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

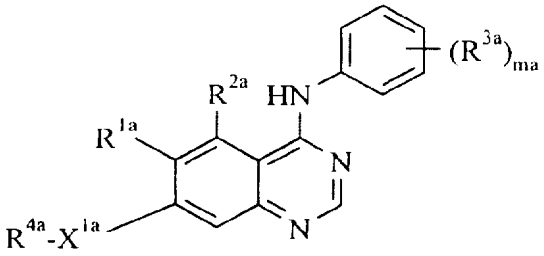
8) C_{2-3}alkylR^{43} (wherein R^{43} is morpholino which may bear one or two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl) with the proviso that when R^4 is C_{2-3}alkylR^{43}, X^1 is -S-, -SO-, $-SO_2-$, $-SO_2NR^9-$ or $-NR^{10}SO_2-$.

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-}

$_2$alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl) with the proviso that when R^{4a} is C_{2-3}alkylR^{32a}, X^{1a} is -S- or -$NR^{6a}SO_2$- (wherein R^{6a} is as defined hereinbefore); and

11) C_{2-3}alkylR^{33a} (wherein R^{33a} is morpholino which bears at least one and optionally two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl);

and an additional value of R^{4a} is C_{2-3}alkylX^{2a}methyl$X^{3a}R^{13a}$ (wherein X^{2a} and X^{3a} are as defined hereinbefore and R^{13a} represents hydrogen or C_{1-3}alkyl);]

and salts thereof.

Preferred compounds of the present invention, by virtue of their substantially equivalent activity against VEGF and EGF receptor tyrosine kinases include:

4-(4-chloro-2-fluoroanilino)-7-(1,3-dioxolan-2-ylmethoxy)-6-methoxyquinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-morpholinobut-2-yn-1-yloxy)quinazoline,

(E)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-morpholinobut-2-en-1-yloxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-7-(3-(2,6-dimethylmorpholino)propoxy)-6-methoxyquinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-([N-methyl-N-methylsulphonyl]amino)propoxy)quinazoline,

7-(2-[N-tert-butoxycarbonylamino]ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline,

4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-([N-methyl-N-methylsulphonyl]amino)propoxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-oxoimidazolidin-1-yl)ethoxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(3-oxomorpholino)ethoxy)quinazoline and

4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(3-oxomorpholino)ethoxy)quinazoline

and salts thereof especially hydrochloride salts thereof.

More preferred compounds of the present invention, by virtue of their substantially equivalent activity against VEGF and EGF receptor tyrosine kinases include:

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-thiomorpholinoethoxy)quinazoline,

(S)-4-(4-bromo-2-fluoroanilino)-7-(3-(2-carbamoylpyrrolidin-1-yl)propoxy)-6-methoxyquinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(2-oxopyrrolidin-1-yl)propoxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-oxopyrrolidin-1-yl)ethoxy)quinazoline,

(S)-7-(3-(2-carbamoylpyrrolidin-1-yl)propoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-morpholinoethoxy)ethoxy)quinazoline and

4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-(2-oxopyrrolidin-1-yl)propoxy)quinazoline

and salts thereof especially hydrochloride salts thereof.

Particularly preferred compounds of the present invention, by virtue of their substantially equivalent activity against VEGF and EGF receptor tyrosine kinases include:

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-2-(2-methoxyethoxy)ethoxyquinazoline and

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-yl)methoxyquinazoline

and salts thereof especially hydrochloride salts thereof.

Additional particularly preferred compounds of the present invention, by virtue of their substantially equivalent activity against VEGF and EGF receptor tyrosine kinases include:

4-(4-bromo-2-fluoroanilino)-7-(3-(1,1-dioxothiomorpholino)propoxy)-6-methoxyquinazoline,

4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(2-methoxyethoxy)ethoxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-pyrrolidin-1-ylethoxy)ethoxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-[4-methylpiperazin-1-yl]ethoxy)ethoxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropylthio)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-([N-methyl-N-methoxyacetyl]amino)ethoxy)quinazoline and

4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(2-oxopyrrolidin-1-yl)ethoxy)quinazoline

and salts thereof especially hydrochloride salts thereof.

Especially preferred compounds of the present invention, by virtue of their substantially equivalent activity against VEGF and EGF receptor tyrosine kinases include:

(E)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-(pyrrolidin-1-yl)but-2-en-1-yloxy)quinazoline,

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(methylsulphonyl)propoxy)quinazoline,

(S)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-yl)methoxyquinazoline

and

(R)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-yl)methoxyquinazoline

and salts thereof especially hydrochloride salts thereof, of which 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(methylsulphonyl)propoxy)quinazoline and salts thereof especially hydrochloride salts thereof, is more especially preferred.

In a particular aspect of the present invention preferred compounds are:

4-(3-acetoxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-7-(2-(1,3-dioxolan-2-yl)ethoxy)-6-methoxyquinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazoline

4-(4-chloro-2-fluoroanilino)-7-(1,3-dioxolan-2-ylmethoxy)-6-methoxyquinazoline

4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-thiomorpholinoethoxy)quinazoline

and salts thereof especially the hydrochloride salts thereof.

For the avoidance of doubt it is to be understood that where in this specification a group is qualified by 'hereinbefore defined' or 'defined hereinbefore' the said group encompasses the first occurring and broadest definition as well as each and all of the preferred definitions for that group.

In this specification unless stated otherwise the term "alkyl" includes both straight and branched chain alkyl groups but references to individual alkyl groups such as "propyl" are specific for the straight chain version only. An analogous convention applies to other generic terms. Unless otherwise stated the term "alkyl" advantageously refers to chains with 1-6 carbon atoms, preferably 1-4 carbon atoms. The term "alkoxy" as used herein, unless stated otherwise includes "alkyl"-O- groups in which "alkyl" is as hereinbefore defined. The term "aryl" as used herein unless stated otherwise includes reference to a C_{6-10} aryl group which may, if desired, carry one or more substituents selected from halogeno, alkyl, alkoxy, nitro, trifluoromethyl and cyano, (wherein alkyl and alkoxy are as hereinbefore defined). The term "aryloxy" as used herein unless otherwise stated includes "aryl"-O-groups in which "aryl" is as hereinbefore defined. The term "sulphonyloxy" as used herein refers to alkylsulphonyloxy and arylsulphonyloxy groups in which "alkyl" and "aryl" are as hereinbefore defined. The term "alkanoyl" as used herein unless otherwise stated includes formyl and alkylC=O groups in which "alkyl" is as defined hereinbefore, for example C_2alkanoyl is ethanoyl and refers to $CH_3C{=}O$, C_1alkanoyl is formyl and refers to CHO. In this specification unless stated otherwise the term "alkenyl" includes both straight and branched chain alkenyl groups but references to

individual alkenyl groups such as 2-butenyl are specific for the straight chain version only. Unless otherwise stated the term "alkenyl" advantageously refers to chains with 2-5 carbon atoms, preferably 3-5 carbon atoms. In this specification unless stated otherwise the term "alkynyl" includes both straight and branched chain alkynyl groups but references to individual alkynyl groups such as 2-butynyl are specific for the straight chain version only. Unless otherwise stated the term "alkynyl" advantageously refers to chains with 2-5 carbon atoms, preferably 3-5 carbon atoms.

In formula I, as hereinbefore defined, hydrogen will be present at positions 2 and 8 of the quinazoline group.

Within the present invention it is to be understood that a compound of the formula I or a salt thereof may exhibit the phenomenon of tautomerism and that the formulae drawings within this specification can represent only one of the possible tautomeric forms. It is to be understood that the invention encompasses any tautomeric form which inhibits VEGF receptor tyrosine kinase activity and is not to be limited merely to any one tautomeric form utilised within the formulae drawings.

It is also to be understood that certain compounds of the formula I and salts thereof can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms which inhibit VEGF receptor tyrosine kinase activity.

For the avoidance of any doubt, it is to be understood that when X^1 is, for example, a group of formula $-NR^7CO-$, it is the nitrogen atom bearing the R^7 group which is attached to the quinazoline ring and the carbonyl (CO) group is attached to R^4, whereas when X^1 is, for example, a group of formula $-CONR^8-$, it is the carbonyl group which is attached to the quinazoline ring and the nitrogen atom bearing the R^8 group is attached to R^4. A similar convention applies to the other two atom X^1 linking groups such as $-NR^{10}SO_2-$ and $-SO_2NR^9-$. When X^1 is $-NR^{11}-$ it is the nitrogen atom bearing the R^{11} group which is linked to the quinazoline ring and to R^4. An analogous convention applies to other groups, thus when R^4 is, for example, a group of the formula $C_{1-5}alkylX^5R^{27}$ and X^5 is a group $-NR^{28}CO-$, it is the nitrogen atom bearing the R^{28} group which is attached to the alkyl chain which is attached to the quinazoline ring and the carbonyl (CO) group is attached to R^{27}, whereas when X^5 is, for example, a group of formula $-CONR^{29}-$, it is the carbonyl group which is attached to the alkyl

chain which is attached to the quinazoline ring and the nitrogen atom bearing the R^{29} group is attached to R^{27}. It is further to be understood that when X^1 represents $-NR^{11}-$ and R^{11} is C_{1-3}alkoxyC_{2-3}alkyl it is the C_{2-3}alkyl moiety which is linked to the nitrogen atom of X^1 and an analogous convention applies to other groups.

For the avoidance of any doubt, it is to be understood that in a compound of the formula I when R^4 is, for example, a group of formula C_{1-5}alkylX^2C_{1-5}alkylX^3R^{16}, it is the terminal C_{1-5}alkyl moiety which is bound to X^1, similarly when R^4 is, for example, a group of formula C_{2-5}alkenylR^{14} it is the C_{2-5}alkenyl moiety which is bound to X^1 and an analgous convention applies to other groups. When R^4 is a group 1-R^{33}prop-1-en-3-yl it is the first carbon to which the group R^{33} is attached and it is the third carbon which is linked to X^1, similarly when R^4 is a group 2-R^{33}pent-3-en-5-yl it is the second carbon to which the group R^{33} is attached and it is the fifth carbon which is linked to X^1, and an analogous convention applies to other groups.

The present invention relates to the compounds of formula I as hereinbefore defined as well as to the salts thereof. Salts for use in pharmaceutical compositions will be pharmaceutically acceptable salts, but other salts may be useful in the production of the compounds of formula I and their pharmaceutically acceptable salts. Pharmaceutically acceptable salts of the invention may, for example, include acid addition salts of the compounds of formula I as hereinbefore defined which are sufficiently basic to form such salts. Such acid addition salts include for example salts with inorganic or organic acids affording pharmaceutically acceptable anions such as with hydrogen halides (especially hydrochloric or hydrobromic acid of which hydrochloric acid is particularly preferred) or with sulphuric or phosphoric acid, or with trifluoroacetic, citric or maleic acid. In addition where the compounds of formula I are sufficiently acidic, pharmaceutically acceptable salts may be formed with an inorganic or organic base which affords a pharmaceutically acceptable cation. Such salts with inorganic or organic bases include for example an alkali metal salt, such as a sodium or potassium salt, an alkaline earth metal salt such as a calcium or magnesium salt, an ammonium salt or for example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

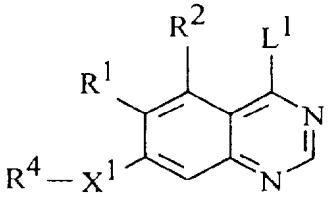
A compound of the formula I, or salt thereof, and other compounds of the invention (as hereinafter defined) may be prepared by any process known to be applicable to the

preparation of chemically-related compounds. Such processes include, for example, those illustrated in European Patent Applications, Publication Nos. 0520722, 0566226, 0602851 and 0635498. Such processes, are provided as a further feature of the invention and are as described hereinafter. Necessary starting materials may be obtained by standard procedures of organic chemistry. The preparation of such starting materials is described within the accompanying non-limiting Examples. Alternatively necessary starting materials are obtainable by analogous procedures to those illustrated which are within the ordinary skill of an organic chemist.

Thus the following processes (a) to (g) and (i) to (v) constitute further features of the present invention.

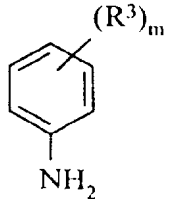
Synthesis of Compounds of Formula I

(a) Compounds of the formula I and salts thereof may be prepared by the reaction of a compound of the formula III:



(III)

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



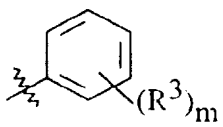
(IV)

(wherein R^3 and m are as defined hereinbefore) whereby to obtain compounds of the formula I and salts thereof. A convenient displaceable moiety L^1 is, for example, a halogeno, alkoxy (preferably C_{1-4}alkoxy), aryloxy or sulphonyloxy group, for example a chloro, bromo, methoxy, phenoxy, methanesulphonyloxy or toluene-4-sulphonyloxy group.

The reaction is advantageously effected in the presence of either an acid or a base. Such an acid is, for example, an anhydrous inorganic acid such as hydrogen chloride. Such a base is, for example, an organic amine base such as, for example, pyridine, 2,6-lutidine, collidine, 4-dimethylaminopyridine, triethylamine, morpholine, N-methylmorpholine or diazabicyclo[5.4.0]undec-7-ene, or for example, an alkali metal or alkaline earth metal carbonate or hydroxide, for example sodium carbonate, potassium carbonate, calcium carbonate, sodium hydroxide or potassium hydroxide. Alternatively such a base is, for example, an alkali metal hydride, for example sodium hydride, or an alkali metal or alkaline earth metal amide, for example sodium amide or sodium bis(trimethylsilyl)amide. The reaction is preferably effected in the presence of an inert solvent or diluent, for example an alkanol or ester such as methanol, ethanol, 2-propanol or ethyl acetate, a halogenated solvent such as methylene chloride, trichloromethane or carbon tetrachloride, an ether such as tetrahydrofuran or 1,4-dioxan, an aromatic hydrocarbon solvent such as toluene, or a dipolar aprotic solvent such as N,N-dimethylformamide, N,N-dimethylacetamide, N-methylpyrrolidin-2-one or dimethylsulphoxide. The reaction is conveniently effected at a temperature in the range, for example, 10 to 150°C, preferably in the range 20 to 80°C.

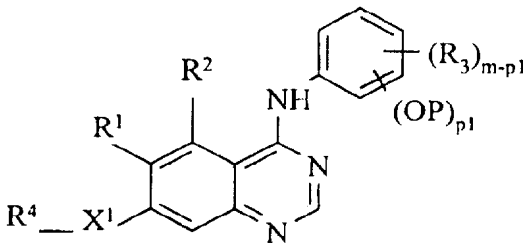
The compound of the invention may be obtained from this process in the form of the free base or alternatively it may be obtained in the form of a salt with the acid of the formula H-L^1 wherein L^1 has the meaning defined hereinbefore. When it is desired to obtain the free base from the salt, the salt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

(b) Where the group of formula IIa:



(IIa)

(wherein R^3 and m are as hereinbefore defined) represents a phenyl group carrying one or more hydroxy groups, a compound of the formula I and salts thereof can be prepared by the deprotection of a compound of formula V:

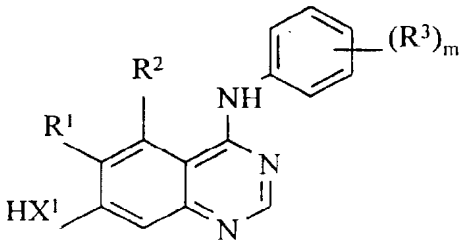


(V)

(wherein X^1, m, R^1, R^2, R^3 and R^4 are as hereinbefore defined, P represents a phenolic hydroxy protecting group and p1 is an integer from 1 to 5 equal to the number of protected hydroxy groups and such that m-p1 is equal to the number of R^3 substituents which are not protected hydroxy). The choice of phenolic hydroxy protecting group P is within the standard knowledge of an organic chemist, for example those included in standard texts such as "Protective Groups in Organic Synthesis" T.W. Greene and R.G.M.Wuts, 2nd Ed. Wiley 1991, including ethers (for example, methyl, methoxymethyl, allyl and benzyl and benzyl substituted with up to two substituents selected from C_{1-4}alkoxy and nitro), silyl ethers (for example, t-butyldiphenylsilyl and t-butyldimethylsilyl), esters (for example, acetate and benzoate) and carbonates (for example, methyl and benzyl and benzyl substituted with up to two substituents selected from C_{1-4}alkoxy and nitro). The removal of such a phenolic hydroxy protecting group may be effected by any of the procedures known for such a transformation, including those reaction conditions indicated in standard texts such as that indicated hereinbefore, or by a related procedure. The reaction conditions preferably being such that the hydroxy derivative is produced without unwanted reactions at other sites within the starting or product compounds. For example, where the protecting group P is acetate, the transformation may conveniently be effected by treatment of the quinazoline derivative with a base as defined hereinbefore and including ammonia, and its mono and di-alkylated derivatives, preferably in the presence of a protic solvent or co-solvent such as water or an alcohol, for example methanol or ethanol. Such

a reaction can be effected in the presence of an additional inert solvent or diluent as defined hereinbefore and at a temperature in the range 0 to 50°C, conveniently at about 20°C.

