diethyl azodicarboxylate (296µl, 1.88mmol) was added dropwise to a solution of 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (350mg, 0.94mmol), (prepared as described for the starting material in

Example 22), triphenylphosphine (492mg, 1.88mmol) and 2-chloro-4-hydroxymethyl-6-methylpyridine (178mg, 1.12mmol) in methylene chloride (30ml) and the mixture stirred for 30 minutes at ambient temperature. The solvent was removed by evaporation and the residue was purified by column chromatography eluting with ethyl acetate/methylene chloride (75/25). The purified product was triturated with ether, the solid collected by filtration, washed with ether and dried under vacuum to give 7-((2-chloro-6-methyl-4-pyridyl)methoxy)-4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-6-methoxyquinazoline (373mg, 78%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.15(s, 3H); 2.5(s, 3H); 3.85(s, 3H); 3.98(s, 3H); 5.35(s, 2H); 7.25(s, 1H); 7.3(d, 1H); 7.35(s, 1H); 7.4(m, 2H); 7.85(s, 1H); 8.35(s, 1H); 9.58(s, 1H)

MS - ESI: 513 $[MH]^+$

Example 44

A mixture of 4-(4-chloro-2-fluorophenoxy)-7-hydroxy-6-methoxyquinazoline (112mg, 0.35mmol), potassium carbonate (138mg, 1mmol) and 4-(chloromethyl)pyridine hydrochloride (59mg, 0.36mmol) in DMF (2ml) was heated at 80°C for 1 hour. The mixture was allowed to cool and partitioned between ethyl acetate and water. 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) to give **4-(4-chloro-2-fluorophenoxy)-6-methoxy-7-((4-pyridyl)methoxy)quinazoline** (115mg, 80%).

m.p. 197-198°C

^{1}H NMR Spectrum: ($DMSOd_6$) 4.03(s, 3H); 5.46(s, 2H); 7.45(d, 1H); 7.49(s, 1H); 7.5(d, 2H); 7.58(t, 1H); 7.62(s, 1H); 7.72(dd, 1H); 8.58(s, 1H); 8.65(d, 2H)

MS - ESI: 412 $[MH]^+$

Elemental Analysis:	Found	C 59.5	H 3.9	N 9.6
$C_{21}H_{15}N_3O_3ClF\ 0.8H_2O$	Requires	C 59.2	H 3.9	N 9.9%

The starting material was prepared as follows:

4-Chloro-2-fluoro-phenol (264mg, 1.8mmol) was added to a solution of 7-benzyloxy-4-chloro-6-methoxyquinazoline hydrochloride (506mg, 1.5mmol), (prepared as described for the starting material in Example 1), 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 hydrochloric acid, 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, 3H); 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 63.4	H 4.1	N 6.8
$C_{22}H_{16}ClFN_2O_3$ 0.06H_2O 0.05CH_2Cl_2	Requires	C 63.6	H 3.9	N 6.7%

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 45

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (320mg, 1mmol), (prepared as described for the starting material in Example 24), potassium carbonate (414mg, 3mmol), potassium iodide (40mg) and 4-(chloromethyl)pyridine hydrochloride (250mg, 1.5mmol) in DMF (15ml) was heated at 60°C for 2 hours. The mixture was allowed to cool and partitioned between ethyl acetate and water. The organic layer was separated, washed with water and brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was suspended in ethanol (20ml) and concentrated hydrochloric acid (0.5ml) was added. The volatiles were removed by evaporation and the solid residue was azeotroped with toluene. The solid product was recrystallised from isopropanol to give **4-(4-**

chloro-2-fluoroanilino)-6-methoxy-7-((4-pyridyl)methoxy)quinazoline hydrochloride (335mg, 70%).

^{1}H NMR Spectrum: (DMSOd_6) 4.1(s, 3H); 5.69(s, 2H); 7.46(dd, 1H); 7.52(s, 1H); 7.62(t, 1H); 7.69(dd, 1H); 8.03(d, 2H); 8.55(s, 1H); 8.83(s, 1H); 8.93(d, 2H)

MS - ESI: 411 $[MH]^+$

Elemental Analysis:	Found	C 51.0	H 3.9	N 11.2
$C_{21}H_{16}N_4O_2ClF$ 0.5H_2O 1.95HCl	Requires	C 51.4	H 3.9	N 11.4%

Example 46

Diethyl azodicarboxylate (261mg, 1.5mmol) was added dropwise to a suspension of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (160mg, 0.5mmol), (prepared as described for the starting material in Example 24), triphenylphosphine (393mg, 1.5mmol) and 2-(N-(2,6-dimethyl-4-pyridyl)-N-methylamino)ethanol (125mg, 0.7mmol) in methylene chloride (5ml) and the mixture stirred for 2 hours at ambient temperature. Methanol (10 drops) was added and the mixture was poured on to a column of neutral aluminium oxide and the product was separated by elution with methylene chloride/acetonitrile/methanol (60/35/35). The purified solid product was triturated with ether and collected by filtration. The solid was dissolved in methylene chloride/methanol and a solution of 3M ethereal hydrogen chloride (1ml) was added. The solid was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-7-(2-(N-(2,6-dimethyl-4-pyridyl)-N-methylamino)ethoxy)-6-methoxyquinazoline** (170mg, 61%).

m.p. 208-212°C

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD) 2.52(s, 6H); 3.26(s, 3H); 3.98(s, 3H); 4.12(t, 2H); 4.46(t, 2H); 6.8(br s, 1H); 7.1(br s, 1H); 7.38(s, 1H); 7.46(dd, 1H); 7.62(t, 1H); 7.67(dd, 1H); 8.18(s, 1H); 8.89(s, 1H)

MS - ESI: 482 $[MH]^+$

Elemental Analysis:	Found	C 52.2	H 5.2	N 12.2
$C_{25}H_{25}N_5O_2ClF$ 1H_2O 2HCl	Requires	C 52.4	H 5.1	N 12.2%

The starting material was prepared as follows:

A solution of 4-chloro-2,6-dimethylpyridine (849mg, 6mmol), (J. Het. Chem. 1990, 1841), in 2-(methylamino)ethanol (1.35g, 18mmol) and 3M ethereal hydrogen chloride (3 drops) was heated at 140°C for 1 hour. The reaction mixture was allowed to cool and was diluted with water. The insolubles were removed by filtration and the aqueous filtrate was poured onto a suspension of magnesium sulphate (50g) in ethyl acetate (100ml). The insolubles were removed by filtration and the filtrate dried ($MgSO_4$) and the solvent removed by evaporation. The solid residue was triturated with ether, collected by filtration and dried under vacuum at 50°C to give 2-(N-(2,6-dimethyl-4-pyridyl)-N-methylamino)ethanol (960mg, 90%).

m.p. 139-144°C

^{1}H NMR Spectrum: ($CDCl_3$) 2.4(s, 6H); 3.0(s, 3H); 3.51(t, 2H); 3.81(t, 2H); 6.26(s, 2H)

MS - ESI: 181 $[MH]^+$

Example 47

Diethyl azodicarboxylate (261mg, 1.5mmol) was added dropwise to a suspension of of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (160mg, 0.5mmol), (prepared as described for the starting material in Example 24), triphenylphosphine (393mg, 1.5mmol) and 2-(N-(4-pyridyl)amino)ethanol (97mg, 0.7mmol) in methylene chloride (8ml) and the mixture stirred for 2 hours at ambient temperature. The mixture was diluted with ethyl acetate (5ml), the solid product was collected by filtration and purified by chromatography on an aluminium oxide column eluting with methylene chloride/acetonitrile/methanol (60/35/5). The purified solid was triturated with ether and collected by filtration. The solid was dissolved in a mixture of methylene chloride/methanol and 3M ethereal hydrogen chloride (0.5ml) 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-chloro-2-fluoroanilino)-6-methoxy-7-(2-(N-(4-pyridyl)amino)ethoxy)quinazoline** hydrochloride (95mg, 37%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.87(t, 2H); 4.00(s, 3H); 4.43(t, 2H); 6.97(dd, 1H); 7.15(dd, 1H); 7.43(s, 1H); 7.46(dd, 1H); 7.66(t, 1H); 7.68(dd, 1H); 8.12(d, 1H); 8.21(s, 1H); 8.34(d, 1H); 8.89(s, 1H)

MS - ESI: 440 $[MH]^+$

Elemental Analysis: Found C 50.0 H 4.3 N 13.2

$C_{22}H_{19}N_5O_2ClF$ $0.8H_2O$ 2HCl Requires C 50.0 H 4.3 N 13.2%

The starting material was prepared as follows:

Using a procedure analogous to that described for the starting material in Example 46, 4-chloropyridine (3g, 20mmol) was treated with aminoethanol (6.1g, 0.1mol) to give 2-(N-(4-pyridyl)aminoethanol (400mg, 25%).