(c) Production of those compounds of formula I and salts thereof wherein the substituent X^1 is -O-, -S- or $-NR^{11}$- or $-SO_2$-, $-CONR^8$- or $-SO_2NR^9$- can be achieved by the reaction, conveniently in the presence of a base as defined hereinbefore, of a compound of the formula VI:



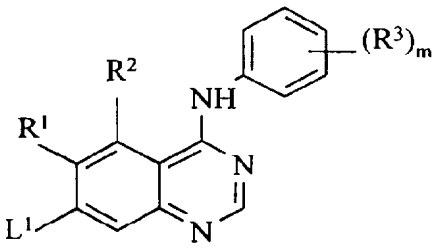
(VI)

(wherein m, X^1, R^1, R^2 and R^3 are as hereinbefore defined) with a compound of formula VII:

$$R^4\text{-}L^1 \qquad \text{(VII)}$$

(wherein R^4 and L^1 are as hereinbefore defined); L^1 is a displaceable moiety for example a halogeno or sulphonyloxy group such as a bromo or methanesulphonyloxy group. Conveniently L^1 is a group $O\text{-}^+P(R^{52})_3$ (wherein R^{52} is butyl or phenyl) and in such cases the compound of formula VII is conveniently formed in situ. The reaction is preferably effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), advantageously at a temperature in the range, for example 10 to 150°C, conveniently at about 50°C.

(d) Compounds of the formula I and salts thereof may be prepared by the reaction of a compound of the formula VIII:



(VIII)

with a compound of the formula IX:

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

(wherein L^1, R^1, R^2, R^3, R^4, m and X^1 are all as hereinbefore defined). The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), advantageously at a temperature in the range, for example 10 to 150°C, conveniently at about 100°C.

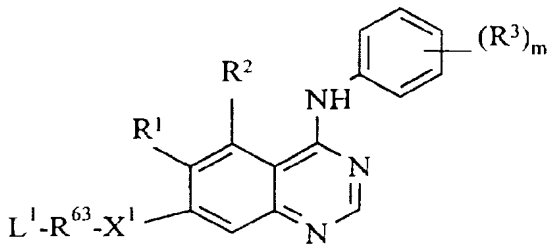
(e) Compounds of the formula I and salts thereof wherein R^4 is $C_{1-5}alkylR^{53}$, [wherein R^{53} is selected from one of the following three groups:

1) X^7R^{27} (wherein X^7 represents -O-, -S-, $-SO_2-$, $-NR^{54}CO-$, $-NR^{55}SO_2-$ or $-NR^{56}-$ (wherein R^{54}, R^{55} and R^{56} each independently represents hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$) and R^{27} is as defined hereinbefore); and

2) $X^8C_{1-5}alkylX^3R^{16}$ (wherein X^8 represents -O-, -S-, $-SO_2-$, $-NR^{57}CO-$, $-NR^{58}SO_2-$ or $-NR^{59}-$ (wherein R^{57}, R^{58} and R^{59} each independently represents hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$) and X^3 and R^{16} are as defined hereinbefore); and

3) $X^9C_{1-5}alkylR^{33}$ (wherein X^9 represents -O-, -S-, $-SO_2-$, $-NR^{60}CO-$, $-NR^{61}SO_2-$ or $-NR^{62}-$ (wherein R^{60}, R^{61} and R^{62} each independently represents hydrogen, $C_{1-3}alkyl$ or $C_{1-3}alkoxyC_{2-3}alkyl$) and R^{33} is as defined hereinbefore);]

may be prepared by reacting a compound of the formula X:



(X)

(wherein L^1, X^1, R^1, R^2, R^3 and m are as hereinbefore defined and R^{63} is C_{1-5}alkyl) with a compound of the formula XI:

$$R^{53}\text{-}H \qquad \text{(XI)}$$

(wherein R^{53} is as defined hereinbefore) to give a compound of the formula I. The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), and at a temperature in the range, for example 0 to 150°C, conveniently at about 50°C.

Compounds of the formula I wherein R^4 is C_{2-5}alkylR^{45}, (wherein R^{45} is as defined hereinbefore), may be prepared by reacting a compound of formula X (wherein R^{63} is C_{2-5}alkyl) with a compound of the formula XIa:

$$R^{45}\text{-}H \qquad \text{(XIa)}$$

(wherein R^{45} is as defined hereinbefore) to give a compound of the formula I. The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), and at a temperature in the range, for example 0 to 150°C, conveniently at about 50°C.

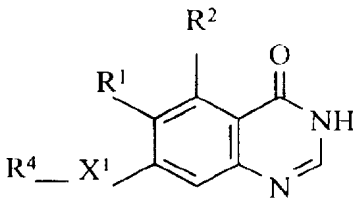
(f) The production of those compounds of the formula I and salts thereof wherein the substituent R^1 is represented by $-NR^5R^6$, where one or both of R^5 and R^6 are C_{1-3}alkyl, may be effected by the reaction of compounds of formula I wherein the substituent R^1 is an amino group and an alkylating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

Such alkylating agents are C_{1-3}alkyl moieties bearing a displaceable moiety as defined hereinbefore such as C_{1-3}alkyl halides for example C_{1-3}alkyl chloride, bromide or iodide. The reaction is preferably effected in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)) and at a temperature in the range, for example, 10 to 100°C, conveniently at about ambient temperature.

(g) The production of compounds of formula I and salts thereof wherein one or more of the substituents R^1, R^2 or R^3 is an amino group may be effected by the reduction of a corresponding compound of formula I wherein the substituent(s) at the corresponding position(s) of the quinazoline and/or aniline ring is/are a nitro group(s). The reduction may conveniently be effected as described in process (i) hereinafter. The production of a compound of formula I and salts thereof wherein the substituent(s) at the corresponding position(s) of the quinazoline and/or aniline ring is/are a nitro group(s) may be effected by the processes described hereinbefore and hereinafter in processes (a-e) and (i-v) using a quinazoline compound selected from the compounds of the formulae (I-XXVII) in which the substituent(s) at the corresponding position(s) of the quinazoline and/or aniline ring is/are a nitro group(s).

Synthesis of Intermediates

(i) The compounds of formula III and salts thereof, constitute a further feature of the present invention. Such compounds in which L^1 is halogeno may for example be prepared by halogenating a compound of the formula XII:



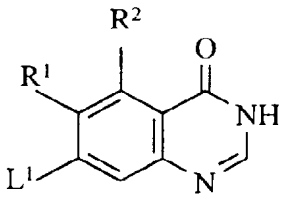
(XII)

(wherein R^1, R^2, R^4 and X^1 are as hereinbefore defined).

Convenient halogenating agents include inorganic acid halides, for example thionyl chloride, phosphorus(III)chloride, phosphorus(V)oxychloride and phosphorus(V)chloride. The halogenation reaction is conveniently effected in the presence of an inert solvent or diluent such

as for example a halogenated solvent such as methylene chloride, trichloromethane or carbon tetrachloride, or an aromatic hydrocarbon solvent such as benzene or toluene. The reaction is conveniently effected at a temperature in the range, for example 10 to 150°C, preferably in the range 40 to 100°C.

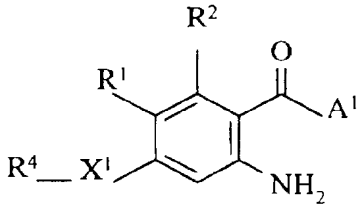
The compounds of formula XII and salts thereof which constitute a further feature of the present invention may for example be prepared by reacting a compound of the formula XIII:



(XIII)

(wherein R^1, R^2 and L^1 are as hereinbefore defined) with a compound of the formula IX as hereinbefore defined. The reaction may conveniently be effected in the presence of a base (as defined hereinbefore in process (a)) and advantageously in the presence of an inert solvent or diluent (as defined hereinbefore in process (a)), advantageously at a temperature in the range, for example 10 to 150°C, conveniently at about 100°C.

The compounds of formula XII and salts thereof may also be prepared by cyclising a compound of the formula XIV:

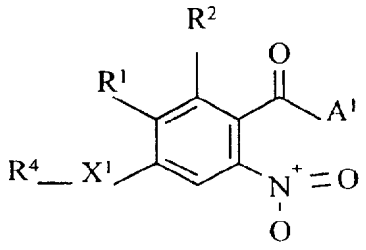


(XIV)

(wherein R^1, R^2, R^4 and X^1, are as hereinbefore defined, and A^1 is an hydroxy, alkoxy (preferably C_{1-4}alkoxy) or amino group) whereby to form a compound of formula XII or salt thereof. The cyclisation may be effected by reacting a compound of the formula XIV, where A^1

is an hydroxy or alkoxy group, with formamide or an equivalent thereof effective to cause cyclisation whereby a compound of formula XII or salt thereof is obtained, such as [3-(dimethylamino)-2-azaprop-2-enylidene]dimethylammonium chloride. The cyclisation is conveniently effected in the presence of formamide as solvent or in the presence of an inert solvent or diluent such as an ether for example 1,4-dioxan. The cyclisation is conveniently effected at an elevated temperature, preferably in the range 80 to 200°C. The compounds of formula XII may also be prepared by cyclising a compound of the formula XIV, where A^1 is an amino group, with formic acid or an equivalent thereof effective to cause cyclisation whereby a compound of formula XII or salt thereof is obtained. Equivalents of formic acid effective to cause cyclisation include for example a tri-C_{1-4}alkoxymethane, for example triethoxymethane and trimethoxymethane. The cyclisation is conveniently effected in the presence of a catalytic amount of an anhydrous acid, such as a sulphonic acid for example p-toluenesulphonic acid, and in the presence of an inert solvent or diluent such as for example a halogenated solvent such as methylene chloride, trichloromethane or carbon tetrachloride, an ether such as diethylether or tetrahydrofuran, or an aromatic hydrocarbon solvent such as toluene. The cyclisation is conveniently effected at a temperature in the range, for example 10 to 100°C, preferably in the range 20 to 50°C.

Compounds of formula XIV and salts thereof, which constitute a further feature of the present invention, may for example be prepared by the reduction of the nitro group in a compound of the formula XV:

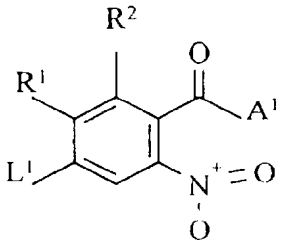


(XV)

(wherein R^1, R^2, R^4, X^1 and A^1 are as hereinbefore defined) to yield a compound of formula XIV as hereinbefore defined. The reduction of the nitro group may conveniently be effected by

any of the procedures known for such a transformation. The reduction may be carried out, for example, by the hydrogenation of a solution of the nitro compound in the presence of an inert solvent or diluent as defined hereinbefore in the presence of a metal effective to catalyse hydrogenation reactions such as palladium or platinum. A further reducing agent is, for example, an activated metal such as activated iron (produced for example by washing iron powder with a dilute solution of an acid such as hydrochloric acid). Thus, for example, the reduction may be effected by heating the nitro compound and the activated metal in the presence of a solvent or diluent such as a mixture of water and alcohol, for example methanol or ethanol, to a temperature in the range, for example 50 to 150°C, conveniently at about 70°C.

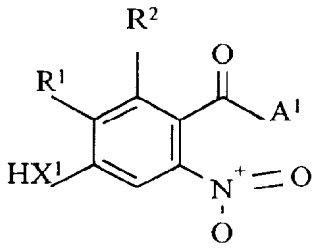
Compounds of the formula XV and salts thereof which constitute a further feature of the present invention, may for example be prepared by the reaction of a compound of the formula XVI:



(XVI)

(wherein R^1, R^2, L^1 and A^1 are as hereinbefore defined) with a compound of the formula IX as hereinbefore defined to give a compound of the formual XV. The reaction of the compounds of formulae XVI and IX is conveniently effected under conditions as described for process (d) hereinbefore.

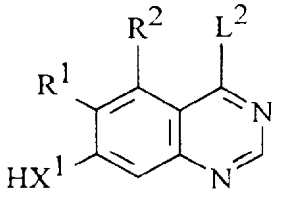
Compounds of formula XV and salts thereof, may for example also be prepared by the reaction of a compound of the formula XVII:



(XVII)

(wherein R^1, R^2, X^1 and A^1 are as hereinbefore defined with the proviso that X^1 is not $-CH_2-$) with a compound of the formula VII as hereinbefore defined to yield a compound of formula XV as hereinbefore defined. The reaction of the compounds of formulae XVII and VII is conveniently effected under conditions as described for process (c) hereinbefore.

The compounds of formula III and salts thereof may also be prepared for example by reacting a compound of the formula XVIII:

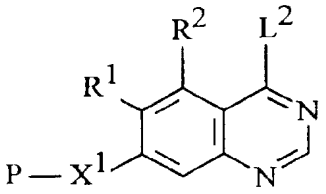


(XVIII)

(wherein R^1, R^2 and X^1 are as hereinbefore defined with the proviso that X^1 is not $-CH_2-$ and L^2 represents a displaceable protecting moiety) with a compound of the formula VII as hereinbefore defined, whereby to obtain a compound of formula III in which L^1 is represented by L^2.

A compound of formula XVIII is conveniently used in which L^2 represents a phenoxy group which may if desired carry up to 5 substituents, preferably up to 2 substituents, selected from halogeno, nitro and cyano. The reaction may be conveniently effected under conditions as described for process (c) hereinbefore.

The compounds of formula XVIII and salts thereof as hereinbefore defined may for example be prepared by deprotecting a compound of the formula XIX:

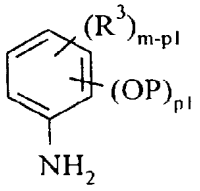


(XIX)

(wherein R^1, R^2, P, X^1 and L^2 are as hereinbefore defined with the proviso that X^1 is not $-CH_2-$). Deprotection may be effected by techniques well known in the literature, for example where P represents a benzyl group deprotection may be effected by hydrogenolysis or by treatment with trifluoroacetic acid.

One compound of formula III may if desired be converted into another compound of formula III in which the moiety L^1 is different. Thus for example a compound of formula III in which L^1 is other than halogeno, for example optionally substituted phenoxy, may be converted to a compound of formula III in which L^1 is halogeno by hydrolysis of a compound of formula III (in which L^1 is other than halogeno) to yield a compound of formula XII as hereinbefore defined, followed by introduction of halide to the compound of formula XII, thus obtained as hereinbefore defined, to yield a compound of formula III in which L^1 represents halogen.

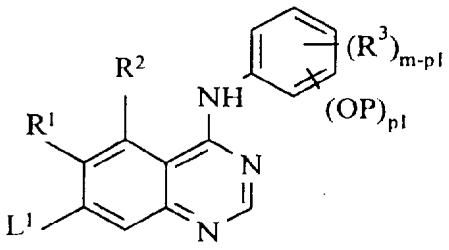
(ii) The compounds of formula V and salts thereof, constitute a further feature of the present invention, and may for example be prepared by the reaction of a compound of formula III as hereinbefore defined with a compound of the formula XX:



(XX)

(wherein R^3, m, p1 and P are as hereinbefore defined). The reaction may for example be effected as described for process (a) hereinbefore.

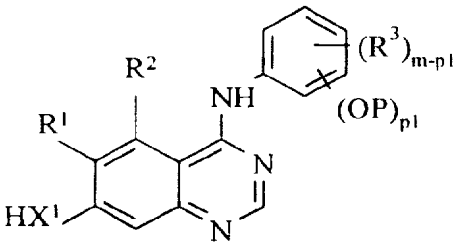
The compounds of formula V and salts thereof may also be prepared by reacting a compound of formula XXI:



(XXI)

(wherein R^1, R^2, L^1, R^3, m, p1 and P are as hereinbefore defined) with a compound of formula IX as hereinbefore defined. The reaction may for example be effected as described for process (d) above.

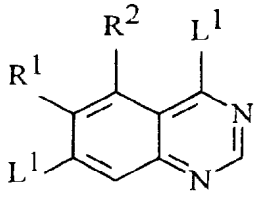
The compounds of formula V and salts thereof may also be prepared by reacting a compound of formula XXII:



(XXII)

(wherein R^1, R^2, R^3, X^1, P, p1 and m are as hereinbefore defined with the proviso that X^1 is not -CH_2-) with a compound of the formula VII as hereinbefore defined. The reaction may for example be effected as described for process (c) hereinbefore.

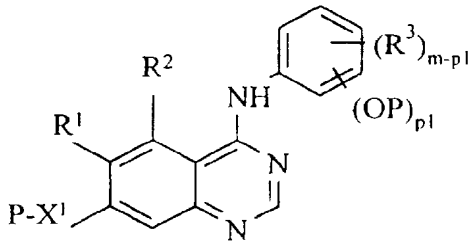
The compounds of formula XXI and salts thereof may for example be prepared by reaction of a compound of formula XXIII:



(XXIII)

(wherein R^1, R^2, and L^1 are as hereinbefore defined, and L^1 in the 4- and 7- positions may be the same or different) with a compound of the formula XX as hereinbefore defined. The reaction may be effected for example by a process as described in (a) above.

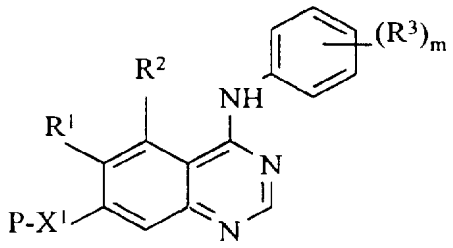
Compounds of the formula XXII and salts thereof may be made by reacting compounds of the formulae XIX and XX as hereinbefore defined, under conditions described in (a) hereinbefore, to give a compound of formula XXIV:



(XXIV)

(wherein R^1, R^2, R^3, P, X^1, p1 and m are as hereinbefore defined with the proviso that X^1 is not -CH_2-) and then deprotecting the compound of formula XXIV for example as described in (i) above.