m.p. 110-111°C

^1H NMR Spectrum: ($CDCl_3$) 3.3(m, 2H); 3.81(m, 2H); 4.94(br s, 1H); 6.44(d, 2H); 8.13(d, 2H)

MS - ESI: 138 $[MH]^+$

Example 48

Using a procedure analogous to that described in Example 47, 3-(N-methyl-N-(4-pyridyl)amino)propanol (116mg, 0.7mmol) was treated with 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (160mg, 0.5mmol), (prepared as described for the starting material in Example 24), to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(N-methyl-N-(4-pyridyl)amino)propoxy)quinazoline** hydrochloride (150mg, 55%).

m.p. 243-248°C

^1H NMR Spectrum: ($DMSOd_6$; CD_3COOD) 2.2(t, 2H); 3.21(t, 3H); 3.82(t, 2H); 4.0(s, 3H); 4.31(t, 2H); 6.95(br s, 1H); 7.2(br s, 1H); 7.39(s, 1H); 7.46(dd, 1H); 7.62(t, 1H); 7.68(dd, 1H); 8.2(s, 1H); 8.3(br s, 2H); 8.87(s, 1H)

MS - ESI: 468 $[MH]^+$

Elemental Analysis: Found C 51.4 H 5.1 N 12.9

$C_{24}H_{23}N_5O_2ClF$ $1.2H_2O$ 1.95HCl Requires C 51.4 H 4.9 N 12.5%

The starting material was prepared as follows:

Using a procedure analogous to that described for the starting material in Example 46, 4-chloropyridine (885mg, 59mmol) and 3-(methylamino)propanol (2.1g, 0.23mmol),

(Tetrahedron Lett. 1994, 35, 1545-1548), were heated for 8 hours to give 3-(N-methyl-N-(4-pyridyl)amino)propanol (979mg, 61%).

^{1}H NMR Spectrum: ($CDCl_3$; CD_3COOD) 1.8-1.9(m, 2H); 3.16(s, 3H); 3.6-3.75(m, 4H); 6.8(br s, 2H); 8.30(d, 2H)

MS - ESI: 166 $[MH]^+$

Example 49

Diethyl azodicarboxylate (261mg, 1.5mmol) was added dropwise to a suspension of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (160mg, 0.5mmol), (prepared as described for the starting material in Example 24), triphenylphosphine (393mg, 1.5mmol) and 1-(2-hydroxyethyl)-2-methylimidazole (88mg, 0.7mmol), (Chem.Abs. 1964, 60, 2949), in methylene chloride (8ml) and the mixture stirred for 2 hours at ambient temperature. The mixture was diluted with ether (8ml) and the solid product was collected by filtration. The solid was dissolved in methylene chloride/methanol and a solution of 3M ethereal hydrogen chloride (0.5ml)was added. 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-methylimidazol-1-yl)ethoxy)quinazoline** hydrochloride (180mg, 72%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.79(s, 3H); 4.02(s, 3H); 4.59(t, 2H); 4.72(t, 2H); 7.40(s, 1H); 7.45(d, 1H); 7.60(s, 1H); 7.62(t, 1H); 7.67(dd, 1H); 7.71(s, 1H); 8.23(s, 1H); 8.89(s, 1H)

MS - ESI: 428 $[MH]^+$

Elemental Analysis:	Found	C 47.9	H 4.7	N 13.3
$C_{21}H_{19}N_4O_2ClF\ 1.4H_2O\ 2.1HCl$	Requires	C 47.6	H 4.6	N 13.2%

Example 50

Diethyl azodicarboxylate (295µl, 1.8mmol) was added dropwise to a solution of 1-(3-hydroxypropyl)imidazole (102mg, 0.81mmol), (EP 0060696 A1), 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (200mg, 0.62mmol), (prepared as described for the starting material in Example 24), and triphenylphosphine (492mg, 1.8mmol) in methylene chloride (4ml) and the mixture stirred for 2 hours at ambient temperature. The solvent was removed by evaporation and the residue was purified by column chromatography

eluting with methylene chloride/acetonitrile/methanol (60/35/5). The purified solid was dissolved in methylene chloride/methanol and 5M ethereal hydrogen chloride (2ml) 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-chloro-2-fluoroanilino)-7-(3-(imidazol-1-yl)propoxy)-6-methoxyquinazoline** hydrochloride (114mg, 36%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.5(m, 2H); 3.99(s, 3H); 4.32(t, 2H); 4.45(t, 2H); 7.39(s, 1H); 7.45(dd, 1H); 7.61(t, 1H); 7.66(dd, 1H); 7.71(s, 1H); 7.84(s, 1H); 8.19(s, 1H); 8.77(s, 1H); 9.20(s, 1H)

MS - ESI: 428 $[MH]^+$

Elemental Analysis:	Found	C 48.2	H 4.5	N 13.2
$C_{21}H_{19}N_5O_2ClF$ $1.2H_2O$ 1.9HCl	Requires	C 48.6	H 4.5	N 13.5%

Example 51

7-(2-Bromoethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (98mg, 0.23mmol) was added to a solution of 4-methyl-4H-1,2,4-triazole-3-thiol (40mg, 0.34mmol) and potassium-t-butoxide (36mg, 0.32mmol) in DMF (1ml) and the mixture heated at 40°C for 30 minutes. The reaction mixture was allowed to cool and was partitioned between ammonium chloride and ethyl acetate. 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 a gradient of methylene chloride/methanol (95/50 to 80/20). The purified solid product was triturated with ether and collected by filtration. The solid was dissolved in methylene chloride/methanol and 3M ethereal hydrogen chloride (0.5ml) was added. The volatiles were removed by evaporation and the residue was crystallised from methylene chloride and ether. The solid was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-methyl-4H-1,2,4-triazol-3-ylthio)ethoxy)-quinazoline** hydrochloride (90mg, 79%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.78(s, 3H); 3.81(t, 2H); 3.99(s, 3H); 4.57(t, 2H); 7.40(s, 1H); 7.46(dd, 1H); 7.62(t, 1H); 7.67(dd, 1H); 8.16(s, 1H); 8.89(s, 1H); 9.68(s, 1H)

MS - ESI: 461 $[MH]^+$

Elemental Analysis:	Found	C 43.7	H 3.9	N 14.9
$C_{20}H_{18}N_6O_2ClFS\ 1H_2O\ 2HCl$	Requires	C 43.5	H 4.0	N 15.2%

The starting material was prepared as follows:

1,2-Dibromoethane (725mg, 4mmol) was added by portions of 70µl every 30 minutes to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (320mg, 1mmol), (prepared as described for the starting material in Example 24), and potassium carbonate (552mg, 4mmol) in DMF (5ml) heated at 35°C. The mixture was stirred for a further 30 minutes after the completion of the addition and then partitioned between ethyl acetate and water. The organic layer was separated, washed with water and brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was triturated with petroleum ether/ether, the solid was collected by filtration and dried under vacuum to give 7-(2-bromoethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (200mg, 47%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.89(t, 2H); 3.96(s, 3H); 4.51(t, 2H); 7.23(s, 1H); 7.35(dd, 1H); 7.55(dd, 1H); 7.59(t, 1H); 7.83(s, 1H); 8.36(s, 1H); 9.57(s, 1H)

MS - ESI: 428 $[MH]^+$

Example 52

Using an analogous procedure to that described in Example 51, 7-(2-bromoethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (98mg, 0.23mmol), (prepared as described for the starting material in Example 51), was treated with 5-mercapto-1-methyltetrazole (40mg, 0.35mmol) to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1-methyltetrazol-5-ylthio)ethoxy)-quinazoline** hydrochloride (50mg, 44%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.8(t, 2H); 3.97(s, 6H); 4.57(t, 2H); 7.35(s, 1H); 7.46(dd, 1H); 7.62(t, 1H); 7.70(dd, 1H); 8.12(s, 1H); 8.87(s, 1H)

MS - ESI: 462 $[MH]^+$

Elemental Analysis:	Found	C 45.1	H 3.7	N 19.3
$C_{19}H_{17}N_7O_2ClFS\ 0.5H_2O\ 1HCl$	Requires	C 45.0	H 3.8	N 19.3%

Example 53

Diethyl azodicarboxylate (295µl, 1.8mmol) was added dropwise to a solution of 2-methyl-1-(3-hydroxypropyl)imidazole (131mg, 0.93mmol), (EP 0060696 A1), triphenylphosphine (492mg, 1.8mmol) and 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (200mg, 0.62mmol), (prepared as described for the starting material in Example 24), in methylene chloride (4ml) and the mixture stirred for 2 hours at ambient temperature. Further 2-methyl-1-(3-hydroxypropyl)imidazole (43mg, 0.31mmol), triphenylphosphine (82mg, 0.31mmol) and diethyl azodicarboxylate (50µl, 0.31mmol) were added and the mixture stirred for a further 3 hours. The volatiles were removed by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol (93/7). The purified solid was dissolved in methylene chloride and 3M ethereal hydrogen chloride (2ml) was added. The volatiles were removed by evaporation and the solid residue was suspended in ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(2-methylimidazol-1-yl)propoxy)quinazoline** hydrochloride (104mg, 32%).