(iii) Compounds of the formula VI as hereinbefore defined and salts thereof may be made by deprotecting the compound of formula XXV:



(XXV)

(wherein R^1, R^2, R^3, P, X^1 and m are as hereinbefore defined) by a process for example as described in (i) above.

Compounds of the formula XXV and salts thereof may be made by reacting compounds of the formulae XIX and IV as hereinbefore defined, under the conditions described in (a) hereinbefore, to give a compound of the formula XXV or salt thereof.

(iv) Compounds of the formula VIII and salts thereof as hereinbefore defined may be made by reacting compounds of the formulae XXIII and IV as hereinbefore defined, the reaction for example being effected by a process as described in (a) above.

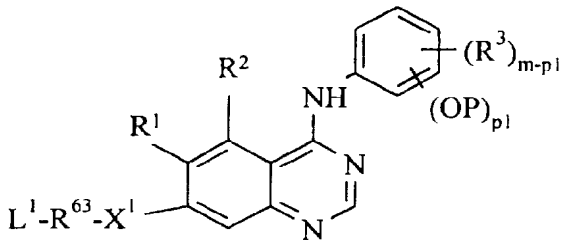
(v) Compounds of the formula X as defined hereinbefore and salts thereof may for example be made by the reaction of a compound of formula VI as defined hereinbefore with a compound of the formula XXVI:

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

(XXVI)

(wherein L^1 and R^{63} are as hereinbefore defined) to give a compound of the formula X. The reaction may be effected for example by a process as described in (c) above.

Compounds of the formula X and salts thereof may also be made for example by deprotecting a compound of the formula XXVII:



(XXVII)

(wherein L^1, R^{63}, X^1, R^1, R^2, R^3, P, m and p1 are as defined hereinbefore) by a process for example as described in (b) above.

Compounds of the formula XXVII and salts thereof may be made for example by reacting compounds of the formulae XXII and XXVI as defined hereinbefore, under the conditions described in (c) above.

When a pharmaceutically acceptable salt of a compound of the formula I is required, it may be obtained, for example, by reaction of said compound with, for example, an acid using a conventional procedure, the acid having a pharmaceutically acceptable anion.

Many of the intermediates defined herein are novel, for example, those of the formulae III, V, XII, XIV and XV, and these are provided as a further feature of the invention.

Intermediates of the formulae VIII, X, XXI, XXII, XXIV, XXV and XXVII are also provided as a further feature of the invention.

The identification of compounds which potently inhibit the tyrosine kinase activity associated with the VEGF receptors such as Flt and/or KDR and which inhibit angiogenesis and/or increased vascular permeability is desirable and is the subject of the present invention. These properties may be assessed, for example, using one or more of the procedures set out below:

(a) In Vitro Receptor Tyrosine Kinase Inhibition Test

This assay determines the ability of a test compound to inhibit tyrosine kinase activity. DNA encoding VEGF or epidermal growth factor (EGF) receptor cytoplasmic domains may be obtained by total gene synthesis (Edwards M, International Biotechnology Lab 5(3), 19-25, 1987) or by cloning. These may then be expressed in a suitable expression system to obtain polypeptide with tyrosine kinase activity. For example VEGF and EGF

receptor cytoplasmic domains, which were obtained by expression of recombinant protein in insect cells, were found to display intrinsic tyrosine kinase activity. In the case of the VEGF receptor Flt (Genbank accession number X51602), a 1.7kb DNA fragment encoding most of the cytoplasmic domain, commencing with methionine 783 and including the termination codon, described by Shibuya et al (Oncogene, 1990, 5: 519-524), was isolated from cDNA and cloned into a baculovirus transplacement vector (for example pAcYM1 (see The Baculovirus Expression System: A Laboratory Guide, L.A. King and R. D. Possee, Chapman and Hall, 1992) or pAc360 or pBlueBacHis (available from Invitrogen Corporation)). This recombinant construct was co-transfected into insect cells (for example Spodoptera frugiperda 21(Sf21)) with viral DNA (eg Pharmingen BaculoGold) to prepare recombinant baculovirus. (Details of the methods for the assembly of recombinant DNA molecules and the preparation and use of recombinant baculovirus can be found in standard texts for example Sambrook et al, 1989, Molecular cloning - A Laboratory Manual, 2nd edition, Cold Spring Harbour Laboratory Press and O'Reilly et al, 1992, Baculovirus Expression Vectors - A Laboratory Manual, W. H. Freeman and Co, New York). For other tyrosine kinases for use in assays, cytoplasmic fragments starting from methionine 806 (KDR, Genbank accession number L04947) and methionine 668 (EGF receptor, Genbank accession number X00588) may be cloned and expressed in a similar manner.

For expression of cFlt tyrosine kinase activity, Sf21 cells were infected with plaque-pure cFlt recombinant virus at a multiplicity of infection of 3 and harvested 48 hours later. Harvested cells were washed with ice cold phosphate buffered saline solution (PBS) (10mM sodium phosphate pH7.4, 138mM sodium chloride, 2.7mM potassium chloride) then resuspended in ice cold HNTG/PMSF (20mM Hepes pH7.5, 150mM sodium chloride, 10% v/v glycerol, 1% v/v Triton X100, 1.5mM magnesium chloride, 1mM ethylene glycol-bis(βaminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), 1mM PMSF (phenylmethylsulphonyl fluoride); the PMSF is added just before use from a freshly-prepared 100mM solution in methanol) using 1ml HNTG/PMSF per 10 million cells. The suspension was centrifuged for 10 minutes at 13,000 rpm at 4°C, the supernatant (enzyme stock) was removed and stored in aliquots at -70°C. Each new batch of stock enzyme was titrated in the assay by dilution with enzyme diluent (100mM Hepes pH 7.4, 0.2mM sodium orthovanadate,

0.1% v/v Triton X100, 0.2mM dithiothreitol). For a typical batch, stock enzyme is diluted 1 in 2000 with enzyme diluent and 50µl of dilute enzyme is used for each assay well.

A stock of substrate solution was prepared from a random copolymer containing tyrosine, for example Poly (Glu, Ala, Tyr) 6:3:1 (Sigma P3899), stored as 1 mg/ml stock in PBS at -20°C and diluted 1 in 500 with PBS for plate coating.

On the day before the assay 100µl of diluted substrate solution was dispensed into all wells of assay plates (Nunc maxisorp 96-well immunoplates) which were sealed and left overnight at 4°C.

On the day of the assay the substrate solution was discarded and the assay plate wells were washed once with PBST (PBS containing 0.05% v/v Tween 20) and once with 50mM Hepes pH7.4.

Test compounds were diluted with 10% dimethylsulphoxide (DMSO) and 25µl of diluted compound was transferred to wells in the washed assay plates. "Total" control wells contained 10% DMSO instead of compound. Twenty five microlitres of 40mM manganese(II)chloride containing 8µM adenosine-5'-triphosphate (ATP) was added to all test wells except "blank" control wells which contained manganese(II)chloride without ATP. To start the reactions 50µl of freshly diluted enzyme was added to each well and the plates were incubated at room temperature for 20 minutes. The liquid was then discarded and the wells were washed twice with PBST. One hundred microlitres of mouse IgG anti-phosphotyrosine antibody (Upstate Biotechnology Inc. product 05-321), diluted 1 in 6000 with PBST containing 0.5% w/v bovine serum albumin (BSA), was added to each well and the plates were incubated for 1 hour at room temperature before discarding the liquid and washing the wells twice with PBST. One hundred microlitres of horse radish peroxidase (HRP)-linked sheep anti-mouse Ig antibody (Amersham product NXA 931), diluted 1 in 500 with PBST containing 0.5% w/v BSA, was added and the plates were incubated for 1 hour at room temperature before discarding the liquid and washing the wells twice with PBST. One hundred microlitres of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) solution, freshly prepared using one 50mg ABTS tablet (Boehringer 1204 521) in 50ml freshly prepared 50mM phosphate-citrate buffer pH5.0 + 0.03% sodium perborate (made with 1 phosphate citrate buffer with sodium perborate (PCSB) capsule (Sigma P4922) per 100ml distilled water), was added to each well. Plates were then incubated for 20-60 minutes at

room temperature until the optical density value of the "total" control wells, measured at 405nm using a plate reading spectrophotometer, was approximately 1.0. "Blank" (no ATP) and "total" (no compound) control values were used to determine the dilution range of test compound which gave 50% inhibtion of enzyme activity.

(b) In Vitro HUVEC Proliferation Assay

This assay determines the ability of a test compound to inhibit the growth factor-stimulated proliferation of human umbilical vein endothelial cells (HUVEC).

HUVEC cells were isolated in MCDB 131 (Gibco BRL) + 7.5% v/v foetal calf serum (FCS) and were plated out (at passage 2 to 8), in MCDB 131 + 2% v/v FCS + 3μg/ml heparin + 1μg/ml hydrocortisone, at a concentration of 1000 cells/well in 96 well plates. After a minimum of 4 hours they were dosed with the appropriate growth factor (i.e. VEGF 3ng/ml, EGF 3ng/ml or b-FGF 0.3ng/ml) and compound. The cultures were then incubated for 4 days at 37°C with 7.5% carbon dioxide. On day 4 the cultures were pulsed with 1μCi/well of tritiated-thymidine (Amersham product TRA 61) and incubated for 4 hours. The cells were harvested using a 96-well plate harvester (Tomtek) and then assayed for incorporation of tritium with a Beta plate counter. Incorporation of radioactivity into cells, expressed as cpm, was used to measure inhibition of growth factor-stimulated cell proliferation by compounds.

(c) In Vivo Rat Uterine Oedema Assay

This test measures the capacity of compounds to reduce the acute increase in uterine weight in rats which occurs in the first 4-6 hours following oestrogen stimulation. This early increase in uterine weight has long been known to be due to oedema caused by increased permeability of the uterine vasculature and recently Cullinan-Bove and Koos (Endocrinology, 1993,133:829-837) demonstrated a close temporal relationship with increased expression of VEGF mRNA in the uterus. We have found that prior treatment of the rats with a neutralising monoclonal antibody to VEGF significantly reduces the acute increase in uterine weight, confirming that the increase in weight is substantially mediated by VEGF.

Groups of 20 to 22-day old rats were treated with a single subcutaneous dose of oestradiol benzoate (2.5μg/rat) in a solvent, or solvent only. The latter served as unstimulated controls. Test compounds were orally administered at various times prior to the administration of oestradiol benzoate. Five hours after the administration of oestradiol benzoate the rats were humanely sacrificed and their uteri were dissected, blotted and weighed. The increase in

uterine weight in groups treated with test compound and oestradiol benzoate and with oestradiol benzoate alone was compared using a Student T test. Inhibition of the effect of oestradiol benzoate was considered significant when $p<0.05$.

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises a compound of the formula I as defined hereinbefore or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable excipient or carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) for example as a sterile solution, suspension or emulsion, for topical administration for example as an ointment or cream or for rectal administration for example as a suppository. In general the above compositions may be prepared in a conventional manner using conventional excipients.

The compositions of the present invention are advantageously presented in unit dosage form. The compound will normally be administered to a warm-blooded animal at a unit dose within the range 5-5000mg per square metre body area of the animal, i.e. approximately 0.1-100mg/kg. A unit dose in the range, for example, 1-100mg/kg, preferably 1-50mg/kg is envisaged and this normally provides a therapeutically-effective dose. A unit dose form such as a tablet or capsule will usually contain, for example 1-250mg of active ingredient.

According to a further aspect of the present invention there is provided a compound of the formula I or a pharmaceutically acceptable salt thereof as defined hereinbefore for use in a method of treatment of the human or animal body by therapy.

We have found that compounds of the present invention inhibit VEGF receptor tyrosine kinase activity and are therefore of interest for their antiangiogenic effects and/or their ability to cause a reduction in vascular permeability.

A further feature of the present invention is a compound of formula I, or a pharmaceutically acceptable salt thereof, for use as a medicament, conveniently a compound of formula I, or a pharmaceutically acceptable salt thereof, for use as a medicament for producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal such as a human being.

Thus according to a further aspect of the invention there is provided the use of a compound of the formula I, or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for use in the production of an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal such as a human being.

According to a further feature of the invention there is provided a method for producing an antiangiogenic and/or vascular permeability reducing effect in a warm-blooded animal, such as a human being, in need of such treatment which comprises administering to said animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof as defined hereinbefore.

As stated above the size of the dose required for the therapeutic or prophylactic treatment of a particular disease state will necessarily be varied depending on the host treated, the route of administration and the severity of the illness being treated. Preferably a daily dose in the range of 1-50mg/kg is employed. However the daily dose will necessarily be varied depending upon the host treated, the particular route of administration, and the severity of the illness being treated. Accordingly the optimum dosage may be determined by the practitioner who is treating any particular patient.

The antiangiogenic and/or vascular permeability reducing treatment defined hereinbefore may be applied as a sole therapy or may involve, in addition to a compound of the invention, one or more other substances and/or treatments. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate administration of the individual components of the treatment. In the field of medical oncology it is normal practice to use a combination of different forms of treatment to treat each patient with cancer. In medical oncology the other component(s) of such conjoint treatment in addition to the antiangiogenic and/or vascular permeability reducing treatment defined hereinbefore may be: surgery, radiotherapy or chemotherapy. Such chemotherapy may cover three main categories of therapeutic agent:

(i) other antiangiogenic agents that work by different mechanisms from those defined hereinbefore (for example linomide, inhibitors of integrin $\alpha v\beta 3$ function, angiostatin, razoxin, thalidomide);

(ii) cytostatic agents such as antioestrogens (for example tamoxifen,toremifene, raloxifene, droloxifene, iodoxyfene), progestogens (for example megestrol acetate), aromatase inhibitors

(for example anastrozole, letrazole, vorazole, exemestane), antiprogestogens, antiandrogens (for example flutamide, nilutamide, bicalutamide, cyproterone acetate), LHRH agonists and antagonists (for example goserelin acetate, luprolide), inhibitors of testosterone 5α-dihydroreductase (for example finasteride), anti-invasion agents (for example metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator receptor function) and inhibitors of growth factor function, (such growth factors include for example platelet derived growth factor and hepatocyte growth factor such inhibitors include growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors and serine/threonine kinase inhibitors); and

(iii) antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as antimetabolites (for example antifolates like methotrexate, fluoropyrimidines like 5-fluorouracil, purine and adenosine analogues, cytosine arabinoside); antitumour antibiotics (for example anthracyclines like doxorubicin, daunomycin, epirubicin and idarubicin, mitomycin-C, dactinomycin, mithramycin); platinum derivatives (for example cisplatin, carboplatin); alkylating agents (for example nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide, nitrosoureas, thiotepa); antimitotic agents (for example vinca alkaloids like vincristine and taxoids like taxol, taxotere); topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan).

As stated above the compounds defined in the present invention are of interest for their antiangiogenic and/or vascular permeability reducing effects. Such compounds of the invention are expected to be useful in a wide range of disease states including cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation and ocular diseases with retinal vessel proliferation. In particular such compounds of the invention are expected to slow advantageously the growth of primary and recurrent solid tumours of, for example, the colon, breast, prostate, lungs and skin. More particularly such compounds of the invention are expected to inhibit the growth of those primary and recurrent solid tumours which are associated with VEGF especially those tumours which are significantly dependent on VEGF for their growth and spread, including for example, certain tumours of the colon, breast, prostate, lung, vulva and skin.

In addition to their use in therapeutic medicine, the compounds of formula I and their pharmaceutically acceptable salts are also useful as pharmacological tools in the development and standardisation of in vitro and in vivo test systems for the evaluation of the effects of inhibitors of VEGF receptor tyrosine kinase activity in laboratory animals such as cats, dogs, rabbits, monkeys, rats and mice, as part of the search for new therapeutic agents.

It is to be understood that where the term "ether" is used anywhere in this specification it refers to diethyl ether.

The invention will now be illustrated in the following non-limiting Examples in which, unless otherwise stated:-

[(i) evaporations were carried out by rotary evaporation in vacuo and work-up procedures were carried out after removal of residual solids such as drying agents by filtration;

(ii) operations were carried out at ambient temperature, that is in the range 18-25°C and under an atmosphere of an inert gas such as argon;

(iii) column chromatography (by the flash procedure) and medium pressure liquid chromatography (MPLC) were performed on Merck Kieselgel silica (Art. 9385) or Merck Lichroprep RP-18 (Art. 9303) reversed-phase silica obtained from E. Merck, Darmstadt, Germany;

(iv) yields are given for illustration only and are not necessarily the maximum attainable;

(v) melting points are uncorrected and were determined using a Mettler SP62 automatic melting point apparatus, an oil-bath apparatus or a Koffler hot plate apparatus.

(vi) the structures of the end-products of the formula I were confirmed by nuclear (generally proton) magnetic resonance (NMR) and mass spectral techniques; proton magnetic resonance chemical shift values were measured on the delta scale and peak multiplicities are shown as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; q, quartet;

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

(viii) the following abbreviations have been used:-

DMF N,N-dimethylformamide

DMSO dimethylsulphoxide

THF tetrahydrofuran

TFA trifluoroacetic acid

NMP 1-methyl-2-pyrrolidinone.]