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD) 2.4(t, 2H); 2.60(s, 3H); 4.0(s, 3H); 4.3-4.4(m, 4H); 7.41(s, 1H); 7.46(dd, 1H); 7.58(s, 1H); 7.62(t, 1H); 7.67(dd, 1H); 7.70(s, 1H); 8.21(s, 1H); 8.88(s, 1H)

MS - ESI: 442 $[MH]^+$

Elemental Analysis:	Found	C 49.8	H 5.0	N 12.5
$C_{22}H_{21}N_5O_2ClF$ $1H_2O$ 2HCl 0.23ether	Requires	C 50.1	H 5.0	N 12.7%

Example 54

A solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(methylaminoethoxy)quinazoline hydrochloride hydrate (135mg, 0.3mmol) and 2-chloropyrimidine (66mg, 0.6mmol) in N,N-diisopropylethylamine (2ml) was heated at reflux for 1 hour. The mixture was allowed to cool and was triturated with ether. The solid product was collected by filtration and purified by column chromatography eluting with methylene chloride/methanol (95/5). The purified oil was crystallised from ether and the solid collected by filtration. The solid was dissolved in methylene chloride/methanol and a solution of 3M ethereal hydrogen chloride (0.5ml) was added. The suspension was diluted with ether, 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-methyl-N-(pyrimidin-2-yl)amino)ethoxy)quinazoline** hydrochloride (52mg, 33%).

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD) 3.36(s, 3H); 3.9(s, 3H); 4.22(t, 2H); 4.51(t, 2H); 6.94(t, 1H); 7.36(s, 1H); 7.46(d, 1H); 7.63(t, 1H); 7.66(dd, 1H); 8.08(s, 1H); 8.62(d, 2H); 8.9(s, 1H)

MS - ESI: 455 $[MH]^+$

Elemental Analysis:	Found	C 49.8	H 4.4	N 15.9
$C_{22}H_{20}N_6O_2ClF$ 1.1H_2O 1.5HCl	Requires	C 49.9	H 4.5	N 15.9%

The starting material was prepared as follows:

A solution of di-t-butyl dicarbonate (4.52g, 20mmol) in THF (10ml) was added to a solution of 2-(methylamino)ethanol (1.5g, 20mmol) in water (10ml) and THF (10ml) and the mixture was stirred for 18 hours at ambient temperature. The organic solvents was removed by evaporation and the residue was partitioned between water and ether. The organic layer was separated, washed with 0.1M hydrochloric acid and brine, dried ($MgSO_4$) and the solvent removed by evaporation to give 2-(N-methyl-N-t-butoxycarbonylamino)ethanol (3g, 85%) as an oil.

^{1}H NMR Spectrum: ($CDCl_3$) 1.46(s, 9H); 2.92(s, 3H); 3.39(t, 2H); 3.74(t, 2H)

MS - ESI: 176 $[MH]^+$

A solution of 2-(N-methyl-N-t-butoxycarbonylamino)ethanol (116mg, 0.7mmol) in methylene chloride (1ml) was added to a suspension of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (160mg, 0.5mmol), (prepared as described for the starting material in Example 24), and triphenylphosphine (393mg, 1.5mmol) in methylene chloride (5ml). Diethyl azodicarboxylate (261mg, 1.5mmol) was then added dropwise and the mixture was stirred at ambient temperature for 4 hours. The reaction mixture was poured onto a column of silica and eluted with a gradient of methylene chloride/acetonitrile/methanol (70/30/0 to 70/20/10). The partially purified product was further purified by column chromatography eluting with a gradient of methylene chloride/ether/methanol (60/40/0 to 60/10/30). The pure oil was crystallised from ether, collected by filtration and washed with

ether to give 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(N-methyl-N-t-butoxycarbonylamino)ethoxy)quinazoline (450mg, 63%).

m.p. 194-196°C

^{1}H NMR Spectrum: ($CDCl_3$) 1.46(s, 9H); 3.05(br s, 3H); 3.72(br s, 2H); 4.02(s, 3H); 4.27(br s, 2H); 7.0(s, 1H); 7.2-7.3(m, 3H); 8.54(t, 1H); 8.69(s, 1H)

MS - ESI: 499 $[MNa]^+$

Elemental Analysis:	Found	C 57.2	H 5.7	N 11.5
$C_{23}H_{26}N_4O_4ClF\ 0.3H_2O$	Requires	C 57.3	H 5.6	N 11.6%

TFA (4ml) was added to a solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(N-methyl-N-t-butoxycarbonylamino)ethoxy)quinazoline (390mg, 0.82mmol) in methylene chloride (4ml) and the mixture stirred for 2 hours at ambient temperature. Toluene was added and the volatiles were removed by evaporation. The residue was dissolved in methylene chloride and 3M ethereal hydrogen chloride (1ml) was added. 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-(methylamino)ethoxy)quinazoline hydrochloride hydrate (290mg; 79%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.74(s, 3H); 3.53(t, 2H); 4.05(s, 3H); 4.53(t, 2H); 7.46(d, 1H); 7.47(s, 1H); 7.6-7.7(m, 2H); 8.24(s, 1H); 8.91(s, 1H)

MS - ESI: 377 $[MH]^+$

Elemental Analysis:	Found	C 45.8	H 5.0	N 12.0
$C_{18}H_{18}N_4O_2ClF\ 1.1H_2O\ 2HCl$	Requires	C 46.0	H 4.8	N 11.9%

Example 55

Isonicotinoyl chloride (36mg, 0.2mmol) was added to a suspension of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(methylamino)ethoxy)quinazoline hydrochloride hydrate (90mg, 0.1mmol), (prepared as described for the starting material in Example 54), in methylene chloride (3ml) and triethylamine (80mg, 0.8mmol) was then added dropwise. The mixture was stirred for 30 minutes at ambient temperature and the solvent was then 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 residue was dissolved in methylene chloride/methanol and 3M ethereal

hydrogen chloride (0.5ml) was added. The suspension was diluted with ether, the 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-(4-pyridylcarbonyl)amino)ethoxy)quinazoline** hydrochloride (75mg, 67%).

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD; 95°C) 3.1(s, 3H); 3.8-3.9(br s, 2H); 4.1(s, 3H); 4.4-4.6(br s, 2H); 7.4-7.45(m, 2H); 7.55(dd, 1H); 7.65(t, 1H); 7.9-8.0(br s, 2H); 8.28(s, 1H); 8.8(s, 1H); 8.95(s, 2H)

MS - ESI: 482 $[MH]^+$

Elemental Analysis:	Found	C 51.7	H 4.6	N 12.0
$C_{24}H_{21}N_6O_3ClF$ $1H_2O$ 1.7HCl 0.1ether	Requires	C 51.5	H 4.6	N 12.3%

Example 56

A mixture of 7-(4-pyridylthio)-3,4-dihydroquinazolin-4-one (100mg, 0.4mmol), thionyl chloride (20ml) and DMF (0.1ml) was heated at reflux for 1.5 hours. The volatiles were removed by evaporation and the residue azeotroped with toluene. A solution of 3-hydroxy-4-methylaniline (53mg, 0.04mmol) in isopropanol (10ml) was added to the solid residue and the mixture was heated at reflux for 2 hours. The mixture was allowed to cool and the precipitated product collected by filtration, washed with isopropanol and dried to give **4-(3-hydroxy-4-methylanilino)-7-(4-pyridylthio)quinazoline** hydrochloride(103mg, 73%).

^{1}H NMR Spectrum: (DMSOd_6) 2.17(s, 3H); 7.05(dd, 1H); 7.17(d, 1H); 7.19(s, 1H); 7.64(d, 2H); 8.00(d, 1H); 8.20(s, 1H); 8.66(d, 2H); 8.92(s, 1H); 9.05(d, 1H)

MS - ESI: 361 $[MH]^+$

Elemental analysis:	Found	C 53.2	H 4.6	N 11.8
$C_{20}H_{16}N_4OS$ $1H_2O$ 2HCl	Requires	C 53.2	H 4.4	N 12.4%

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 hydride (3.3g of a 50% suspension in mineral oil, 69mmol) was added to a solution of 4-mercaptopyridine (8.12g, 73mmol) in DMF (100ml) and the mixture stirred for 30 minutes. 7-Fluoro-3,4-dihydroquinazolin-4-one (1.5g, 9mmol) was added and the reaction heated at 100°C for 4 hours. The mixture was allowed to cool, diluted with water and extracted with ethyl acetate. The organic extracts were 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 (97/3) to give 7-(4-pyridylthio)-3,4-dihydroquinazolin-4-one (500mg, 6%).