Example 1

Potassium carbonate (2.2g, 16mmol) was added to a solution of 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline (1.51g, 4mmol) in DMF (30ml) and the mixture stirred for 15 minutes. 2-Bromoethyl methyl ether (667mg, 4.8mmol) was then added dropwise. The mixture was stirred for 1 hour at ambient temperature, then heated at 60°C for 17 hours and finally allowed to cool. The insoluble material was removed by filtration and the filter pad washed with DMF. The filtrate was partitioned between ethyl acetate and water, the organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (95/5 followed by 93/7). The purified product was triturated with ether to give **4-(3-acetoxy-4-methylanilino)-6-methoxy-7-(2-methoxyethoxy)quinazoline** (1.34g, 84%) as a white powder.

m.p. 180-183°C

^{1}H NMR Spectrum: ($CDCl_3$) 2.16(s, 3H); 2.34(s, 3H); 3.47(s, 3H); 3.87(t, 2H); 3.99(s, 3H); 4.31(t, 2H); 6.98(s, 1H); 7.21(d, 1H); 7.24(d, 1H); 7.42(d, 1H); 7.50(s, 1H); 8.64(s, 1H)

MS - ESI: 420 $[MNa]^+$

Elemental analysis:	Found	C 63.1	H 6.1	N 10.4
$C_{21}H_{23}N_3O_5$	Requires	C 63.5	H 5.8	N 10.6%

The starting material was prepared as follows:

A mixture of 2-amino-4-benzyloxy-5-methoxybenzamide (J. Med. Chem. 1977, vol 20, 146-149, 10g, 0.04mol) and Gold's reagent (7.4g, 0.05mol) in dioxane (100ml) was stirred and heated at reflux for 24 hours. Sodium acetate (3.02g, 0.037mol) and acetic acid (1.65ml, 0.029mol) were added to the reaction mixture and it was heated for a further 3 hours. The mixture was evaporated, water was added to the residue, the solid was filtered off, washed with

water and dried ($MgSO_4$). Recrystallisation from acetic acid gave 7-benzyloxy-6-methoxy-3,4-dihydroquinazolin-4-one (8.7g, 84%).

A mixture of 7-benzyloxy-6-methoxy-3,4-dihydroquinazolin-4-one (2.82g, 0.01mol), thionyl chloride (40ml) and DMF (0.28ml) was stirred and heated to reflux for 1 hour. The mixture was evaporated, the residue was taken up in toluene and evaporated to dryness to give 7-benzyloxy-4-chloro-6-methoxyquinazoline (3.45g).

Acetic anhydride (1.9ml, 20.3mmol) was added to a mixture of 2-methyl-5-nitrophenol (2.5g, 16.3mmol) and 1M aqueous sodium hydroxide (24.5ml) at ambient temperature. The mixture was stirred for 40 minutes, the solid was removed by filtration and the filtrate extracted with ethyl acetate. The organic layers were combined, washed with an aqueous saturated sodium chloride solution, dried ($MgSO_4$) and evaporated to yield 2-acetoxy-4-nitrotoluene (3.1g, 100%). A mixture of this material (3.1g, 15.9mmol) and 10% palladium-on-charcoal catalyst (0.12g) in ethyl acetate (50ml) was stirred at ambient temperature under an atmosphere of hydrogen for 2 hours. The catalyst was removed by filtration and the filtrate evaporated to give 3-acetoxy-4-methylaniline (2.45g, 94%).

A mixture of 7-benzyloxy-4-chloro-6-methoxyquinazoline (2.18g, 7.25mmol), 3-acetoxy-4-methylaniline (1.32g, 8mmol) and 2-propanol (50ml) was stirred and heated to reflux for 1 hour. The mixture was cooled to ambient temperature. The precipitate was collected by filtration, washed with 2-propanol and ether to give 4-(3-acetoxy-4-methylanilino)-7-benzyloxy-6-methoxyquinazoline. A mixture of 4-(3-acetoxy-4-methylanilino)-7-benzyloxy-6-methoxyquinazoline (2.68g, 5.75mmol), 10% palladium-on-charcoal catalyst (0.27g) in methanol (50ml), DMF (12ml) and trichloromethane (50ml) was stirred at ambient temperature under 1.5 atmospheres of hydrogen for 30 minutes. The catalyst was removed by filtration and the filtrate evaporated. The residual solid was triturated in ether, collected by filtration and dried under vacuum at 50°C to give 4-(3-acetoxy-4-methylanilino)-7-hydroxy-6-methoxyquinazoline (2.1g, 100%).

Example 2

A solution of 2-(2-bromoethyl)-1,3-dioxolane (258mg, 1.4mmol) in DMF (0.5ml) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (329mg, 1.02mmol) and potassium carbonate (264mg, 2mmol) in DMF (2ml). The mixture

was heated at 100°C for 3 hours and allowed to cool. The volatiles were removed by evaporation, and the residue partitioned between aqueous sodium hydrogen carbonate solution and methylene chloride. The organic phase was separated and passed through phase separating paper. The solvent was removed by evaporation, and the residue was purified by column chromatography eluting with methylene chloride/methanol (95/5) to give **4-(4-chloro-2-fluoroanilino)-7-(2-(1,3-dioxolan-2-yl)ethoxy)-6-methoxyquinazoline** (71mg, 17%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.1(m, 2H,); 3.8(m, 2H); 3.95(m, 5H); 4.25(t, 2H); 5.05(t, 1H); 7.18(s, 1H); 7.3(m, 1H); 7.55(m, 2H); 7.8(s, 1H); 8.35(s, 1H); 9.5(s, 1H)

MS - ESI: 420 $[MH]^+$

Elemental analysis:	Found	C 57.4	H 4.7	N 9.1
$C_{20}H_{19}N_3O_4ClF$	Requires	C 57.2	H 5.6	N 10.0%

The starting material was prepared as follows:

A solution of 7-benzyloxy-4-chloro-6-methoxyquinazoline (1.2g, 4mmol), (prepared as described for the starting material in Example 1), and 4-chloro-2-fluoroaniline (444µl, 4mmol) in 2-propanol (40ml) was refluxed for 1.5 hours. After cooling, the precipitate was collected by filtration, washed with 2-propanol then ether and dried under vacuum to give 7-benzyloxy-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (1.13g, 64%).

m.p. 239-242°C

^{1}H NMR Spectrum: ($DMSOd_6$) 4.0(s, 3H); 5.36(s, 2H); 7.39-7.52(m, 9H); 8.1(s, 1H); 8.75(s, 1H)

MS - ESI: 410 $[MH]^+$

Elemental analysis:	Found	C 59.2	H 4.3	N 9.4
$C_{22}H_{17}N_3O_2ClF$ 1HCl	Requires	C 59.2	H 4.1	N 9.41%

A solution of 7-benzyloxy-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (892mg, 2mmol) in TFA (10ml) was refluxed for 50 minutes. After cooling, the mixture was poured onto ice. The precipitate was collected by filtration, dissolved in methanol (10ml) and basified to pH11 with aqueous ammonia. After concentration by evaporation, the solid product was collected by filtration, washed with water then ether and dried under vacuum to give 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline as a yellow solid (460mg, 72%).

m.p. 141-143°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.95(s, 3H); 7.05(s, 1H); 7.35(d, 1H); 7.54-7.59(m, 2H); 7.78(s, 1H); 8.29(s, 1H)

MS - ESI: 320-322 $[MH]^+$

Example 3

1-(2-Chloroethyl)pyrrolidine hydrochloride (200mg, 1.2mmol) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (403mg, 1.26mmol), (prepared as described for the starting material in Example 2), and potassium carbonate (650mg, 4.7mmol) in DMF (4ml). The mixture was heated to 100°C and further portions of 1-(2-chloroethyl)pyrrolidine hydrochloride (800mg in total) were added periodically over 4 hours while the reaction mixture was maintained at 100°C. The reaction was then allowed to cool and volatiles were removed by evaporation. The residue was partitioned between methylene chloride and water, separated and the organic phase passed through phase separating paper. Column chromatography eluting with methylene chloride/methanol (95/5) gave **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)quinazoline** (50mg, 10%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.8-2.1(m, 4H); 3.1(m, 2H); 3.55-3.7(m, 4H); 4.05(s, 3H); 4.6(t, 2H); 7.4(m, 2H); 7.58(d, 1H); 7.65(dt, 1H); 8.5(s, 1H); 8.8(s, 1H)

MS - ESI: 417 $[MH]^+$

Elemental analysis:	Found	C 60.2	H 5.4	N 12.3
$C_{21}H_{22}N_4O_2ClF$	Requires	C 60.5	H 5.3	N 13.4%

Example 4

A solution of 1-(3-chloropropyl)pyrrolidine (230mg, 0.96mmol) was added to 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (295mg, 0.92mmol), (prepared as described for the starting material in Example 2), and potassium carbonate (130mg, 0.94mmol) in DMF (8ml). The mixture was heated at 100°C for 90 minutes and allowed to cool. The volatiles were removed by evaporation and the residues were partitioned between water and methylene chloride. The organic phase was separated and passed through phase separating paper, and the solvent was removed under reduced pressure. The residue was

dissolved in acetone and hydrogen chloride in ether (2ml of a 1M solution, 2mmol) was added. The mixture was stirred at ambient temperature for 30 minutes and the resulting precipitate was collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazoline** hydrochloride hydrate (320mg, 67%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.8-2.0(m, 6H); 3-3.6(m, 6H); 4.05(s, 3H); 4.3(t, 2H); 7.4(m, 2H); 7.55(d, 1H); 7.6(m, 1H); 8.4(s, 1H); 8.8(s, 1H)

MS - ESI: 431 $[MH]^+$

Elemental analysis:	Found	C 51.0	H 5.9	N 10.6	
$C_{22}H_{24}N_4O_2ClF\ 1.8H_2O\ 1.5HCl$	Requires	C 51.0	H 5.7	N 10.8%	

The starting material was prepared as follows:

Pyrrolidine (3g, 42mmol) was added to a solution of 1-bromo-3-chloropropane (3.2g, 20mmol) in toluene (20ml). The mixture was stirred at ambient temperature overnight and then heated at 60°C for 4 hours. The mixture was allowed to cool and the precipitate removed by filtration. The bulk of toluene was removed by evaporation to give an oil. ^{1}H NMR indicated the oil was a 1:1 mol:mol mixture of toluene and 1-(3-chloropropyl)pyrrolidine. This material was used without further purification.

^{1}H NMR Spectrum: ($CDCl_3$) 1.75(m, 4H); 2.0(q, 2H); 2.35(s, 3H, toluene); 2.45-2.6(m, 6H); 3.6(t, 2H); 7.15-7.3(m, 5H, toluene)

Example 5

A solution of 2-(2-methoxyethoxy)ethanol (90mg, 0.75mmol) in methylene chloride (1ml) was added to tributylphosphine (320mg, 1.58mmol) and 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (200mg, 0.63mmol), (prepared as described for the starting material in Example 2), in methylene chloride (6ml) at 0°C under argon. To the resulting mixture 1,1'-(azodicarbonyl)dipiperidine (400mg, 1.6mmol) was added in portions. The mixture was allowed to warm to ambient temperature and stirred under argon for 2 hours. Ether (5ml) was added, and the precipitated solids were removed by filtration. The volatiles were removed from the filtrate by evaporation, and the residue was purified by column chromatography eluting with methylene chloride/methanol (90/10). The resulting partially purified product was dissolved in acetone, and ethereal hydrogen chloride (0.6ml of a 1M

solution, 0.6mmol) added. The resulting precipitated product was collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-methoxyethoxy)ethoxy)quinazoline** hydrochloride (128mg, 44%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.25(s, 3H); 3.45(dd, 2H); 3.6(dd, 2H); 3.8(t, 2H); 4.0(s, 3H); 4.3(t, 2H); 7.4(s, 1H); 7.45(dd, 1H); 7.55-7.7(m, 2H); 8.3(s, 1H); 8.75(s, 1H); 11.5(br s, 1H)

MS - ESI: 422 $[MH]^+$

Elemental analysis :	Found	C 52.3	H 4.7	N 9.1
$C_{20}H_{21}N_3O_4ClF$ 1HCl	Requires	C 52.4	H 4.8	N 9.2%

Example 6

A solution of 2-(bromomethyl)-1,3-dioxolane (190mg, 1.1mmol) in DMF (1ml) was added to 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (258mg, 0.81mmol), (prepared as described for the starting material in Example 2), and potassium carbonate (200mg, 1.5mmol) in DMF (2ml). The mixture was heated at 100°C for 4 hours and then allowed to cool. The volatiles were removed by evaporation and the residue partitioned between water and methylene chloride. The organic phase was separated, passed through phase separating paper and purified by column chromatography eluting with methylene chloride/methanol (95/5) to give **4-(4-chloro-2-fluoroanilino)-7-(1,3-dioxolan-2-ylmethoxy)-6-methoxyquinazoline** (130mg, 38%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.8-4.1(m, 7H); 4.15(d, 2H); 5.30(t, 1H); 7.22(s, 1H); 7.30(m, 1H); 7.55(m, 2H); 7.80(s, 1H); 8.35(s, 1H); 9.55(s, 1H). Also contained 0.3 methanol.

MS - ESI: 406 $[MH]^+$

Elemental analysis:	Found	C 55.1	H 4.5	N 9.5
$C_{19}H_{17}N_3O_4ClF$ $0.3H_2O$	Requires	C 55.1	H 4.5	N 10.0%

0.3Methanol

Example 7

A mixture of 6,7-dimethoxy-5-nitro-3,4-dihydroquinazolin-4-one (1.75g, 7.0mmol) and thionyl chloride (25ml) and DMF (3 drops) was heated at reflux for 2 hours. Excess thionyl chloride was removed by evaporation and the residue azeotroped with toluene. 3-Hydroxy-4-methylaniline (0.94g, 7.6mmol) in 2-propanol (40ml) was added to the residue

and the mixture heated at reflux for 2 hours. The mixture was allowed to cool, the precipitate collected by filtration, washed with 2-propanol and dried to give **6,7-dimethoxy-4-(3-hydroxy-4-methylanilino)-5-nitroquinazoline** hydrochloride (2.02g, 81%).

m.p. 206-208°C

^{1}H NMR Spectrum: (DMSOd_6) 3.90(s, 3H); 4.05(s, 1H); 6.50(d,1H); 6.65(s,1H); 6.97(d, 1H); 7.57(s, 1H); 8.15(s, 1H)

MS - ESI: 357 $[MH]^+$

Elemental analysis:	Found	C 52.0	H 4.3	N 13.9
$C_{17}H_{16}N_4O_5$ 1HCl	Requires	C 52.0	H 4.3	N 14.3%

The starting material was prepared as follows:

A mixture of 4,5-dimethoxyanthranilic acid (19.7g) and formamide (10ml) was stirred and heated at 190°C for 5 hours. The mixture was allowed to cool to approximately 80°C and water (50ml) was added. The mixture was stored at ambient temperature for 3 hours. The precipitate was isolated, washed with water and dried to give 6,7-dimethoxy-3,4-dihydroquinazolin-4-one (3.65g).

Fuming nitric acid (47ml) was added to 6,7-dimethoxy-3,4-dihydroquinazolin-4-one (10g, 48mmol), in water (40ml). The reaction mixture was heated at 120°C for 1 hour, then allowed to cool and diluted with water. The resulting precipitate was collected by filtration, washed with water and dried to give 6,7-dimethoxy-5-nitro-3,4-dihydroquinazolin-4-one (3.9g, 32%).

^{1}H NMR Spectrum: (DMSOd_6) 3.87(s, 3H); 4.05(s, 1H); 7.42(s, 1H); 8.13(s, 1H)

MS - ESI: 251 $[MH]^+$

Example 8

Sodium (148mg, 6.4mmol) was added to 2-methoxyethanol (10ml), the mixture stirred for 15 minutes to give a complete solution and the volatiles removed by evaporation. The residue was dissolved in DMSO (5ml) and 7-chloro-4-(4-chloro-2-fluoroanilino)-6-nitroquinazoline hydrochloride (500mg, 1.3mmol) was added. The mixture was stirred at ambient temperature for 18 hours then diluted with a solution of acetic acid (1ml) in water (20ml). The resulting precipitate was collected by filtration, washed with water, dried and

purified by column chromatography eluting with methylene chloride/methanol (96/4). The purified product was recrystallised from methylene chloride/isohexane to give **4-(4-chloro-2-fluoroanilino)-7-(2-methoxyethoxy)-6-nitroquinazoline** (304mg, 60%) as a yellow solid.

^{1}H NMR Spectrum: ($DMSOd_6$) 3.15(s, 3H); 3.60(m, 2H); 4.31(m, 2H); 7.24(m, 1H); 7.4-7.5(m, 3H); 8.42(s, 1H); 9.03(s, 1H)

MS - ESI: 393 $[MH]^+$

Elemental analysis:	Found	C 51.8	H 3.7	N 14.1
$C_{17}H_{14}N_4O_4ClF$	Requires	C 52.0	H 3.6	N 14.3%

The starting material was prepared as follows:

A mixture of 7-chloro-6-nitro-3,4-dihydroquinazolin-4-one (40g, 0.18mol), (J. Org. Chem. 1975, 40, 356), phosphorus oxychloride (50ml) and DMF (1ml) in thionyl chloride (300ml) was heated at reflux for 4 hours. The reaction mixture was allowed to cool and the volatiles removed by evaporation and by azeotroping with toluene. The residue was basified with aqueous sodium hydrogen carbonate solution and extracted with methylene chloride (4x100ml). The extracts were combined, washed with brine and filtered through phase separating paper. The solvent was removed by evaporation and the residue triturated with ether/isohexane to give 4,7-dichloro-6-nitroquinazoline (35.2g, 81%) as a pale yellow solid.