^{1}H NMR Spectrum: ($DMSOd_6$) 7.24(d, 2H); 7.54(dd, 1H); 7.70(d, 1H); 8.10(s, 1H); 8.14(d, 1H); 8.44(d, 2H)

MS - ESI: 256 [MH]$^+$

Example 57

A mixture of 4-chloro-2-fluoro-3-hydroxyaniline (118mg, 0.7mmol), (EP 061741 A2), and 4-chloro-6-methoxy-7-((4-pyridyl)methoxy)quinazoline (200mg, 0.7mmol), (prepared as described for the starting material in Example 13), in isopropanol (10ml) and ethereal hydrogen chloride (5ml) was heated at 80°C for 2 hours and the mixture was allowed to cool. The precipitated product was collected by filtration, washed with isopropanol and dried to give **4-(4-chloro-2-fluoro-5-hydroxyanilino)-6-methoxy-7-((4-pyridyl)methoxy)quinazoline** hydrochloride (110mg, 31%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.96(s, 3H); 5.38(s, 2H); 7.14(d, 1H); 7.24(s, 1H); 7.38(d, 1H); 7.48(d, 2H); 7.82(s, 1H); 8.32(s, 1H); 8.58(d, 2H0; 9.48(s, 1H)

MS - ESI: 427 [MH]$^+$

Example 58

A mixture of 7-((2-chloro-4-pyridyl)methoxy)-6-methoxy-3,4-dihydroquinazolin-4-one (150mg, 0.47mmol), phosphoryl chloride (0.2ml) and N,N-dimethylaniline (0.2ml) in toluene (5ml) was heated at reflux for 1 hour. The volatiles were removed by evaporation and the residue was partitioned between ethyl acetate and saturated sodium hydrogen carbonate solution. The organic layer was separated, dried ($MgSO_4$) and the solvent removed by evaporation. A solution of 2-fluoro-5-hydroxy-4-methylaniline (67mg, 0.47mmol), (prepared

as described for the starting material in Example 13), in isopropanol (10ml) was added to the solid residue and the mixture was heated at reflux for 2 hours. The mixture was allowed to cool and the precipitated product collected by filtration, washed with acetone and dried to give **7-((2-chloro-4-pyridyl)methoxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline** hydrochloride (70mg, 30%).

m.p. 245-250°C

^{1}H NMR Spectrum: (DMSOd_6) 2.30(s, 3H); 4.10(s, 3H); 5.45(s, 2H); 6.90(d, 1H); 7.10(d, 1H); 7.35(s, 1H); 7.50(d, 1H); 7.65(s, 1H); 8.25(s, 1H); 8.45(d, 1H); 8.75(s, 1H); 9.60(br s, 1H); 11.30(s, 1H)

MS - ESI: 441 $[MH]^+$

Elemental analysis:	Found	C 53.7	H 4.0	N 10.9
$C_{22}H_{18}N_4O_3FCl\ 1H_2O\ 1HCl$	Requires	C 53.4	H 4.3	N 11.3%

The starting material was prepared as follows:

Oxalyl chloride (0.3ml) was added to a mixture of 4-(2-chloropyridine)carboxylic acid (950mg, 6mmol) and DMF (0.05ml) in methylene chloride (20ml) and the mixture strirred at ambient temperature for 1 hour. The volatiles were removed by evaporation and ethanol (10ml) was added to the residue and the mixture stirred at ambient temperature for 18 hours. Water was added and the mixture was extracted with ethyl acetate (3x25ml). The extracts were combined, dried ($MgSO_4$) and the solvent removed by evaporation to give ethyl 4-(2-chloropyridine)carboxylate (700mg, 63%) as a brown oil.

^{1}H NMR Spectrum: (DMSOd_6) 1.30(t, 3H); 4.37(q, 2H); 7.80(m, 2H); 8.60(d, 1H)

Lithium aluminium hydride (5ml of a 1M solution in ether, 5mmol) was added dropwise to a stirred solution of ethyl 4-(2-chloropyridine)carboxylate (700mg, 3.8mmol) in ether (10ml) at 0°C. The mixture was allowed to warm to ambient temperature, wet ether and 2M sodium hydroxide solution (2ml). The insolubles were removed by filtration, the organic phase was separated and the aqueous layer was extracted with ether (3x25ml). The extracts were combined, dried ($MgSO_4$) and the solvent removed by evaporation to give 2-chloro-4-hydroxymethylpyridine (180mg, 33%) as a brown oil which crystallised on standing.

^{1}H NMR Spectrum: (DMSOd_6) 4.55(s, 2H); 5.50(br s, 1H); 7.32(d, 1H); 7.20(s, 1H); 8.30(d, 1H)

A mixture of 2-chloro-4-hydroxymethylpyridine (180mg, 1.25mmol), thionyl chloride (0.2ml) in toluene (10ml) was stirred at ambient tempertaure for 1 hour. The volatiles were removed by evaporation to give 2-chloro-4-chloromethylpyridine hydrochloride (180mg, 0.9mmol). A mixture of 7-hydroxy-6-methoxy-4-phenoxyquinazoline (268mg, 1mmol), (prepared as described for the starting material in Example 13), potassium carbonate (680mg, 5mmol) and DMF (10ml) was added to this crude product and the mixture was heated at 90°C for 1 hour. The mixture was allowed to cool, diluted with water and extracted with ethyl acetate (3x70ml). The extracts were combined, washed with water (x3) and brine, dried ($MgSO_4$) and the solvent removed by evaporation to give 7-((2-chloro-4-pyridyl)methoxy)-6-methoxy-4-phenoxyquinazoline (260mg, 66%) as a solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 4.00(s, 3H); 5.45(s, 2H); 7.30(m, 3H); 7.42(s, 1H); 7.4-7.5(m, 3H); 7.60(s, 1H); 7.62(s, 1H); 8.44(d, 1H); 8.52(s, 1H)

MS - ESI: 394 $[MH]^+$

A mixture of 7-((2-chloro-4-pyridyl)methoxy)-6-methoxy-4-phenoxyquinazoline (260mg, 0.7mmol) and 2M hydrochloric acid (15ml) was heated at 85°C for 2 hours. The mixture was allowed to cool and adjusted to pH6-7 with sodium hydrogen carbonate solution. The resulting precipitate was collected by filtration and dried to give 7-((2-chloro-4-pyridyl)methoxy)-6-methoxy-3,4-dihydroquinazolin-4-one (160mg, 76%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.90(s, 3H); 5.36(s, 2H); 7.18(s, 1H); 7.45(m, 2H); 7.46(s, 1H); 7.59(s, 1H); 8.42(d, 1H)

MS - ESI: 318 $[MH]^+$

Example 59

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (950mg, 3mmol), (prepared as described for the starting material in Example 24), 2-bromo-4-bromomethylpyridine (765mg, 3mmol) and potassium carbonate (2.38g 17mmol) in DMF (10ml) was heated at 80°C for 2 hours. The mixture was allowed to cool, poured into water and extracted with ethyl acetate. The combined extracts were dried ($MgSO_4$) and the solvent removed by evaporation and azeotroped with toluene. The residue was triturated with ethyl acetate/hexane and the solid product collected by filtration, washed with ethyl acetate/hexane

and dried to give **7-((2-bromo-4-pyridyl)methoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline** (647mg, 44%).

m.p. 210-212°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.98(s, 3H); 5.40(s, 2H); 7.25(s, 1H); 7.30(d, 1H); 7.50(s, 1H); 7.50(d, 1H); 7.55(m, 2H); 7.74(s, 1H); 7.86(s, 1H); 8.35(br s, 1H); 8.42(d, 1H); 9.56(s, 1H)

MS - ESI: 489 $[MH]^+$

Elemental analysis:	Found	C 52.0	H 3.2	N 11.2
$C_{21}H_{15}N_4O_2BrClF$	Requires	C 51.5	H 3.1	N 11.4%

The starting material was prepared as follows:

A mixture of 2-bromo-4-methylpyridine (12.2g), N-bromosuccinimide (30g) and 2,2'-azobis(2-methylpropionitrile) (100mg) in carbon tetrachloride (200ml) was heated at reflux for 2.5 hours. The mixture was allowed to cool and the insoluble material removed by filtration. The solvent was removed from the filtrate by evaporation and the residue was purified by filtration through a silica pad eluting with ethyl acetate/hexane (10/1) to give 2-bromo-4-bromomethylpyridine.

^{1}H NMR Spectrum: ($DMSOd_6$) 4.65(s, 2H); 7.50(d, 1H); 7.42(s, 1H); 7.70(s, 1H); 8.35(d, 1H)

MS - ESI: 250 $[MH]^+$

Example 60

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (600mg, 2mmol), (prepared as described for the starting material in Example 24), 4-chloromethyl-2-cyanopyridine hydrochloride (620mg, 3mmol) and potassium carbonate (1.0g 7mmol) in DMF (8ml) was heated at 80°C for 30 minutes. The mixture was allowed to cool, poured into water and extracted with ethyl acetate. The combined extracts were dried ($MgSO_4$) and the solvent removed by evaporation and azeotroped with toluene. The residue was triturated with ethyl acetate/hexane, the solid product collected by filtration and purified by column chromatography eluting with ethyl acetate and further chromatography eluting with methylene chloride/methanol (99/1). The purified product was recrystallised from ethyl

acetate/hexane to give **4-(4-chloro-2-fluoroanilino)-7-((2-cyano-4-pyridyl)methoxy)-6-methoxyquinazoline** (35mg, 4%).

m.p. 209-213°C

^{1}H NMR Spectrum: (DMSOd_6) 3.98(s, 3H); 5.44(s, 2H); 7.26(s, 1H); 7.34(dd, 1H); 7.53(dd, 1H); 7.58(d, 1H); 7.80(d, 1H); 7.85(s, 1H); 8.27(s, 1H); 8.35(s, 1H); 8.80(d, 1H); 9.60(s, 1H)

MS - ESI: 436 [MH]$^+$

Elemental analysis:	Found	C 60.3	H 3.4	N 16.1
$C_{22}H_{15}N_5O_2ClF$	Requires	C 60.6	H 3.5	N 16.1%

The starting material was prepared as follows:

Tetrabutyl ammonium fluoride (9ml of a 1M solution in THF, 9mmol) was added to a solution of 2-cyano-4-dimethyl-t-butylsilyloxymethylpyridine (1.4g, 5.6mmol), (J.Het. Chem. 1993, **30**, 631), in THF (15ml) and the mixture was stirred for 2 hours at ambient temperature. Water was added and the volatiles were removed by evaporation. The residue was partitioned between ethyl acetate and water. The organic layer was separated and the aqueous layer extracted with ethyl acetate. The combined extracts were dried ($MgSO_4$) and the solvent removed by evaporation to give 2-cyano-4-hydroxymethylpyridine (0.55g, 73%).

^{1}H NMR Spectrum: (DMSOd_6) 4.65(s, 2H); 5.70(t, 1H); 7.70(d, 1H); 7.95(s, 1H); 8.75(d, 1H)

A mixture of 2-cyano-4-hydroxymethylpyridine (0.51g, 3.8mmol) and thionyl chloride (0.6ml) in toluene (20ml) was stirred at room temperature for 1 hour. The volatiles were removed by evaporation and the residue azeotroped with toluene to give 4-chloromethyl-2-cyanopyridine hydrochloride (620mg, 86%).

^{1}H NMR Spectrum: (DMSOd_6) 4.75(s, 2H); 7.75(dd, 1H); 8.05(s, 1H); 8.34(d, 1H)

Example 61

A mixture of 7-((6-chloro-2-oxo-1,2-dihydropyrid-4-yl)methoxy)-6-methoxy-3,4-dihydroquinazolin-4-one (190mg, 0.4mmol), thionyl chloride (5ml) and DMF (0.1ml) was heated at reflux for 2 hours. The volatiles were removed by evaporation and the residue azeotroped with toluene. A solution of 4-chloro-2-fluoroaniline (1ml) in isopropanol (15ml) was added to the solid residue and the mixture was heated at reflux for 3 hours. The mixture was allowed to cool and the precipitated product collected by filtration, washed with

isopropanol and dried to give **4-(4-chloro-2-fluoroanilino)-7-((6-chloro-2-oxo-1,2-dihydropyrid-4-yl)methoxy)-6-methoxyquinazoline** hydrochloride (110mg, 41%).

m.p. 271-273°C (decomp.)

^{1}H NMR Spectrum: ($DMSOd_6$) 4.08(s, 3H); 5.35(s, 2H); 6.70(s, 1H); 7.00(s, 1H); 7.30(s, 1H); 7.40(d, 1H); 7.60(m, 2H); 8.30(s, 1H); 8.75(s, 1H)

MS - ESI: 461 $[MH]^+$

The starting material was prepared as follows:

A mixture of 2,6-dichloro-4-hydroxymethylpyridine (1.72g, 16mmol) and 40% aqueous sodium hydroxide solution (5ml) in methanol (50ml) was heated at reflux for 24 hours. The mixture was allowed to cool and the volatiles removed by evaporation. The residue was extracted with ethyl acetate and the solvent removed from the extracts by evaporation. The residue was recrystallised from ethyl acetate/hexane to give 2-chloro-4-hydroxymethyl-6-methoxypyridine (490mg, 28%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.80(s, 3H); 4.45(d, 2H); 5.45(t, 1H); 6.70(s, 1H); 6.98(s, 1H)

Thionyl chloride (1.0ml) was added to a solution of 2-chloro-4-hydroxymethyl-6-methoxypyridine (0.9g, 5.2mmol) in toluene (10ml) and the mixture stirred at ambient temperature for 1 hour. The volatiles were removed by evaporation, the residue was azeotroped with toluene and dried under vacuum to give 2-chloro-4-chloromethyl-6-methoxypyridine hydrochloride (0.88g, 74%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.85(s, 3H); 4.70(s, 2H); 6.90(s, 1H); 7.15(s, 1H)

A mixture of 7-hydroxy-6-methoxy-4-phenoxyquinazoline (1.1g, 4.1mmol), (prepared as described for the starting material in Example 13), 2-chloro-4-chloromethyl-6-methoxypyridine hydrochloride (0.88g, 3.9mmol) and potassium carbonate (2.0g, 14mmol) in DMF (20ml) was heated at 80°C for 1 hour. The mixture was allowed to cool, diluted with water and the precipitated product collected by filtration, washed with water and dried to give 7-((2-chloro-6-methoxy-4-pyridyl)methoxy)-6-methoxy-4-phenoxyquinazoline (1.38g, 79%).

^{1}H NMR Spectrum: ($CDCl_3$) 3.95(s, 3H); 4.04(s, 3H); 5.20(s, 2H); 6.70(s, 1H); 6.95(s, 1H); 7.18(m, 3H); 7.30(t, 1H); 7.40(t, 2H); 7.58(s, 1H); 8.52(s, 1H)

MS - ESI: 424 $[MH]^+$

A mixture of 7-((2-chloro-6-methoxy-4-pyridyl)methoxy)-6-methoxy-4-phenoxyquinazoline (400mg, 0.95mmol) and 2M hydrochloric acid (20ml) was heated at reflux for 3 hours. The mixture was allowed to cool and adjusted to pH6-7 with aqueous ammonia solution. The resulting precipitate was collected by filtration washed with water and dried to give crude 7-((6-chloro-2-oxo-1,2-dihydropyrid-4-yl)methoxy)-6-methoxy-3,4-dihydroquinazolin-4-one (190mg, 60%).

Example 62

Thionyl chloride (0.6ml) was added to a solution of 4-hydroxymethyl-2-methoxypyridine (0.59g, 4.2mmol) in toluene (10ml) and the mixture stirred at ambient temperature for 1.5 hours. The volatiles were removed by evaporation and the residue was azeotroped with toluene and dried under vacuum to give crude 4-chloromethyl-2-methoxypyridine hydrochloride (0.50g, 2.6mmol) which was used directly. This product was then added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (420mg, 1.3mmol), (prepared as described for the starting material in Example 24), and potassium carbonate (1.0g 7mmol) in DMF (8ml) and the resulting mixture was heated at 75°C for 2 hours. The mixture was allowed to cool, diluted with water and the precipitated solid collected by filtration, washed with water and dried to give **4-(4-chloro-2-fluoroanilino)-7-((2-methoxy-4-pyridyl)methoxy)-6-methoxyquinazoline** (140mg, 25%).

m.p. 202-204°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.85(s, 3H); 3.98(s, 3H); 5.35(s, 2H); 6.88(s, 1H); 7.05(d, 1H); 7.24(s, 1H); 7.35(dd, 1H); 7.54(dd, 1H); 7.58(t, 1H); 7.84(s, 1H); 8.11(d, 1H); 8.35(s, 1H); 9.58(br s, 1H)

MS - ESI: 441 $[MH]^+$

Elemental analysis:	Found	C 59.9	H 4.1	N 12.4
$C_{22}H_{18}N_4O_3ClF$	Requires	C 59.9	H 4.1	N 12.7%

The starting material was prepared as follows:

A mixture of ethyl 2-hydroxy-pyridine-4-carboxylate (1.0g, 6mmol), (Chem. Abs. 1957, 8740c), methyl iodide (1ml) and silver(I)carbonate (1.64g) in toluene (20ml) was heated at reflux for 2 hours. The mixture was allowed to cool and the insolubles removed by

filtration through diatomaceous earth and the pad was washed through with ethyl acetate. The filtrate was washed with water, dried ($MgSO_4$) and the solvent removed by evaporation to give ethyl 2-methoxy-pyridine-4-carboxylate (0.93g, 86%) as a yellow oil.