A mixture of 4,7-dichloro-6-nitroquinazoline (24.4g, 0.1mol), 4-chloro-2-fluoroaniline and ethereal hydrogen chloride (100ml of a 1M solution) in 2-propanol (600ml) was heated at reflux for 1.5 hours. The mixture was allowed to cool and diluted with acetone. The solid product was collected by filtration, washed with acetone and dried to give 7-chloro-4-(4-chloro-2-fluoroanilino)-6-nitroquinazoline hydrochloride (35.0g, 90%) as a yellow powder.

MS - ESI: 353 $[MH]^+$

Example 9

Triphenylphosphine (410mg, 1.5mmol) and 1-methyl-3-pyrrolidinol (0.128ml, 1.5mmol) were added to a solution of 4-(chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (250mg, 0.78mmol), (prepared as described for the starting material in Example 2), in methylene chloride (4ml). Diethyl azodicarboxylate (0.246ml, 1.5mmol) was

added dropwise and the reaction mixture was stirred for 1 hour at ambient temperature. Additional triphenylphosphine (61mg, 0.23 mmol) followed by diethyl azodicarboxylate (37µl, 0.23mmol) was added and the mixture was stirred for 15 minutes at ambient temperature. The solvent was removed by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol (80/20) followed by methylene chloride/methanol/triethylamine (80/20/0.5). The purified product was dissolved in methylene chloride/methanol and the insolubles were removed by filtration. A solution of hydrogen chloride in 2-propanol (0.5ml of a 5M solution) was added to the filtrate and the volatiles were removed by evaporation. The residue was triturated with 2-propanol and ether, collected by filtration and dried to give **4-(4-chloro-2-fluoranilino)-6-methoxy-7-(1-methylpyrrolidin-3-yloxy)quinazoline** hydrochloride hydrate (149mg, 40%).

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD) 2.13-2.83(m, 2H); 2.92(s, 3H); 2.99(s, 3H); 3.20-3.32(m, 1H); 3.44-3.59(m, 1H); 3.72-3.81(m, 1H); 3.96-4.14(m, 2H); 4.01 (s, 3H); 5.35-5.43(m, 1H); 7.42-7.47(m, 2H); 7.58-7.63(m, 2H); 8.21(s, 1H); 8.88(s, 1H)

MS - ESI: 403 $[MH]^+$

Elemental analysis:	Found	C 48.8	H 5.2	N 11.0
$C_{20}H_{20}N_4O_2ClF$ $1H_2O$ 2HCl	Requires	C 48.7	H 4.9	N 11.4%

Example 10

4-(Chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (250mg, 0.78mmol), (prepared as described for the starting material in Example 2), and triphenylphosphine (512mg, 1.9mmol) were added to a stirred solution of 4-morpholino-2-butyn-1-ol (182mg, 1.1mmol), (J. Am. Chem. Soc. 1957, 79, 6184), in methylene chloride (4ml) under argon. Diethyl azodicarboxylate (0.307ml, 1.9mmol) was added dropwise and the reaction mixture was stirred for 30 minutes at ambient temperature. Additional 4-morpholino-2-butyn-1-ol (60mg, 0.39mmol), triphenylphosphine (102mg, 0.39mmol) and followed by diethyl azodicarboxylate (61µl, 0.39mmol) were added and the mixture was stirred for a further 15 minutes at ambient temperature. The solvent was removed by evaporation and the residue was purified by column chromatography eluting with methylene chloride/acetonitrile/methanol (60/37/3 followed by 60/35/5 and 55/37/8). The resulting purified oil was dissolved in a mixture of methylene chloride and methanol and ethereal

hydrogen chloride (1ml of a 2.9M solution) was added. The volatiles were removed by evaporation, the solid residue suspended in ether and collected by filtration. The product was recrystallised from 2-propanol/methanol/ether, collected by filtration, washed with 2-propanol and ether and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-morpholinobut-2-yn-1-yloxy)quinazoline** hydrochloride hydrate (75mg, 18%).

m.p. 175-178°C

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.10(m, 2H); 3.46(m, 2H); 3.72(m, 2H); 3.99(m, 2H); 4.03(s, 3H); 4.29(s, 2H); 5.28(s, 2H); 7.47(dd, 2H); 7.62(s, 1H); 7.62(t, 1H); 7.69(dd, 1H); 8.29(s, 1H); 8.89(s, 1H)

MS - ESI: 457 $[MH]^+$

Elemental analysis:	Found	C 50.8	H 4.9	N 10.3
$C_{23}H_{22}N_4O_3ClF\ 1H_2O\ 2HCl$	Requires	C 50.4	H 4.7	N 10.2%

Example 11

Tetrakis(triphenylphosphine)palladium(0) (23mg, 0.02mmol) followed by a solution of sodium triisopropylsilylthiolate (102mg, 0.48mmol), (Tetrahedron.Lett. 1994, 35, 3221), in THF (2ml) was added to a stirred solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(trifluoromethylsulphonyloxy)quinazoline (180mg, 0.4mmol) in THF (2ml) and benzene (2ml) under argon. The reaction mixture was heated at reflux for 2 hours and then allowed to cool. 2-Bromoethyl methyl ether (83mg, 0.6mmol) in DMF (1ml) and then a solution of tetrabutylammonium fluoride in THF (0.5ml of a 1M solution, 0.5 mmol) were added dropwise and the reaction mixture was stirred at ambient temperature for 30 minutes. The mixture was diluted with ethyl acetate, washed with water and brine, dried ($MgSO_4$) and the volatiles removed by evaporation. The residue was purified by column chromatography on neutral alumina eluting with methylene chloride/acetone (95/5). The purified product was triturated with ether, collected by filtration and dissolved in methylene chloride (4ml). Ethereal hydrogen chloride (0.4ml of 3M solution) was added, the solution was diluted with ether and the resulting precipitate was collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethylthio)quinazoline** hydrochloride (80mg, 46%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.33(t, 2H); 3.34(s, 3H); 3.71(t, 2H); 4.07(s, 3H); 7.48(dd, 1H); 7.64(t, 1H); 7.69(dd, 1H); 7.73(s, 1H); 8.10(s, 1H); 8.89(s, 1H).

MS - ESI: 394 $[MH]^+$

Elemental analysis:	Found	C 50.1	H 4.3	N 9.8	S 7.3	
$C_{18}H_{17}N_3O_2ClFS$ 1HCl	Requires	C 50.2	H 4.2	N 9.8	S 7.4%	

The starting material was prepared as follows:

Trifluoromethanesulphonic anhydride (0.55ml, 3.3mmol) was added to a stirred suspension of 4-(chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (959mg, 3mmol), (prepared as described for the starting material in Example 2), in methylene chloride (2.2ml) and pyridine (2.2ml) under argon at 0°C. The reaction mixture was stirred for 1 hour at 0°C, allowed to warm to ambient temperature and stirred for a further 1.5 hours. The volatiles were removed by evaporation, the residue was dissolved in ethyl acetate, washed with dilute hydrochloric acid and brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was triturated with ether/petroleum ether to give 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(trifluoromethylsulphonyloxy)quinazoline (270mg, 60%) as a beige solid.

^1H NMR Spectrum: ($DMSOd_6$) 4.07(s, 3H); 7.39(dd, 1H); 7.57-7.62(m, 2H); 7.92(s, 1H); 8.21(s, 1H); 8.49(s, 1H)

MS - ESI: 452 $[MH]^+$

Example 12

4-(2-Hydroxyethyl)thiomorpholine (114mg, 0.78mmol), (J. Am. Chem. Soc. 1934, 56, 1720), in methylene chloride (1ml) followed by 1,1'-(azodicarbonyl)dipiperidine (525mg, 2.08mmol) were added to a stirred solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (225mg, 0.70mmol), (prepared as described for the starting material in Example 2), and tributylphosphine (0.51ml, 2.08mmol) in methylene chloride (10ml) under nitrogen. The mixture was stirred for 3.5 hours and allowed to stand for a further 18 hours. Ether (8ml) was added, the precipitate removed by filtration and the solvent removed from the filtrate by evaporation. The residue was dissolved in acetone and ethereal hydrogen chloride (2.5ml of a 1M solution) added. The precipitated product was collected by filtration and purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (150/8/1). The purified product was triturated with ether to give 4-(4-chloro-2-

fluoroanilino)-6-methoxy-7-(2-thiomorpholinoethoxy)quinazoline (70mg, 22%) as a pale yellow solid.

m.p. 181-182°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.56(t, 2H); 3.92(s, 3H); 4.59(t, 2H); 7.31(dd, 1H); 7.35(s, 1H); 7.46(d, 1H); 7.53(dd, 1H); 8.33(s, 1H); 8.68(s, 1H); 11.7(br s, 1H)

MS - ESI: 449 $[MH]^+$

Elemental analysis:	Found	C 56.4	H 5.1	N 12.3	
$C_{21}H_{22}N_4O_2ClFS$	Requires	C 56.2	H 4.9	N 12.5%	

Example 13

A solution of (R)-(1-methylpiperidin-3-yl)methanol (2.29g, 18mmol) in methylene chloride (10ml) was added to a stirred mixture of 4-(chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (4.0g, 12.5mmol), (prepared as described for the starting material in Example 2), and triphenylphosphine (9.81g, 37.5mmol) in methylene chloride (200ml). Diethyl azodicarboxylate (5.87ml, 37mmol) was added dropwise and the reaction mixture was stirred for 18 hours at ambient temperature. The volatiles were removed by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (a gradient from 100/0/0 to 85/15/0.1). The purified product was triturated with ethyl acetate, collected by filtration, washed with ethyl acetate and dried to give **(R)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-yl)methoxyquinazoline** (2.78g, 52%).

$[\alpha]_D$ +11.7°

^{1}H NMR Spectrum: ($DMSOd_6$) 1.08(m, 1H); 1.50(m, 1H); 1.64(m, 1H); 1.80(m, 3H); 2.07(m, 1H); 2.16(s, 3H); 2.62(d, 1H); 2.81(d, 1H); 3.92(s, 3H); 4.02(d, 2H); 7.18(s, 1H); 7.32(d, 1H); 7.55(m, 2H); 7.79(s, 1H); 8.34(s, 1H); 9.50(s, 1H)

MS - ESI: 431 $[MH]^+$

Elemental analysis:	Found	C 60.7	H 5.4	N 13.3	
$C_{22}H_{24}N_4O_2ClF$	Requires	C 61.3	H 5.6	N 13.0%	

The starting material was prepared as follows:

(R)-Ethyl nipecotate (5.7g 365mmol), (prepared by resolution of ethyl nipecotate by treatment with L(+)-tartaric acid as described in J. Org. Chem. 1991, (56), 1168), was dissolved in 38.5% aqueous formaldehyde solution (45ml) and formic acid (90ml) and the mixture heated at reflux for 18 hours. The mixture was allowed to cool and added dropwise to cooled saturated aqueous sodium hydrogen carbonate solution. The mixture was adjusted to pH12 by addition of sodium hydroxide and the mixture was extracted with methylene chloride. The organic extract was washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation to give (R)-ethyl 1-methylpiperidine-3-carboxylate (4.51g, 73%) as a colourless oil.

MS - ESI: 172 $[MH]^+$

A solution of (R)-ethyl 1-methylpiperidine-3-carboxylate (5.69g, 33mmol) in ether (20ml) was added dropwise to a stirred solution of lithium aluminium hydride (36.6ml of a 1M solution in THF, 36.6mmol) in ether (85ml) cooled to maintain a reaction temperature of 20°C. The mixture was stirred for 1.5 hours at ambient temperature and then water (1.4ml), 15% aqueous sodium hydroxide solution (1.4ml) and then water (4.3ml) were added. The insolubles were removed by filtration and the volatiles removed from the filtrate by evaporation to give (R)-(1-methylpiperidin-3-yl)methanol (4.02g, 94%) as a colourless oil.

^1H NMR Spectrum: ($DMSOd_6$) 1.06(q, 1H); 1.51-1.94(m, 5H); 2.04(s, 3H); 2.34(br s, 1H); 2.62(m, 1H); 2.78(d, 1H); 3.49(m, 1H); 3.59(m, 1H)

MS - ESI: 130 $[MH]^+$

Example 14

Using a method analogous to that in Example 13, (S)-(1-methylpiperidin-3-yl)methanol (185g, 1.1mmol), (prepared as described for the starting material in Example 13 but resolving with D(-)-tartaric acid), was treated with 4-(chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (319mg, 1mmol), (prepared as described for the starting material in Example 2), triphenylphosphine (785mg, 3mmol) and diethyl azodicarboxylate (0.475ml, 3mmol) to give, after work-up and purification, **(S)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-yl)methoxyquinazoline** (187mg, 44%).

Example 15

The final compounds in Examples 13 and 14 may be mixed, in any relative proportions, for example to give a racemic mixture.

Alternatively the racemate may be made as follows:

1,1'-(Azodicarbonyl)dipiperidine (560mg, 2.2mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (240mg, 0.75mmol), (prepared as described for the starting material in Example 2), 1-methyl-3-piperidinemethanol (115mg, 0.89mmol) and tributylphosphine (440mg, 2.2mmol) in methylene chloride (10ml). The mixture was stirred for 18 hours, diluted with ether and the resulting precipitate was removed by filtration. The volatiles were removed from the filtrate by evaporation, and the residue was dissolved in acetone and ethereal hydrogen chloride (1.5ml of a 1M solution, 1.5mmol) was added. The precipitated product was collected by filtration and purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (75/8/1). The purified solid product was triturated with ether collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-ylmethoxy)quinazoline** (105mg, 33%).

m.p. 211-212°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.08(m, 1H); 1.50(m, 1H); 1.78(m, 4H); 2.08(m, 1H); 2.16(m, 3H); 2.62(m, 1H); 2.82(m, 1H); 3.95(s, 3H); 4.00(d, 2H); 7.18(s, 1H); 7.32(m, 1H); 7.52(dd, 1H); 7.58(t, 1H); 7.79(s, 1H); 8.35(s, 1H); 9.52(s, 1H)

MS - ESI: 431 $[MH]^+$

Elemental analysis:	Found	C 59.9	H 5.5	N 12.9
$C_{22}H_{24}N_4O_2ClF\ 0.5H_2O$	Requires	C 60.0	H 5.7	N 12.7%

Example 16

3-(Methylsulphonyl)propan-1-ol (0.6g, 4.3mmol) followed by 1,1'-(azodicarbonyl)dipiperidine (4.2g, 16mmol) in portions were added to a stirred solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (1.5g, 4.7mmol), (prepared as described for the starting material in Example 2), and tributylphosphine (4.0ml, 16mmol) in methylene chloride (50ml) under nitrogen. The mixture was stirred for 18 hours, the resulting precipitate was collected by filtration and dried to give crude product (1.36g). The solvent was

removed from the filtrate by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol (a gradient from 100/0 to 90/10). The semi-purified product was triturated with acetone and the solid product collected by filtration and dried to give further crude product (0.53g). The filtrate from the trituration was repurified by column chromatography as before to give further crude product (0.23g). The crude products were combined and dissolved in acetone/methanol/methylene chloride and ethereal hydrogen chloride (6ml of a 1M solution) added. The precipitated product was collected by filtration and recrystallised from methanol/methylene chloride/hexane to give 4-**(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(methylsulphonyl)propoxy)quinazoline** hydrochloride (640mg, 29%).

m.p. >250°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.25(q, 2H); 3.02(s, 3H); 3.36(t, 2H); 4.00(s, 3H); 4.30(t, 2H); 7.35(s, 1H); 7.42(d, 1H); 7.60(t, 1H); 7.65(d, 1H); 8.25(s, 1H); 8.78(s, 1H); 11.5(br s, 1H)

MS - ESI: 440 $[MH]^+$

Elemental analysis:	Found	C 47.8 H 4.2 N 8.8 S 6.7
$C_{19}H_{19}N_3O_4ClFS$ 1HCl	Requires	C 47.4 H 4.2 N 9.0 S 6.8%

The starting material was prepared as follows:

A solution of 3-(methylthio)propan-1-ol (5.3g, 50mmol) in methanol (500ml) was added to a solution of OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc), (30g) in water (150ml) and the mixture stirred at ambient temperature for 24 hours. The precipitated solid was removed by filtration and the methanol removed from the filtrate by evaporation. The aqueous residue was saturated with sodium chloride and extracted with methylene chloride (4x25ml). The aqueous residue was then saturated with ammonium chloride and extracted with ethyl acetate (4x25ml). The extracts were combined, dried ($MgSO_4$) and the solvent removed by evaporation to give 3-(methylsulphonyl)propan-1-ol (610mg, 9%) as an oil.