^{1}H NMR Spectrum: ($CDCl_3$) 1.30(t, 3H); 3.90(s, 3H); 4.30(q, 2H); 7.24(s, 1H); 7.35(d, 1H); 8.20(d, 1H)

MS - ESI: 182 $[MH]^+$

A solution of ethyl 2-methoxy-pyridine-4-carboxylate (0.93g, 5mmol) in ether (5ml) was added to lithium aluminium hydride (0.3g, 8mmol) in ether (10ml) cooled to 5°C and the mixture stirred for 2 hours. Water was added, the mixture was filtered through diatomaceous earth and the pad was washed through with ethyl acetate. The filtrate was extracted with ethyl acetate and the combined extracts were washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation to give 4-hydroxymethyl-2-methoxypyridine (0.64g, 89%) as a yellow oil.

^{1}H NMR Spectrum: ($CDCl_3$) 3.86(s, 3H); 4.62(s, 2H); 6.65(s, 1H); 6.76(d, 1H); 8.05(d, 1H)

MS - ESI: 140 $[MH]^+$

Example 63

Thionyl chloride (0.3ml) was added to a solution of 4-hydroxymethyl-2-methylpyridine (240mg, 1.9mmol) in toluene (10ml) and the mixture stirred at ambient temperature for 2 hours. The volatiles were removed by evaporation, the residue azeotroped with toluene and dried under vacuum to give crude 4-chloromethyl-2-methylpyridine hydrochloride which was used directly. This product was then added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (510mg, 1.6mmol), (prepared as described for the starting material in Example 24), and potassium carbonate (1.4g 10mmol) in DMF (8ml) for 90 hours. The mixture was diluted with water and the precipitated solid collected by filtration, washed with water and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-((2-methyl-4-pyridyl)methoxy)quinazoline** (290mg, 43%), a sample was recrystallised from ethyl acetate/hexane.

m.p. 221-224°C

^{1}H NMR Spectrum: ($CDCl_3$) 2.50(s, 3H); 4.00(s, 3H); 5.20(s, 2H); 6.98(s, 1H); 7.15(d, 1H); 7.2(m, 4H); 8.45(m, 2H); 8.60(s, 1H)

Elemental analysis:	Found	C 61.7	H 4.2	N 13.2
$C_{22}H_{18}N_4O_2ClF$	Requires	C 62.2	H 4.3	N 13.2%

The starting material was prepared as follows:

Oxalyl chloride (1.9g, 15mmol) was added to 2-chloro-6-methyl-pyridine-4-carboxylic acid (1.7g, 10mmol) in methylene chloride (30ml) and the mixture stirred for 2 hours. The volatiles were removed by evaporation and methanol (20ml) added to the residue. The mixture was stirred for 1 hour and the volatiles removed by evaporation to give methyl 2-chloro-6-methyl-pyridine-4-carboxylate (1.85g, 100%) as an off-white solid.

^{1}H NMR Spectrum: ($CDCl_3$) 2.55(s, 3H); 3.90(s, 3H); 7.55(s, 1H); 7.60(s, 1H);
MS - ESI: 186 $[MH]^+$

A mixture of methyl 2-chloro-6-methyl-pyridine-4-carboxylate (1.8g, 10mmol) and 10% palladium-on-charcoal catalyst (200mg) in methanol (100ml) was stirred under hydrogen at 5 atmospheres pressure. The catalyst was removed by filtration and the volatiles removed from the filtrate by evaporation. The residue was treated with 10% aqueous sodium hydroxide solution and extracted with ether (3x30ml). The combined extracts were dried ($MgSO_4$) and the solvent removed by evaporation to give methyl 2-methyl-pyridine-4-carboxylate (800mg, 53%) as an oil.

A solution of methyl 2-methyl-pyridine-4-carboxylate (800mg, 6mmol) in ether (5ml) was added to lithium aluminium hydride (340mg, 9mmol) in ether (10ml) cooled to 5°C and the mixture stirred for 2 hours. Water was added, the mixture was filtered through diatomaceous earth and the pad was washed through with ethyl acetate. The filtrate was extracted with ethyl acetate and the combined extracts were washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation to give 4-hydroxymethyl-2-methylpyridine (240mg, 38%) as a yellow oil.

^{1}H NMR Spectrum: ($CDCl_3$) 2.48(s, 3H); 5.44(s, 2H); 7.00(d, 1H); 7.10(s, 1H); 8.40(d, 1H)
MS - ESI: 124 $[MH]^+$

Example 64

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (350mg, 0.9mmol), (prepared as described for the starting material in Example 24), 2-(2-

chloroethylthio)-1-methylimidazole hydrochloride (203mg, 0.95mmol) and potassium carbonate (303mg 2.2mmol) in NMP (20ml) was heated at 90°C for 2 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 the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (100/0 increasing to 90/10) to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(1-methylimidazol-2-ylthio)ethoxy)quinazoline** (75mg, 17%) as a solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.46(s, 3H); 3.93(s, 3H); 4.39-4.44(m, 4H); 7.13(dd, 2H); 7.23(s, 1H); 7.31(dd, 1H); 7.49-7.60(m, 2H); 7.79(s, 1H); 8.37(s, 1H); 9.51(s, 1H)

MS - ESI: 460 $[MH]^+$

Elemental analysis:	Found	C 52.8	H 4.0	N 14.3
$C_{21}H_{19}N_4O_2ClFS\ 1H_2O$	Requires	C 52.8	H 4.4	N 14.7%

The starting material was prepared as follows:

2-Chloroethanol (3g, 37mmol) was added to a solution of 2-mercapto-1-methylimidazole (3.45g, 30mmol) in 2M aqueous sodium hydroxide solution (30ml) and the mixture heated at 100°C for 2 hours. The mixture was allowed to cool and extracted with ethyl acetate. The combined extracts were dried ($MgSO_4$) and the solvent removed by evaporation to give 2-(2-hydroxyethylthio)-1-methylimidazole (3.9g, 82%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.04(t, 2H); 3.30(s, 3H); 3.54(t, 2H); 5.00(s, 1H); 6.87(s, 1H); 7.20(s, 1H)

Thionyl chloride (1.41ml, 19mmol) was slowly added to a solution of 2-(2-hydroxyethylthio)-1-methylimidazole (1.81g, 11mmol) in trichloromethane (20ml) at 5°C. The mixture was stirred for 1 hour at 5°C and then for 3 hours at ambient temperature. The volatiles were removed by evaporation and the residue azeotroped with toluene to give 2-(2-chloroethylthio)-1-methylimidazole hydrochloride (1.5g, 77%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.58 (t, 2H); 3.78(s, 3H); 3.80(t, 2H); 7.78(d, 1H); 7.83(d, 1H)

Example 65

Thionyl chloride (0.55ml, 7.5mmol) was added to a solution of 1-(3-hydroxypropyl)-1.2dihyro-2-pyridone (770mg, 5mmol) in trichloromethane (15ml) at 5°C. The mixture was

stirred at 5°C for 1 hour and then at ambient temperature for 2 hours. The volatiles were removed by evaporation, the residue azeotroped with toluene and dried under vacuum to give crude 1-(3-chloropropyl)-1,2-dihydro-2-pyridone (500mg) which was used directly. Part of this product (206mg, 1.2mmol) was then added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (350mg, 1.0mmol), (prepared as described for the starting material in Example 24), and potassium carbonate (303mg, 2.2mmol) in NMP (20ml) and the reaction mixture was heated at 90°C for 2 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 the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol mixtures (100/0 increasing to 95/5) to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(2-oxo-1,2-dihydro-1-pyridyl)propoxy)quinazoline** (194mg, 50%).

m.p. 216-218°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.18(m, 2H); 3.90(s, 3H); 4.06(t, 2H); 4.15(t, 2H); 6.18(t, 1H); 6.38(d, 1H); 7.15(s, 1H); 7.30-7.42(m, 2H); 7.50-7.64(m, 3H); 7.79(s, 1H); 8.34(s, 1H); 9.50(s, 1H)

MS - ESI: 455 $[MH]^+$

Elemental analysis:	Found	C 59.4	H 4.6	N 12.1
$C_{23}H_{20}N_4O_3ClF\ 0.5H_2O$	Requires	C 59.6	H 4.6	N 12.1%

The starting material was prepared as follows:

Sodium hydride (1.31g of a 50% suspension in mineral oil, 27mmol) was added to a solution of 2-hydroxypyridine (2.35g, 24mmol) in DMF (50ml) and the mixture stirred for 30 minutes. 2-(3-Bromopropoxy)tetrahydropyran (5.0g, 22.5mmol), (J. Chem. Soc. 1963, 3440), was added and the mixture heated at 100°C for 3 hours and then stirred at ambient temperature for 18 hours. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined extracts were washed with water, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol mixtures (100/0 increasing to 97/3) to give:

1-(3-(2-tetrahydropyranyloxy)propyl)-1,2-dihydro-2-pyridone (1.6g, 30%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.39-1.75(m, 6H); 1.85(m, 2H); 3.24-3.42(m, 3H); 3.58-3.74(m, 2H); 3.90(t, 2H); 4.52(s, 1H); 6.18(t, 1H); 6.35(d, 1H); 7.38(dd, 1H); 7.60(dd,1H)
MS - ESI: 238 $[MH]^+$
and
2-(3-(2-tetrahydropyranyloxy)propyloxy)pyridine (1.43g, 27%).
^{1}H NMR Spectrum: ($DMSOd_6$) 1.38-1.70(m, 6H); 1.90(m, 2H); 3.30(m, 3H); 3.34-3.50(m, 2H); 3.62-3.80(m, 2H); 4.30(t, 2H); 4.52(s, 1H); 6.78(d, 1H); 6.92(dd, 1H); 7.64(m, 1H); 8.15(dd,1H)
MS - ESI: 238 $[MH]^+$

A solution of 1-(3-(2-tetrahydropyranyloxy)propyl)-1,2-dihydro-2-pyridone (1.0g, 4.5mmol) in acetic acid (8ml), THF (4ml) and water (2ml) was heated at 50°C for 4 hours. The volatiles were removed by evaporation and the residue azeotroped with toluene to give 1-(3-hydroxypropyl)-1,2-dihydro-2-pyridone (680mg, 99%) as an off-white solid.
^{1}H NMR Spectrum: ($DMSOd_6$) 1.74(m, 2H); 3.38(m, 2H); 3.90(t, 2H); 4.58(s, 1H); 6.18(dd, 1H); 6.38(d, 1H); 7.38(m, 1H); 7.60(dd, 1H)

Example 66

Thionyl chloride (0.80ml, 11mmol) was added to a solution of 2-(3-hydroxypropylthio)-1-methylimidazole (1.25g, 7.3mmol) in trichloromethane (25ml) at 5°C. The mixture was stirred at 5°C for 1 hour and then at ambient temperature for 2 hours. The volatiles were removed by evaporation, the residue azeotroped with toluene and dried under vacuum to give crude 2-(3-chloropropylthio)-1-methylimidazole hydrochloride (1.0g) which was used directly. Part of this product (226mg, 1.0mmol) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (350mg, 1.0mmol), (prepared as described for the starting material in Example 24), and potassium carbonate (303mg 2.2mmol) in NMP (20ml) and the mixture was heated at 90°C for 2 hours. The mixture was allowed to cool, diluted with water and extracted with ethyl acetate. The combined extracts were wahed with water, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (100/0 increasing to 95/5) to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(1-methylimidazol-2-ylthio)propoxy)quinazoline** (29mg, 6%).

m.p. 199-201°C

^{1}H NMR Spectrum: (DMSOd_6) 2.22(m, 2H); 3.44(s, 3H); 3.94(s, 3H); 4.10(m, 4H); 7.10(d, 2H); 7.30(dd, 1H); 7.50-7.60(m, 2H); 7.79(s, 1H); 8.34(s, 1H); 9.50(s, 1H)

MS - ESI: 474 [MH]$^+$

Elemental analysis:	Found	C 50.9	H 4.8	N 13.2
$C_{22}H_{21}N_5O_2ClFS$ 2.5H_2O	Requires	C 50.9	H 5.1	N 13.5%

The starting material was prepared as follows:

Sodium hydride (0.95g of a 50% suspension in mineral oil, 20mmol) was added to a solution of 2-mercapto-1-methylimidazole (2.26g, 19mmol) in DMF (100ml) and the mixture stirred for 30 minutes. 2-(3-Bromopropoxy)tetrahydropyran (5.0g, 22.5mmol), (J. Chem. Soc. 1963, 3440), was added and the mixture heated at 100°C for 3 hours and then stirred at ambient temperature for 18 hours. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined extracts were washed with water, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol mixtures (100/0 increasing to 97/3) to give 1-methyl-2-(3-(2-tetrahydropyranyloxy)propylthio)imidazole (2.5g, 55%).

^{1}H NMR Spectrum: (DMSOd_6) 1.38-1.72(m, 6H); 1.80(m, 2H); 3.0(t, 2H); 3.36-343(m, 2H); 3.58(s, 3H); 3.62-3.78(m, 2H); 4.50(s, 1H); 6.90(s, 1H); 7.21(s, 1H)

A solution of 1-methyl-2-(3-(2-tetrahydropyranyloxy)propylthio)imidazole (2.0g, 7.8mmol) in acetic acid (8ml), THF (4ml) and water (2ml) was heated at 50°C for 4 hours. The volatiles were removed by evaporation and the residue azeotroped with toluene to give 2-(3-hydroxypropylthio)-1-methylimidazole (1.3g, 100%) as an off-white solid.

^{1}H NMR Spectrum: (DMSOd_6) 1.68(m, 2H); 2.98(t, 2H); 3.42(t, 2H); 3.57(s, 3H); 4.10(s, 1H); 6.90(d, 1H); 7.20(d, 1H)

Example 67

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (350mg, 1.0mmol), (prepared as described for the starting material in Example 24), 4-(3-chloropropoxy)pyridine hydrochloride (206mg, 1.0mmol) and potassium carbonate (303mg, 2.2mmol) in NMP (20ml) was heated at 90°C for 2 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 the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol mixtures (100/0 increasing to 95/5) to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(4-pyridyloxy)propoxy)quinazoline** (257mg, 56%).

m.p. 138-140°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.25(m, 2H); 3.92(s, 3H); 4.24(t, 2H); 4.30(t, 2H); 6.98(dd, 2H); 7.20(s, 1H); 7.31(dd, 1H); 7.55(dd, 2H); 7.79(s, 1H); 8.32-8.38(m, 3H); 9.50(s, 1H)

MS - ESI: 455 $[MH]^+$

Elemental analysis:	Found	C 58.4	H 4.7	N 11.8
$C_{23}H_{20}N_4O_3ClF\ 1H_2O$	Requires	C 58.4	H 4.7	N 11.8%

The starting material was prepared as follows:

A mixture of 4-chloropyridine (7g, 47mmol), ethylene glycol (17.9g, 235mmol) and sodium hydroxide (4.67g, 195mmol) in DMSO (80ml) was heated at 100°C for 24 hours. Most of the solvent was removed by evaporation and the residue was diluted with ice water. The aqueous mixture was extracted with ethyl acetate, the extracts combined, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol mixtures (100/0 increasing to 97/3) to give 4-(3-hydroxypropoxy)pyridine (3.2g, 45%).

Thionyl chloride (2.2ml, 30mmol) was added slowly to a solution of 4-(3-hydroxypropoxy)pyridine (3.1g, 20mmol) in trichloromethane (40ml) at 5°C. The mixture was stirred at 5°C for 1 hour and then at ambient temperature for 2 hours. The volatiles were removed by evaporation, the residue azeotroped with toluene and dried under vacuum to give 4-(3-chloropropoxy)pyridine hydrochloride (3.81g, 91%) as a solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 2.22(m, 2H); 3.80(t, 2H); 4.42(t, 2H); 7.55(d, 2H); 8.72(d, 2H)

MS - ESI: 172 $[MH]^+$

Example 68

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (350mg, 1.0mmol), (prepared as described for the starting material in Example 24), 4-(2-chloroethylthio)pyridine hydrochloride (252mg, 1.2mmol) and potassium carbonate (454mg, 3.3mmol) in NMP (30ml) was heated at 90°C for 2 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 the solvent removed by evaporation. The residue was purified by column chromatography eluting with ethyl acetate/methanol mixtures (100/0 increasing to 75/25) to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(4-pyridylthio)ethoxy)quinazoline** (13mg, 3%).

m.p. 182-186°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.58(t, 2H); 3.90(s, 3H); 4.40(t, 2H); 7.20(s, 1H); 7.32(d, 1H); 7.40(d, 2H); 7.50-7.60(m, 2H); 7.80(s, 1H); 8.32(s, 1H); 8.38(d, 2H); 9.57(s, 1H)

MS - ESI: 457 [MH]$^+$

The starting material was prepared as follows:

Sodium hydride (890mg of a 50% suspension in mineral oil, 19mmol) was added to a solution of 4-mercaptopyridine (2.34g, 21mmol) in DMF (75ml) and the mixture stirred for 30 minutes. 2-(2-Bromoethoxy)tetrahydropyran (4.0g, 19mmol), (J. Am. Chem. Soc. 1948, **70**, 4187), was added and the mixture heated at 100°C for 3 hours and then stirred at ambient temperature for 18 hours. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined extracts were washed with water, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol mixtures (100/0 increasing to 97/3) to give 4-(2-(tetrahydropyran-2-yloxy)ethylthio)pyridine (2.8g, 56%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.35-1.64(m, 6H); 3.35-3.42(m, 1H); 3.58-3.82(m, 3H); 4.60(s, 1H); 7.30(dd, 2H); 8.33(dd, 2H)

A solution of 4-(2-(tetrahydropyran-2-yloxy)ethylthio)pyridine (2.73g, 11mmol) in acetic acid (8ml), THF (4ml) and water (2ml) was heated at 50°C for 4 hours. The volatiles were removed by evaporation and the residue azeotroped with toluene to give 4-(2-hydroxyethylthio)pyridine (1.39g, 79%) as an off-white solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.10(t, 2H); 3.60(q, 2H); 5.00(t, 1H); 7.22(d, 2H); 8.30(d, 2H)

Thionyl chloride (0.98ml, 13.5mmol) was added slowly to a solution of 4-(2-hydroxyethylthio)pyridine (1.39g, 9.0mmol) in trichloromethane (25ml) at 5°C. The mixture was stirred at 5°C for 1 hour and then at ambient temperature for 2 hours. The volatiles were removed by evaporation, the residue azeotroped with toluene and dried under vacuum to give 4-(2-chloroethylthio)pyridine hydrochloride (500mg, 26%) as a solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.65(t, 2H); 3.90(t, 2H); 7.90(d, 2H); 8.60(d, 2H)

MS - ESI: 174 $[MH]^+$

Example 69

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (350mg, 1.0mmol), (prepared as described for the starting material in Example 24), 3-(2-chloroethoxy)pyridine hydrochloride (234mg, 1.2mmol) and potassium carbonate (456mg, 3.3mmol) in NMP (20ml) was heated at 90°C for 2 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 the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol mixtures (100/0 increasing to 95/5) to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(3-pyridyloxy)ethoxy)quinazoline** (95mg, 20%).

m.p. 188-190°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.90(s, 3H); 4.45(m, 4H); 7.24(s, 1H); 7.18(dd, 1H); 7.42-7.60(m, 3H); 7.80(s, 1H); 8.20(d, 1H); 8.35(s, 2H); 9.50(s, 1H)

MS - ESI: 441 $[MH]^+$

Elemental analysis:	Found	C 55.0	H 3.9	N 11.8
$C_{22}H_{18}N_4O_3ClF\ 2H_2O$	Requires	C 55.4	H 4.6	N 11.7%

The starting material was prepared as follows:

Sodium hydride (1.02g of a 50% suspension in mineral oil, 42mmol) was added to a solution of 3-hydroxypyridine (2.01g, 21mmol) in DMF (50ml) and the mixture stirred for 30 minutes. 2-(2-Bromoethoxy)tetrahydropyran (4.0g, 19mmol), (J. Am. Chem. Soc. 1948, **70**, 4187), was added and the mixture heated at 100°C for 3 hours and then stirred at ambient

temperature for 18 hours. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined extracts were washed with water, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol mixtures (100/0 increasing to 97/3) to give 3-(2-(tetrahydropyran-2-yloxy)ethoxy)pyridine (2.28g, 48%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.38-1.65(m, 6H); 3.40(m, 1H); 3.65-3.79(m, 2H); 3.85-3.95(m, 1H); 4.20(t, 2H); 4.62(s, 1H); 7.30(dd, 1H); 7.39(dd, 1H); 8.15(d, 1H); 8.28(d, 1H)

MS - ESI: 224 $[MH]^+$

A solution of 3-(2-(tetrahydropyran-2-yloxy)ethoxy)pyridine (1.54g, 7mmol) in acetic acid (8ml), THF (4ml) and water (2ml) was heated at 50°C for 4 hours. The volatiles were removed by evaporation and the residue azeotroped with toluene to give 3-(2-hydroxyethoxy)pyridine (820mg, 86%) as an off-white solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.70(t, 2H); 4.05(t, 2H); 4.85(s, 1H); 7.25(dd, 1H); 7.37(dd, 1H); 8.10(d, 1H); 8.24(d, 1H)

MS - ESI: 140 $[MH]^+$

Thionyl chloride (0.89ml, 12mmol) was added slowly to a solution of 3-(2-hydroxyethoxy)pyridine (1.13g, 8mmol) in trichloromethane (20ml) at 5°C. The mixture was stirred at 5°C for 1 hour and then at ambient temperature for 2 hours. The volatiles were removed by evaporation, the residue azeotroped with toluene and dried under vacuum to give 3-(2-chloroethoxy)pyridine hydrochloride (300mg, 19%) as a solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.99(t, 2H); 4.42(t, 2H); 7.82(dd, 1H); 8.05(dd, 1H); 8.42(d, 1H); 8.62(s, 1H)

Example 70

2-Fluoro-5-hydroxy-4-methylaniline (170mg, 1.2mmol), (prepared as described for the starting material in Example 13), was added to a solution of 7-benzyloxy-4-chloroquinazoline hydrochloride (307mg, 1mmol) in 2-pentanol (5ml) and the mixture stirred at 120°C for 2 hours. The mixture was allowed to cool and the resulting precipitate was collected by filtration, washed with isopropanol and then ether and dried under vacuum at 70°C to give **7-benzyloxy-4-(2-fluoro-5-hydroxy-4-methylanilino)quinazoline** hydrochloride (331mg, 80%).

^{1}H NMR Spectrum: (DMSOd_6) 2.16(s, 3H); 5.36(s, 2H); 6.88(d, 1H); 7.12(d, 1H); 7.3-7.65(m, 7H); 8.68(d, 1H); 8.82(s, 1H); 9.68(s, 1H); 11.4(s, 1H)

MS - ESI: 376 $[MH]^+$

Elemental analysis:	Found	C 63.7	H 4.8	N 10.0
$C_{25}H_{18}N_3O_2F$ 1HCl	Requires	C 64.2	H 4.7	N 10.2%

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) and DMF (100µl) in thionyl chloride (20ml, 0.27mmol) 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 $[MH]^+$

Example 71

Using an analogous procedure to that described in Example 70, 7-benzyloxy-4-chloroquinazoline hydrochloride (307mg, 1mmol), (prepared as described for the starting material in Example 70), was treated with 4-chloro-2-fluoro-5-hydroxyaniline (193mg, 1.2mmol), (EP 061741 A2), to give **7-benzyloxy-4-(4-chloro-2-fluoro-5-hydroxyanilino)quinazoline** hydrochloride (407mg, 94%).

m.p. 253-257°C

^{1}H NMR Spectrum: (DMSOd_6) 5.37(s, 2H); 7.16(d, 1H); 7.32-7.5(m, 4H); 7.54(s, 1H); 7.56(d, 2H); 7.59(dd, 1H); 8.73(d, 1H); 8.86(s, 1H); 10.63(br s, 1H); 11.6(br s, 1H)

MS - ESI: 396 [MH]$^+$

Elemental analysis:	Found	C 57.8	H 3.8 N 9.7
$C_{21}H_{15}N_3O_2ClF$ 0.3H_2O 1HCl	Requires	C 57.6	H 3.8 N 9.6%

Example 72

Using an analogous procedure to that described in Example 36, 4-(2-fluoro-5-methoxycarbonyloxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline hydrochloride (224mg, 0.6mmol), (prepared as described for the starting material in Example 22), was treated with 4-bromomethyl-1,2-difluorobenzene (149mg, 0.72mmol) to give **7-(3,4-difluorobenzyloxy)-4-(2-fluoro-5-hydroxy-4-methylanilino)-6-methoxyquinazoline** hydrochloride (90mg, 31%).

^{1}H NMR Spectrum: (DMSOd_6) 2.17(s, 3H); 4.0(s, 3H); 5.33(s, 2H); 6.88(d, 1H); 7.11(d,1H); 7.38(s, 1H); 7.41(m, 1H); 7.55(m, 1H); 7.62(m, 1H); 8.17(s, 1H); 8.75(s, 1H); 9.68(s, 1H); 11.15(s, 1H)

MS - ESI: 442 [MH]$^+$

Elemental analysis:	Found	C 58.0	H 4.3 N 8.7
$C_{23}H_{18}N_3O_3F_3$ 0.9HCl 0.08 isopropanol	Requires	C 58.3	H 4.1 N 8.8

Example 73

Tetrabutylammonium fluoride (563µl of a 1M solution in THF, 0.62mmol) was added to a solution of 4-(4-chloro-5-diphenyl-t-butylsilyloxy-2-fluoroanilino)-6-methoxy-7-((1-methylimidazol-2-yl)methoxy)quinazoline (207mg, 0.31mmol) in THF (5ml) cooled at