^{1}H NMR Spectrum: ($CDCl_3$) 2.10(m, 2H); 2.96(s, 3H); 3.20(t, 2H); 3.80(t, 2H)

MS - ESI: 139 $[MH]^+$

Example 17

Diethyl azodicarboxylate (5.91ml, 37mmol) was added dropwise to a stirred mixture of (E)-4-(pyrrolidin-1-yl)but-2-en-1-ol (3.97g, 28mmol), 4-(chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (3.0g, 9mmol), (prepared as described for the starting material in Example 2), and triphenylphosphine (9.84g, 38mmol) in methylene chloride (300ml). The reaction mixture was stirred for 18 hours at ambient temperature. The volatiles were removed by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol (a gradient from 80/20 to 70/30). The purified product was dissolved in methylene chloride/methanol and 1M ethereal hydrogen chloride (25ml) was added. The precipitated product was collected by filtration, washed with ether and dried to give **(E)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-(pyrrolidin-1-yl)but-2-en-1-yloxy)quinazoline** hydrochloride (1.62g, 33%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.85-1.95(m, 2H); 2.0-2.15(m, 2H); 3.0-3.1(m, 2H); 3.5-3.6(m, 2H); 3.95(d, 2H); 4.1(s, 3H); 4.95(d, 2H); 6.1(td, 1H); 6.35(td, 1H); 7.4(s, 1H); 7.45(dd, 1H); 7.6-7.7(m, 2H); 8.15(s, 1H); 8.90(s, 1H)

MS - EI: 443 $[MH]^+$

Elemental analysis:	Found	C 52.7	H 5.3	N 10.8	
$C_{21}H_{24}N_4O_2ClF$ 0.6H_2O 1.85HCl	Requires	C 53.0	H 5.2	N 10.7%	

The starting material was prepared as follows:

Thionyl chloride (9.3ml, 128mmol) was added dropwise to a stirred solution of 2-butyne-1,4-diol (10g, 116mmol) in toluene (15ml) and pyridine (10.3ml) cooled at 0°C. The mixture was stirred for 3.5 hours at ambient temperature and then poured onto ice water. The mixture was extracted with ether, the organic layer was washed with saturated aqueous sodium hydrogen carbonate solution and then brine, dried ($MgSO_4$) and the volatiles removed by evaporation. The residue was purified by column chromatography eluting with petroleum ether/ether (7/3) to give 4-chlorobut-2-yn-1-ol (4.74g, 39%).

^{1}H NMR Spectrum: ($CDCl_3$) 1.68(t, 1H); 4.18(d, 2H); 4.33(d, 2H)

Pyrrolidine (7.8ml, 94mmol) was added dropwise to a solution of 4-chlorobut-2-yn-1-ol (4.74g, 45mmol) in toluene (40ml) and the mixture stirred and heated at 60°C for 1 hour. The volatiles were removed by evaporation and the residue was purified by chromatography

eluting with methylene chloride/methanol (96/4) to give 4-(pyrrolidin-1-yl)but-2-yn-1-ol (4.3g, 69%).

^{1}H NMR Spectrum: ($CDCl_3$) 1.82(t, 4H); 2.63(t, 4H); 3.44(t, 2H), 4.29(t, 2H)

A solution of 4-(pyrrolidin-1-yl)but-2-yn-1-ol (4.3g, 31mmol) in THF (20ml) was added dropwise to a suspension of lithium aluminium hydride (2.35g, 62mmol) in anhydrous THF (8ml) and the mixture stirred and heated at 60°C for 2 hours. The mixture was cooled to 5°C and 2M aqueous sodium hydroxide solution (28ml) was added dropwise. The resulting suspension was filtered and the volatiles removed from the filtrate by evaporation. The residue was dissolved in a mixture of methylene chloride/ethyl acetate, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography on aluminum oxide eluting with methylene chloride/methanol (97/3) to give (E)-4-(pyrrolidin-1-yl)but-2-en-1-ol (3.09g, 70%).

^{1}H NMR Spectrum: ($CDCl_3$) 1.82(m, 4H); 2.61(m, 4H); 3.17(m, 2H); 4.13(s, 2H); 5.84(m, 2H)

Example 18

A solution of 4-(4-bromo-2-fluoroanilino)-7-(3-chloropropoxy)-6-methoxyquinazoline (150mg, 0.34mmol) in 1-(2-hydroxyethyl)piperazine (5ml) was heated at 100°C for 30 minutes. The reaction mixture was allowed to cool and made basic with aqueous sodium hydrogen carbonate solution and extracted with ethyl acetate (3x50ml). The extracts were combined, washed twice with water, then brine and dried ($MgSO_4$). The volatiles were removed by evaporation and the residue dissolved in acetone/methanol (10/1) (50ml) and ethereal hydrogen chloride added. The resulting precipitate was collected by filtration, washed with ether and hexane and dried under vacuum to give **4-(bromo-2-fluoroanilino)-7-(3-[4-(2-hydroxyethyl)piperazinyl]propoxy)-6-methoxyquinazoline** hydrochloride (180mg, 80%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.35(br t, 2H); 3.2-3.8(br m, 12H); 3.80(t, 2H); 4.02(s, 3H); 4.35(t, 2H); 7.45(s, 1H); 7.30(s, 1H); 7.50-7.58(m, 2H); 7.76(dd, 1H); 8.42(s, 1H); 8.80(s, 1H); 11.82(br s, 1H)

MS - ESI: 534 $[MH]^+$

The starting material was prepared as follows:

A mixture of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (1.2g, 3.3mmol), (prepared as described for the starting material in Example 48), 1-bromo-3-chloropropane (1.6ml, 16mmol) and potassium carbonate (2.1g, 15mmol) in DMF (25ml) was heated at 45°C for 3 hours. The mixture was allowed to cool, was diluted with water and extracted with ethyl acetate (3x70ml). The organic extracts were combined, washed with water and brine, dried ($MgSO_4$) and the volatiles were removed by evaporation. The residue was triturated with hexane/ethyl acetate, collected by filtration and dried under vacuum to give 4-(4-bromo-2-fluoroanilino)-7-(3-chloropropoxy)-6-methoxyquinazoline (492mg, 34%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.24(m, 2H); 3.80(t, 2H); 3.95(s, 3H); 4.26(t, 2H); 7.20(s, 1H); 7.42-7.55(m, 2H); 7.63(dd, 1H); 7.80(s, 1H); 8.35(s, 1H); 9.52(s, 1H)

MS - ESI: 440 $[MH]^+$

Example 19

A solution of OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc), (390mg) in water (2ml) was added to a solution of 4-(chloro-2-fluoroanilino)-7-(3-(ethylthio)propoxy)-6-methoxyquinazoline (75mg, 0.18mmol) in methanol (10ml) and the mixture stirred for 18 hours at ambient temperature. The reaction mixture was basified with aqueous sodium hydrogen carbonate solution and extracted with ethyl acetate (3x25ml). The extracts were combined, washed twice with water and then with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The solid residue was recrystallised from ethyl acetate/hexane to give **4-(chloro-2-fluoroanilino)-7-(3-(ethylsulphonyl)propoxy)-6-methoxyquinazoline** (35mg, 43%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.24 (t, 3H); 2.22(m, 2H); 3.15(q, 2H); 3.95(s, 3H); 4.25(t, 2H); 7.20(s, 1H); 7.35(dd, 1H); 7.5-7.6(m, 2H); 7.80(s, 1H); 8.35(s, 1H); 9.54(s, 1H)

MS - ESI: 454 $[MH]^+$

Elemental analysis:	Found	C 51.7	H 4.6	N 9.2
$C_{20}H_{21}N_3O_4ClFS\ 0.5H_20$	Requires	C 51.9	H 4.8	N 9.1%

The starting material was prepared as follows:

A mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (957mg, 3mmol), (prepared as described for the starting material in Example 2), 1-bromo-3-

chloropropane (2.36g, 15mmol) and potassium carbonate (2.1g, 15mmol) in DMF (20ml) was heated at 40°C for 1.5 hours. The mixture was allowed to cool, was diluted with water and extracted with ethyl acetate (3x50ml). The organic extracts were combined, washed with water and brine, dried ($MgSO_4$) and the volatiles were removed by evaporation. The residue was triturated with hexane/ethyl acetate, collected by filtration and dried under vacuum to give 4-(4-chloro-2-fluoroanilino)-7-(3-chloropropoxy)-6-methoxyquinazoline (650mg, 55%).
^{1}H NMR Spectrum: ($DMSOd_6$) 2.26(m, 2H); 3.82(t, 2H); 3.95(s, 3H); 4.26(t, 2H); 7.20(s, 1H); 7.32(dd, 1H); 7.48-7.60(m, 2H); 7.80(s, 1H); 8.35(s, 1H); 9.52(s, 1H)
MS - EI: 396 $[MH]^+$

A mixture of sodium ethanethiolate (120mg, 1.5mmol) and 4-(4-chloro-2-fluoroanilino)-7-(3-chloropropoxy)-6-methoxyquinazoline (227mg, 0.57mmol) in DMF (10ml) was stirred and heated at 70°C for 3 hours. The reaction mixture was allowed to cool, was diluted with water and extracted with ethyl acetate (3x75ml). The extracts were combined, washed with water (x2), and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was recrystallised from ethyl acetate/hexane to give 4-(chloro-2-fluoroanilino)-7-(3-(ethylthio)propoxy)-6-methoxyquinazoline (86mg, 40%).
^{1}H NMR Spectrum: ($DMSOd_6$) 1.20(t, 3H); 2.03(m, 2H); 2.66(t, 2H); 3.95(s, 3H); 4.20(t, 2H); 7.18(s, 1H); 7.33(dd, 1H); 7.5-7.6(m, 2H); 7.78(s, 1H); 8.35(s, 1H); 9.52(s, 1H)
MS - ESI: 422 $[MH]^+$

Example 20

A mixture of 4-(chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (3.28g, 10mmol), (prepared as described for the starting material in Example 2), 1-bromo-3-tetrahydropyranyloxypropane (2.5g, 11mmol) and potassium carbonate (5.0g, 36mmol) in DMF (50ml) was stirred and heated at 90°C for 3 hours. The reaction mixture was allowed to cool, was diluted with water (500ml) and extracted with ethyl acetate (3x100ml). The extracts were combined, washed with water (x3), and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was purified by column chromatography eluting with ethyl acetate. The purified product was recrystallised from ethyl acetate/hexane to give **4-(chloro-2-fluoroanilino)-6-methoxy-7-(3-tetrahydropyran-2-yloxypropoxy)quinazoline** (2.25g, 49%).

m.p. 184-185°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.35-1.54(m, 4H); 1.55-1.75(m, 2H); 2.05(m, 2H); 3.35-3.45(m,1H); 3.66-3.84(m, 2H); 3.95(s, 3H); 4.23(t, 2H); 4.60(s, 1H); 7.18(s, 1H); 7.32(dd, 1H); 7.5-7.6(m, 2H); 7.78(s, 1H); 8.35(s, 1H); 9.53(s, 1H)

MS - ESI: 462 $[MH]^+$

Elemental analysis:	Found	C 59.6	H 5.3	N 9.1
$C_{23}H_{25}N_3O_4ClF$	Requires	C 59.9	H 5.4	N 9.4%

Example 21

A mixture of sodium methanethiolate (70mg, 1mmol) and 4-(4-chloro-2-fluoroanilino)-7-(3-chloropropoxy)-6-methoxyquinazoline (200mg, 0.5mmol), (prepared as described for the starting material in Example 19), in DMF (10ml) was stirred and heated at 70°C for 1 hour. The reaction mixture was allowed to cool, was diluted with water and extracted with ethyl acetate (3x25ml). The extracts were combined, washed with water (x2), and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was recrystallised from ethyl acetate/hexane to give **4-(chloro-2-fluoroanilino)-6-methoxy-7-(3-methylthiopropoxy)quinazoline** (143mg, 35%).

m.p. 169-170°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.0-2.12(m, 2H); 2.08(s, 3H); 2.64(t, 2H); 3.93(s, 3H); 4.21(t, 2H); 7.18(s, 1H); 7.33(d, 1H); 7.50-7.61(m, 2H); 7.78(s, 1H); 8.34(s, 1H); 9.53(s, 1H)

MS - ESI: 408 $[MH]^+$

Example 22

A mixture of 4-(bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (250mg, 0.7mmol), (prepared as described for the starting material in Example 48), 2-chloroethyl methyl sulphide (0.1ml, 1mmol) and potassium carbonate (1.0g, 7mmol) in DMF (10ml) was stirred and heated at 50°C for 4 hours. The reaction mixture was allowed to cool, was diluted with water and extracted with ethyl acetate (3x25ml). The extracts were combined, washed with water (x2), and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was purified by column chromatography eluting with ethyl acetate. The

purified product was recrystallised from ethyl acetate/hexane to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-methylthioethoxy)quinazoline** (100mg, 34%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.20(s, 3H); 2.90(t, 2H); 3.92(s, 3H); 4.30(t, 2H); 7.20(s, 1H); 7.42-7.54(m, 2H); 7.62(dd, 1H); 7.80(s, 1H); 8.36(s, 1H); 9.54(s, 1H)

MS - ESI: 438 $[MH]^+$

Elemental analysis:	Found	C 48.8	H 3.9	N 9.8	S 7.3	
$C_{18}H_{17}N_3O_2BrFS$	Requires	C 49.3	H 3.9	N 9.6	S 7.3%	

Example 23

A solution of 7-(2-bromoethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (130mg, 0.36mmol), (prepared as described for the starting material in Example 62), in 1-ethoxycarbonylpiperazine (1.5ml) was stirred and heated at 100°C for 2 hours. The mixture was allowed to cool, diluted with water and extracted with ethyl acetate (3x25ml). The extracts were combined, washed with water (x2) and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was dissolved in acetone and 1M ethereal hydrogen chloride (2ml) was added. The resulting precipitate was collected by filtration and then purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (94/5/1). The purified product was dissolved in acetone and 1M ethereal hydrogen chloride (2ml) was added. The resulting precipitate was collected by filtration, washed with ether and dried to give **4-(4-chloro-2-fluoroanilino)-7-(2-(4-ethoxycarbonylpiperazin-1-yl)ethoxy)-6-methoxyquinazoline** hydrochloride (85mg, 46%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.20(t, 3H); 3.1-3.6(m, 8H); 3.66(br s, 2H); 4.00(s, 3H); 4.08(q, 2H); 4.65(br s, 2H); 7.40(m, 2H); 7.90(t, 1H); 7.65(dd, 1H); 8.40(s, 1H); 8.80(s, 1H); 11.66(br s, 1H)

MS - ESI: 504 $[MH]^+$

Elemental analysis:	Found	C 48.6	H 5.0	N 12.2
$C_{24}H_{27}N_5O_4ClF\ 1H_2O\ 2HCl$	Requires	C 48.5	H 5.2	N 11.8%

Example 24

A mixture of 4-(bromo-2-fluoroanilino)-7-hydroxy -6-methoxyquinazoline (306mg, 0.84mmol), (prepared as described for the starting material in Example 48), 2-chloroethyl

ethyl sulphide (0.15ml, 1.3mmol) and potassium carbonate (0.5g, 3.6mmol) in DMF (10ml) was stirred and heated at 50°C for 1 hour. The reaction mixture was allowed to cool, was diluted with water and extracted with ethyl acetate (3x25ml). The extracts were combined, washed with water (x2), and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was purified by column chromatography eluting with ethyl acetate. The purified product was recrystallised from ethyl acetate/hexane to give **4-(4-bromo-2-fluoroanilino)-7-(2-ethylthioethoxy)-6-methoxyquinazoline** (221mg, 58%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.24(t, 3H); 2.66(q, 2H); 2.94(t, 2H); 3.95(s, 3H); 4.30(t, 2H); 7.20(s, 1H); 7.45(t, 1H); 7.52(d, 1H); 7.65(dd, 1H); 7.80(s, 1H); 9.55(s, 1H)

MS - ESI: 452 $[MH]^+$

Example 25

A solution of OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc), (150mg) in water (2ml) was added to a solution of 4-(4-bromo-2-fluoroanilino)-7-(2-ethylthioethoxy)-6-methoxyquinazoline (125mg, 0.28mmol), (prepared as described in Example 24), in methanol (10ml). The reaction mixture was stirred for 16 hours at ambient temperature, the methanol was removed by evaporation, the aqueous residue was basified with sodium hydrogen carbonate solution and then extracted with ethyl acetate (3x30ml). The extracts were combined, washed with water (x2), and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was purified by column chromatography eluting with ethyl acetate and then with methylene chloride/methanol (9/1) to give **4-(4-bromo-2-fluoroanilino)-7-(2-ethylsulphinylethoxy)-6-methoxyquinazoline** (32mg, 31%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.21(t, 3H); 2.72-2.84(m, 1H); 2.86-2.96(m, 1H); 3.04-3.12(m, 1H); 3.94(s, 3H); 4.42-4.58(m, 2H); 7.26(s, 1H); 7.42-7.55(m, 2H); 7.64(dd, 1H); 7.82(s, 1H); 8.35(s, 1H); 9.55(s, 1H)

MS - ESI: 468 $[MH]^+$

Example 26

Using a method analogous to that in Example 25, 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-methylthiopropoxy)quinazoline (250mg, 0.6mmol), (prepared as described in Example 21), was treated with OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc),

(84mg) and the product was purified and isolated to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-methylsulphinylpropoxy)quinazoline** (75mg, 29%).

^{1}H NMR Spectrum: ($DMSOd_6$) 2.18(t, 2H); 2.60(s, 3H); 2.78-2.98(m, 2H); 3.95(s, 3H); 4.25(t, 2H); 7.20(s, 1H); 7.35(dd, 1H); 7.50-7.61(m, 2H); 7.80(s, 1H); 8.53(s, 1H); 9.55(s, 1H)

MS - ESI: 424 [MH]$^+$

Elemental analysis:	Found	C 53.4	H 4.5	N 9.8
$C_{19}H_{19}N_3O_4ClFS$	Requires	C 53.9	H 4.5	N 9.8%

Example 27

A solution of OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc), (800mg) in water (3ml) was added to a solution of 4-(4-bromo-2-fluoroanilino)-7-(2-ethylthioethoxy)-6-methoxyquinazoline (320mg, 0.7mmol), (prepared as described in Example 24), in methanol (10ml). The reaction mixture was stirred for 20 hours at ambient temperature, the methanol was removed by evaporation, the aqueous residue was basified with sodium hydrogen carbonate solution, saturated with sodium chloride and then extracted with ethyl acetate (3x50ml). The extracts were combined, dried ($MgSO_4$) and the solvent was removed by evaporation. The residue was dissolved in acetone/methanol and 1M ethereal hydrogen chloride (2ml) was added. The volatiles were removed by evaporation, the residue was triturated with 2-propanol/hexane, collected by filtration and dried to give **4-(4-bromo-2-fluoroanilino)-7-(2-ethylsulphonylethoxy)-6-methoxyquinazoline** hydrochloride (200mg, 55%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.28(t, 3H); 3.25(q, 2H); 3.74(t, 2H); 4.00(s, 3H); 4.54(t, 2H); 7.43(s, 1H); 7.54(m, 1H); 7.56(s, 1H); 7.75(d, 1H); 8.36(s, 1H); 8.78(s, 1H); 11.61(br s, 1H)

MS - ESI: 484 [MH]$^+$

Example 28

A solution of OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc), (800mg) in water (3ml) was added to a solution of 4-(4-chloro-2-fluoroanilino)-7-(2-ethylthioethoxy)-6-methoxyquinazoline (220mg, 0.56mmol) in methanol (10ml). The reaction mixture was stirred for 20 hours at ambient temperature, the methanol was removed by evaporation, the aqueous residue was basified with sodium hydrogen carbonate solution, saturated with sodium

chloride and then extracted with ethyl acetate (3x50ml). The extracts were combined, dried ($MgSO_4$) and the solvent was removed by evaporation. The residue was dissolved in acetone/methanol and 1M ethereal hydrogen chloride (1.2ml) was added. The volatiles were removed by evaporation, the residue was triturated with 2-propanol, collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-7-(2-ethylsulphonylethoxy)-6-methoxyquinazoline** hydrochloride (24mg, 9%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.25(t, 3H); 3.30(q, 2H); 3.75(t, 2H); 4.00(s, 3H); 4.55(t, 2H); 7.36(s, 1H); 7.41(dd, 1H); 7.58(t, 1H); 7.64(dd, 1H); 8.22(s, 1H); 8.78(s, 1H)

MS - ESI: 440 $[MH]^+$

The starting material was prepared as follows:

A mixture of 4-(chloro-2-fluoroanilino)-7-hydroxy -6-methoxyquinazoline (450mg, 1.4mmol), (prepared as described for the starting material in Example 2), 2-chloroethyl ethyl sulphide (0.2ml, 1.7mmol) and potassium carbonate (1.5g, 11mmol) in DMF (10ml) was stirred and heated at 50°C for 2 hours. The reaction mixture was allowed to cool, was diluted with water and extracted with ethyl acetate (3x50ml). The extracts were combined, washed with 0.1M sodium hydroxide solution (x2), water and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation to give crude 4-(4-chloro-2-fluoroanilino)-7-(2-ethylthioethoxy)-6-methoxyquinazoline (230mg, 57%) which was used directly.

Example 29

A mixture of 4-(chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (400mg, 1.3mmol), (prepared as described for the starting material in Example 2), 2-chloroethyl methyl sulphide (0.168ml, 1.7mmol) and potassium carbonate (347mg, 2.5mmol) in NMP (10ml) was stirred and heated at 90°C for 1 hour, then allowed to cool and stirred for 16 hours at ambient temperature. The reaction mixture was diluted with water and extracted with ethyl acetate. The extracts were combined, washed with water, and then brine, and dried ($MgSO_4$). The solvent was removed by evaporation, the residue was triturated with ethyl acetate/hexane and collected by filtration to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylthioethoxy)quinazoline** (220mg, 44%).

m.p. 174-176°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.20(s, 3H); 2.92(t, 2H); 3.94(s, 3H); 4.32(t, 2H); 7.20(s, 1H); 7.32(d, 1H); 7.49-7.6(m, 2H); 7.80(s, 1H); 8.36(s, 1H); 9.55(s, 1H)

MS - ESI: 452 $[MH]^+$

Example 30

A solution of OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc), (652mg) in water (1.6ml) was added to a solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylthioethoxy)quinazoline (200mg, 0.5mmol), (prepared as described in Example 29), in methanol (10ml) and the mixture was stirred for 18 hours at ambient temperature. The mixture was diluted with methylene chloride, was washed with aqueous sodium hydrogen carbonate solution, dried ($MgSO_4$) and the solvent was removed by evaporation. The residue was triturated with ethyl acetate/hexane, collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylsulphonylethoxy)quinazoline** (172mg, 80%).

m.p. 227-230°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.18(s, 3H); 3.70(t, 2H); 3.92(s, 3H); 4.50(t, 2H); 7.22-7.38(m, 2H); 7.42(s, 1H); 7.48-7.60(m, 2H); 8.37(s, 1H); 9.55(s, 1H)

MS - ESI: 426 $[MH]^-$

Elemental analysis:	Found	C 46.0	H 3.6	N 8.7
$C_{18}H_{17}N_3O_4ClFS\ 2.2H_2O$	Requires	C 46.4	H 4.1	N 9.0%

Example 31

1,1'-(Azodicarbonyl)dipiperidine (1.56g, 6.2mmol) followed by 3-(methylthio)-1-propanol (0.32ml, 3mmol) was added to a mixture of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (225mg, 7.0mmol), (prepared as described for the starting material in Example 48), and tributylphosphine (1.42ml, 6.1mmol) in methylene chloride (20ml) at 5°C. The mixture was stirred at 5°C for 1 hour and then for 18 hours at ambient temperature. The insolubles were removed by filtration and the volatiles were removed from the filtrate by evaporation. The residue was purified by column chromatography eluting with ethyl acetate/methanol (100/0 increasing to 95/5) to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-methylthiopropoxy)quinazoline** (400mg, 50%).

m.p. 250-252°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.08(t, 2H); 2.64(t, 2H); 4.00(s, 3H); 4.28(t, 2H); 7.40(s, 1H); 7.48-7.58(m, 2H); 7.78(d, 1H); 8.30(s, 1H); 8.80(s, 1H)

MS - ESI: 452 $[MH]^+$

Example 32

A solution of OXONE, (trade mark of E.I. du Pont de Nemours & Co.,Inc), (800mg) in water (4.5ml) was added to a solution of 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-methylthiopropoxy)quinazoline (300mg, 0.66mmol), (prepared as described in Example 31), in methanol (15ml) and the mixture was stirred for 4 hours at ambient temperature. The mixture was diluted with methylene chloride, was washed with aqueous sodium hydrogen carbonate solution, dried ($MgSO_4$) and the solvent was removed by evaporation. The residue was triturated with ethyl acetate/hexane, collected by filtration and dried to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-methylsulphonylpropoxy)quinazoline** (235mg, 73%).

m.p. >250°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.30(t, 2H); 3.20(s, 3H); 3.30(t, 2H); 4.10(s, 3H); 4.30(t, 2H); 7.38(s, 1H); 7.5-7.6(m, 2H); 7.78(d, 1H); 8.30(s, 1H); 8.80(s, 1H)

MS - ESI: 484 $[MH]^+$

Elemental analysis:	Found	C 42.8	H 3.8	N 7.8
$C_{19}H_{19}N_3O_4BrFS\ 0.5H_2O$	Requires	C 43.1	H 3.9	N 7.9%

Example 33

1,1'-(Azodicarbonyl)dipiperidine (355mg, 1.4mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (150mg, 0.47mmol), (prepared as described for the starting material in Example 2), 2-(cyclopentyloxy)ethanol (91mg, 0.7mmol), (US Patent Number 4,515,814), and tributylphosphine (284mg, 1.4mmol) in methylene chloride (6ml) at 0°C. The mixture was then allowed to warm to ambient temperature and stirred for 3.5 hours. Ether (3ml) was added and the insolubles were removed by filtration and the volatiles were removed from the filtrate by evaporation. The residue was dissolved in acetone and 1M ethereal hydrogen chloride (0.6ml) was added. The mixture was left to stand for 60 hours and the precipitate was collected by filtration, washed with acetone and dried to give **4-(4-chloro-2-**

fluoroanilino)-7-(2-cyclopentyloxyethoxy)-6-methoxyquinazoline hydrochloride (130mg, 60%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.4-1.8(m, 8H); 3.75(t, 2H); 4.00(s, 4H); 4.30(t, 2H); 7.37(s, 1H); 7.42(dd, 1H); 7.60(t, 1H); 7.64(dd, 1H); 8.25(s, 1H); 8.78(s, 1H)

MS - ESI: 432 $[MH]^+$

Elemental analysis:	Found	C 55.8	H 5.0	N 8.8
$C_{22}H_{23}N_3O_3ClF$ $1H_2O$ 1HCl	Requires	C 56.0	H 5.2	N 8.9%

Example 34

Diethyl azodicarboxylate (0.94ml, 6mmol) was added dropwise to a mixture of triphenylphosphine (1.57g, 6mmol), 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (640mg, 2mmol), (prepared as described for the starting material in Example 2), and N-(tert-butoxycarbonyl)ethanolamine (0.354g, 2.2mmol) in methylene chloride (20ml) at 0°C. The reaction mixture was allowed to warm to ambient temperature and stirred for 4 hours. The reaction mixture was diluted with methylene chloride, washed with aqueous sodium hydrogen carbonate solution, water and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (100/8/1). The product was recrystallised from acetonitrile, collected by filtration, washed with ethyl acetate and dried to give 7-**(2-[N-tert-butoxycarbonylamino]ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline** (235mg, 25%).

m.p. 190-191°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.36(s, 9H); 3.34(q, 2H); 3.91(s, 3H); 4.15(t, 2H); 6.98(t, 1H); 7.19(s, 1H); 7.33(dd, 1H); 7.56(m, 2H); 7.78(s, 1H); 8.34(s, 1H); 9.51(s, 1H)

MS - ESI: 463 $[MH]^+$

Elemental analysis:	Found	C 57.0	H 5.1	N 12.5
$C_{22}H_{24}N_4O_4ClF$	Requires	C 57.1	H 5.1	N 12.1%

Example 35

Sodium hydride (55mg of a 60% dispersion in mineral oil, 1.1mmol) was added to a solution of glutarimide (120mg, 1.06mmol) in DMF (5ml) at ambient temperature under

argon and the mixture stirred for 30 minutes. 7-(2-Bromoethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (428mg, 1mmol), (prepared as described for the starting material in Example 62), in DMF (2ml) was added and the resulting pale green solution was stirred for 18 hours and then quenched with water . The volatiles were removed by evaporation, and the residue was partitioned between water and ethyl acetate. The organic phase was separated and washed with water and then dried ($MgSO_4$). The solvent was removed by evaporation, and the residue was purified by column chromatography eluting with ethyl acetate then ethyl acetate/methanol (9/1). The purified product was recrystallised from ethyl acetate and hexane, collected by filtration and washed with ether to give **4-(4-chloro-2-fluoroanilino)-7-(2-(2,6-dioxopiperidino)ethoxy)-6-methoxyquinazoline** (252mg, 55%).

m.p. 202-203°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.84(m, 2H); 2.63(t, 4H); 3.91(s, 3H); 4.08(t, 2H); 4.17(t, 2H); 7.10(s, 1H); 7.34(dd, 1H); 7.55(m, 2H); 7.79(s, 1H); 8.34(s, 1H); 9.52(s, 1H)

MS - ESI: 459 $[MH]^+$

Elemental analysis:	Found	C 57.2	H 4.2	N 11.9
$C_{22}H_{20}N_4O_4ClF$	Requires	C 57.6	H 4.3	N 12.2%

Example 36

Isobutyl chloroformate (88mg, 5.9mmol) was added to a stirred solution of 7-(3-aminopropoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline trifluoroacetate (151mg, 0.4mmol) and triethylamine (0.2ml, 1.4mmol) in THF (15ml). The reaction mixture was stirred at ambient temperature for 30 minutes and the volatiles were removed by evaporation. The residue was dissolved in methylene chloride, the solution was washed with aqueous sodium hydrogen carbonate solution and then brine, dried ($MgSO_4$) and the solvent was removed by evaporation. The residue was recrystallised from acetonitrile to give **4-(4-chloro-2-fluoroanilino)-7-(3-[N-isobutoxycarbonylamino]propoxy)-6-methoxyquinazoline** (41.2mg, 20%) as a white solid.

m.p. 136-137°C

^{1}H NMR Spectrum: ($DMSOd_6$) 0.87(d, 6H); 1.80(m, 1H); 1.93(t, 2H); 3.16(q, 2H); 3.71(d, 2H); 3.94(s, 3H); 4.15(t, 2H); 7.16(s, 2H); 7.32(dd, 1H); 7.55(m, 2H); 7.79(s, 1H); 8.34(s, 1H); 9.50(s, 1H)

MS - ESI: 477 [MH]$^+$

Elemental analysis:	Found	C 57.1	H 4.9	N 11.6
$C_{23}H_{26}N_4O_4ClF$	Requires	C 57.1	H 5.5	N 11.6%

The starting material was prepared as follows:

A solution of di-tert-butyl dicarbonate (32g, 148mmol) in methylene chloride (70ml) was added dropwise to a stirred solution of 3-amino-1-propanol (10.1g, 134mmol) in methylene chloride (100ml). The reaction mixture was stirred overnight and was then washed with saturated aqueous sodium hydrogen carbonate solution, water and then brine. The organic layer was dried ($MgSO_4$) and the volatiles were removed by evaporation to give 3-(N-tert-butoxycarbonylamino)-1-propanol (23.3g, 100%) as a colourless oil.

^{1}H 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%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.36(s, 9H); 1.89(t, 2H); 3.11(q, 2H); 3.91(s, 3H); 4.14(t, 2H); 6.89(m, 1H); 7.16(s, 1H); 7.31(dd, 1H); 7.56(m, 2H); 7.77(s, 1H); 8.32(s, 1H); 9.51(s, 1H)

MS - ESI: 477 [MH]$^+$

7-(3-(N-tert-butoxycarbonylamino)propoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (610mg, 1.28mmol) was added slowly to TFA (10ml). The reaction was stirred at ambient temperature for 2 hours and the volatiles were removed by evaporation and by azeotroping with toluene to give 7-(3-aminopropoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline trifluoroacetate (455mg, 94%) as an oil.

Example 37

A mixture of 7-(2-bromoethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (425mg, 1mmol), (prepared as described for the starting material in Example 62), and 1-methyl-4-(methylamino)piperidine (128mg, 1mmol) in N,N-dimethylacetamide (2ml) was stirred at 65°C for 3 hours. The volatiles were removed by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (75/8/1). The purified product was triturated with ether, collected by filtration, washed with ether and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-([N-methyl-N-(1-methylpiperidin-4-yl)]amino)ethoxy)quinazoline** (180mg, 38%) as a pale yellow powder.

m.p. 191-192°C

^{1}H NMR Spectrum: (DMSOd_6) 1.44(m, 2H); 1.70(m, 2H); 1.86(m, 2H); 2.15(s, 3H); 2.30(s, 3H); 2.78(m, 2H); 2.88(t, 2H); 3.94(s, 3H); 4.18(t, 2H); 7.19(s, 1H); 7.33(m, 1H); 7.52(m, 1H); 7.58(t, 1H); 7.78(s, 1H); 8.34(s, 1H); 9.48(s, 1H)

MS - ESI: 474 $[MH]^+$

Elemental analysis:	Found	C 60.9	H 6.3	N 14.7
$C_{24}H_{29}N_5O_2ClF$	Requires	C 60.8	H 6.2	N 14.8%

Example 38

1,1'-(Azodicarbonyl)dipiperidine (560mg, 2.2mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (240mg, 0.75mmol), (prepared as described for the starting material in Example 2), tetrahydro-3-furanmethanol (90mg, 0.88mmol) and tributylphosphine (440mg, 2.2mmol) in methylene chloride (12ml) and the mixture stirred for 18 hours. The mixture was diluted with ether, and the resulting precipitate was removed by filtration. The solvent was removed from the filtrate by

evaporation, and the residue was dissolved in acetone and ethereal hydrogen chloride (0.75ml of a 1M solution, 0.75mmol) was added. The mixture was diluted with ether and the resulting precipitate was collected by filtration. The solid was purified by column chromatography eluting with methylene chloride/acetonitrile/methanol (a gradient from 50/50/1 to 50/50/2). The purified product was triturated with ether, collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(tetrahydrofuran-3-ylmethoxy)quinazoline** (93mg, 31%).

m.p. 201-202°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.70(m, 1H); 2.05(m, 1H); 2.72(m, 1H); 3.56(m, 1H); 3.66(q, 1H); 3.79(m, 2H); 3.94(s, 3H); 4.08(m, 2H); 7.20(s, 1H); 7.32(m, 1H); 7.52(dd, 1H); 7.58(t, 1H); 7.78(t, 1H); 8.35(s, 1H); 9.52(s, 1H)

MS - ESI: 404 $[MH]^+$

Elemental analysis:	Found	C 59.2	H 4.6	N 10.6
$C_{20}H_{19}N_3O_3ClF$	Requires	C 59.5	H 4.7	N 10.4%

Example 39

1,1'-(Azodicarbonyl)dipiperidine (5.6g, 22mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (2.4g, 7.5mmol), (prepared as described for the starting material in Example 2), tributylphosphine (4.4g, 22mmol) and 1-(2-hydroxyethyl)-2-pyrrolidinone (1.1g, 8.5mmol) in methylene chloride (105ml). The mixture was stirred for 18 hours, diluted with ether (100ml) and the resulting precipitate was removed by filtration. The volatiles were removed from the filtrate by evaporation, and the residue was dissolved in acetone and ethereal hydrogen chloride (15ml of a 1M solution, 15mmol) was added. The solid was collected by filtration and was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (150/8/1). The purified product was dissolved in acetone and ethereal hydrogen chloride (15ml of a 1M solution, 15mmol) was added. The resulting precipitate was collected by filtration, washed with ether and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-oxopyrrolidin-1-yl)ethoxy)quinazoline** hydrochloride (2.1g, 60%).

m.p. 250-252°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.92(m, 2H); 2.22(t, 2H); 3.52(t, 2H); 3.68(t, 2H); 4.02(s, 3H); 4.30(t, 2H); 7.38(s, 1H); 7.42(m, 1H); 7.58(t, 1H); 7.66(dd, 1H); 8.35(s, 1H); 8.79(s, 1H); 11.69(br s, 1H)

MS - ESI: 431 $[MH]^+$

Elemental analysis:	Found	C 53.5	H 4.4	N 12.2	
$C_{21}H_{20}N_4O_3ClF$ 0.1H_2O 1HCl	Requires	C 53.8	H 4.6	N 11.9%	

Example 40

1,1'-(Azodicarbonyl)dipiperidine (525mg, 2.1mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (225mg, 7.0mmol), (prepared as described for the starting material in Example 2), tributylphosphine (420mg, 2.1mmol) and 1-(2-hydroxyethyl)-2-imidazolidinone (100mg, 7.7mmol) in methylene chloride (10ml). The mixture was stirred for 18 hours, diluted with ether and the resulting precipitate was removed by filtration. The volatiles were removed from the filtrate by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (150/8/1). The purified product was triturated with ether collected by filtration, washed with ether and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-oxoimidazolidin-1-yl)ethoxy)quinazoline** (19mg, 6%).

m.p. >250°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.27(t, 2H); 3.53(m, 4H); 3.97(s, 3H); 4.27(t, 2H); 6.39(s, 1H); 7.26(s, 1H); 7.35(m, 1H); 7.57(dd, 1H); 7.61(t, 1H); 7.82(s, 1H); 8.38(s, 1H); 9.55(s, 1H)

MS - ESI: 432 $[MH]^+$

Elemental analysis:	Found	C 53.7	H 4.4	N 15.4
$C_{20}H_{19}N_5O_3ClF$ 1H_2O	Requires	C 53.4	H 4.7	N 15.6%

Example 41

1,1'-(Azodicarbonyl)dipiperidine (525mg, 2.1mmol) was added in portions to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (225mg, 7.0mmol), (prepared as described for the starting material in Example 2), tributylphosphine (420mg, 2.1mmol) and 4-(2-hydroxyethyl)-1,1-dioxothiomorpholine (140mg, 7.8mmol) in methylene chloride (10ml). The mixture was stirred for 18 hours, diluted with ether and the resulting

precipitate was removed by filtration. The volatiles were removed from the filtrate by evaporation, and the residue was dissolved in acetone and ethereal hydrogen chloride (14ml of a 1M solution, 14mmol) and the precipitate was collected by filtration. The residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (150/8/1). The purified product was triturated with ether/methylene chloride collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-7-(2-(1,1-dioxothiomorpholino)ethoxy)-6-methoxyquinazoline** (120mg, 36%).

m.p. 246-249°C

^{1}H NMR Spectrum: ($DMSOd_6$) 3.03(t, 2H); 3.10(br s, 8H); 3.95(s, 3H); 4.27(t, 2H); 7.24(s, 1H); 7.38(m, 1H); 7.53(dd, 1H); 7.58(t, 1H); 7.80(s, 1H); 8.35(s, 1H); 9.52(s, 1H)

MS - ESI: 481 $[MH]^+$

Elemental analysis:	Found	C 52.0	H 4.6	N 11.9	S 6.6
$C_{21}H_{22}N_4O_4ClFS$	Requires	C 52.4	H 4.6	N 11.6	S 6.7%

Example 42

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (94mg, 4.9mmol) was added to a mixture of 7-(3-carboxypropoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (164mg, 0.4mmol), morpholine (0.11g; 1.26mmol) and 4-dimethylaminopyridine (200mg, 1.64mmol) in DMF (5ml). The reaction mixture was stirred at ambient temperature for 24 hours and the volatiles were removed by evaporation. Water was added to the residue and the aqueous mixture was extracted with methylene chloride (3x30ml). The extracts were combined and the solvent removed by evaporation. The residue was triturated with ether and the precipitate was collected by filtration. The solid was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (100/8/1). The purified product was triturated with acetone, collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinocarbonylpropoxy)quinazoline** (88mg, 46%).

m.p. 216-217°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.02(m, 2H); 2.5(m, 2H); 3.45(m, 4H); 3.55(m, 4H); 3.92(s, 3H); 4.15(t, 2H); 7.18(s, 1H); 7.32(d, 1H); 7.55(m, 2H); 7.78(s, 1H); 8.34(s, 1H); 9.52(s, 1H)

MS - ESI: 475 $[MH]^+$

Elemental analysis:	Found	C 58.2	H 5.2	N 12.2
$C_{23}H_{24}N_4O_4ClF$	Requires	C 58.2	H 5.1	N 11.8%

The starting material was prepared as follows:

Ethyl 4-chlorobutyrate (0.154ml, 1.1mmol) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (319.5mg, 1mmol), (prepared as described for the starting material in Example 2), and anhydrous potassium carbonate (690mg, 5mmol) in DMF (10ml). The mixture was stirred and heated at 105°C for 4 hours then allowed to cool. The mixture was diluted with methylene chloride and the insolubles were removed by filtration. The solvent was removed from the filtrate by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (100/8/1). The purified product was triturated with ether collected by filtration and dried to give 4-(4-chloro-2-fluoroanilino)-7-(3-ethoxycarbonylpropoxy)-6-methoxyquinazoline (230mg, 53%).

^{1}H NMR Spectrum: ($DMSOd_6$) 1.18(t, 3H); 2.02(m, 2H); 2.48(m, 2H); 3.94(s, 3H); 4.06(q. 2H); 4.15(t, 2H); 7.18(s, 1H); 7.32(m, 1H); 7.54(m, 2H); 7.78(s, 1H); 8.34(s, 1H); 9.52(s, 1H)
MS - ESI: 434 $[MH]^+$

Elemental analysis:	Found	C 58.0	H 4.8	N 9.8
$C_{21}H_{21}N_3O_4ClF$	Requires	C 58.1	H 4.9	N 9.7%

A mixture of 4-(4-chloro-2-fluoroanilino)-7-(3-ethoxycarbonylpropoxy)-6-methoxyquinazoline (220mg, 0.5mmol) in aqueous sodium hydroxide solution (4ml of a 2M solution, 8mmol), water (2ml) and methanol (0.5ml) was stirred and heated at 40°C for 3 hours. The mixture was allowed to cool and was then acidified with 2M hydrochloric acid. The resulting white precipitate was collected by filtration and washed with acetone and water to give 7-(3-carboxypropoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (170mg, 83%).

Example 43

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (145mg, 0.75mmol) was added to a mixture of 7-(3-carboxypropoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (250mg, 0.62mmol), (prepared as described for the starting material in Example 42), 1-methylpiperazine (0.21ml, 2.32mmol) and 4-dimethylaminopyridine (300mg,

2.46mol) in DMF (7.5ml). The reaction mixture was stirred at ambient temperature for 24 hours and the volatiles were removed by evaporation. Water was added to the residue and the aqueous mixture was extracted with methylene chloride (3x30ml). The combined organic extracts were washed with brine and the solvent was removed by evaporation. The residue was triturated with ether and the precipitate was collected by filtration. The solid was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (100/8/1). The purified product was triturated with ether collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(4-methylpiperazin-1-ylcarbonyl)propoxy)quinazoline** (133mg, 44%).

m.p. 248-250°C

^{1}H NMR Spectrum: ($DMSOd_6$) 2.00(t, 2H); 2.15(s, 3H); 2.25(m, 4H); 2.45(m, 2H); 3.45(m, 4H); 3.92(s, 3H); 4.15(t, 2H); 7.18(s, 1H); 7.30(d, 1H); 7.55(m, 2H); 7.78(s, 1H); 8.34(s, 1H); 9.52(s, 1H)

MS - ESI: 488 $[MH]^+$

Elemental analysis:	Found	C 58.6	H 5.5	N 13.9
$C_{24}H_{27}N_5O_3ClF\ 0.2H_2O$	Requires	C 58.6	H 5.6	N 14.3%

Example 44

Oxalyl chloride (0.4ml, 2.2mmol) was added to a suspension of 7-(3-carboxypropoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (260mg, 0.64mmol), (prepared as described for the starting material in Example 42), in methylene chloride (25ml) followed by 1 drop of DMF. The mixture was stirred at ambient temperature for 2.5 hours and the volatiles were removed by evaporation. A solution of pyrrolidine (0.13ml, 2.1mmol) in N,N-dimethylacetamide (8ml) was added to the solid residue and the mixture was stirred at ambient temperature for 2 hours. The volatiles were removed by evaporation and the residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (100/8/1). The purified product was triturated with acetone, collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-pyrrolidin-1-ylcarbonylpropoxy)quinazoline** (206mg, 70%).

m.p. 254-256°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.76(m, 2H); 1.85(m, 2H); 2.02(m, 2H); 2.41(t, 2H); 3.26(t, 2H); 3.38(t, 2H); 3.95(s, 3H); 4.15(t, 2H); 7.18(s, 1H); 7.32(d, 1H); 7.55(m, 2H); 7.78(s, 1H); 8.34(s, 1H); 9.52(s, 1H)

MS - ESI: 459 $[MH]^+$

Elemental analysis:	Found	C 59.9	H 5.3	N 12.0
$C_{23}H_{24}N_4O_3ClF$	Requires	C 60.2	H 5.3	N 12.2%

Example 45

A mixture of 4-(4-chloro-2-fluoroanilino)-7-(2,2-dimethoxyethoxy)-6-methoxyquinazoline (210mg, 0.52mmol), water (5ml) and TFA (5ml) was stirred at ambient temperature for 3 hours then heated at 60°C for 1 hour. The solution was allowed to cool, then diluted with water and the resulting precipitate was collected by filtration and dried. The solid was dissolved in methanol (10ml) and cyclopentylamine (0.057ml, 0.57mmol) and dried 3Å molecular sieves (2.5g) were added. The mixture was stirred for 30 minutes, glacial acetic acid (0.20ml, 3.2mmol) and sodium cyanoborohydride (150mg, 2.4mmol) were added and the reaction stirred for 4 hours then left to stand for 18 hours. The insolubles were removed by filtration and the solvent was removed from the filtrate by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (100/8/1). The purified product was triturated with ether/hexane, collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-7-(2-cyclopentylaminoethoxy)-6-methoxyquinazoline** (80mg, 36%).

m.p. 171-173°C

^{1}H NMR Spectrum: ($DMSOd_6$) 1.55(m, 8H); 2.94(t, 2H); 3.08(m, 1H); 3.94(s, 3H); 4.19(t, 2H); 7.19(s, 1H); 7.33(m, 1H); 7.52(dd, 1H); 7.59(t, 1H); 7.78(s, 1H); 8.34(s, 1H); 9.50(s, 1H)

MS - ESI: 431 $[MH]^+$

The starting material was prepared as follows:

Bromoacetaldehyde dimethyl acetal (0.74ml, 3.1mmol) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (1.0g, 3.13mmol), (prepared as described for the starting material in Example 2), and anhydrous potassium carbonate (2.16g, 15.6mmol) in DMF (30ml). The mixture was stirred and heated at 110°C for 4 hours, then

allowed to cool and the volatiles were removed by evaporation. Water was added to the residue and the aqueous mixture was extracted with methylene chloride (x4). The extracts were combined, washed with brine and dried by filtration through phase separating paper. The volatiles were removed by evaporation, the residue was triturated with ether, collected by filtration and dried to give 4-(4-chloro-2-fluoroanilino)-7-(2,2-dimethoxyethoxy)-6-methoxyquinazoline (440mg, 35%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.36(s, 6H); 3.94(s, 3H); 4.05(d, 2H); 4.75(t, 1H); 7.22(s, 1H); 7.32(m, 1H); 7.52(m, 1H); 7.58(t, 1H); 7.80(s, 1H); 8.35(s, 1H); 9.52(s, 1H)

MS - ESI: 408 $[MH]^+$

Example 46

Diethyl azodicarboxylate (1.55ml, 9.89mmol), 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (1.2g, 3.3mmol), (prepared as described for the starting material in Example 48), and a solution of (E)-4-(pyrrolidin-1-yl)but-2-en-1-ol (697mg, 4.9mmol), (prepared as described for the starting material in Example 17), in methylene chloride (5ml) were added successively to a solution of triphenylphosphine (2.59g, 9.89mmol) in methylene chloride (150ml) cooled at 5°C. The mixture was stirred at ambient temperature for 10 minutes then methylene chloride (100ml) was added followed successively by triphenylphosphine (432mg, 1.6mmol), (E)-4-(pyrrolidin-1-yl)but-2-en-1-ol (232mg, 1.6mmol) and diethyl azodicarboxylate (246µl, 1.6mmol). The mixture was stirred at ambient temperature for 30 minutes and then the solvent was removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (8/2 followed by 7/3 and 6/4). The semi-purified product was repurified by column chromatography eluting with methylene chloride/methanol (8/2 followed by 7.5/2.5). The purified product was dissolved in methylene chloride, 3.7M ethereal hydrogen chloride (3ml) was added and the volatiles were removed by evaporation. The residue was triturated with ether, collected by filtration and dried under vacuum to give **(E)-4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(4-pyrrolidin-1-ylbut-2-en-1-yloxy)quinazoline** hydrochloride (600mg, 32%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.8-1.9(m, 2H); 2.0-2.1(m, 2H); 3.0-3.1(m, 2H); 3.45-3.55(m, 2H); 3.88(d, 2H); 4.01(s, 3H); 4.9(d, 2H); 6.0(td, 1H); 6.3(td, 1H); 7.41(s, 1H); 7.5-7.65(m, 2H); 7.82(d, 1H); 8.13(s, 1H); 8.88(s, 1H)

MS - (EI): 487 $[M.]^+$

Elemental analysis: Found C 48.2 H 4.9 N 9.6

$C_{23}H_{24}N_4O_2BrF\ 0.5H_2O\ 2HCl$ Requires C 48.5 H 4.8 N 9.8%

Example 47

Diethyl azodicarboxylate (261mg, 1.5mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (160mg, 0.5mmol), (prepared as described for the starting material in Example 2), triphenylphosphine (393mg, 1.5mmol) and 1-(3-hydroxypropyl)-2-pyrrolidinone (107mg, 0.75mmol) in methylene chloride (5ml) under nitrogen. The mixture was stirred for 20 minutes at ambient temperature and then purified by pouring directly onto a column of silica eluting with methylene chloride/ethyl acetate/methanol (60/35/5 followed by 60/30/10). The purified product was triturated with ether and collected by filtration. The solid was dissolved in ethyl acetate and treated with 3M hydrogen chloride in ethyl acetate (0.4ml). The precipitate was collected by filtration, washed with ethyl acetate and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(2-oxopyrrolidin-1-yl)propoxy)quinazoline** hydrochloride (170mg, 70%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.9-2.0(m, 2H); 2.0-2.1(m, 2H); 2.21(t, 2H); 3.4-3.5(m, 4H); 4.02(s, 3H); 4.20(s, 3H); 4.20(t, 2H); 7.32(s, 1H); 7.46(dd, 1H); 7.63(t, 1H); 7.71(dd, 1H); 8.17(s, 1H); 8.87(s, 1H)

MS - ESI: 445 $[MH]^+$

Elemental analysis : Found C 54.9 H 4.7 N 11.6

$C_{22}H_{22}N_4O_3ClF\ 0.3H_2O\ 0.85HCl$ Requires C 54.9 H 4.9 N 11.6%

The starting material was prepared as follows:

A solution of γ-butyrolactone (8.6g, 0.1mol) and 3-amino-1-propanol (9g, 0.12mol) was heated at reflux for 18 hours. The crude product mixture was distilled under reduced pressure to give 1-(3-hydroxypropyl)-2-pyrrolidinone (2.5g, 17%).

b.p. ~ 130°C under ~0.05mmHg

^{1}H NMR Spectrum: ($CDCl_3$) 1.7-1.8(m, 3H); 2.0-2.15(m, 2H); 2.44(t, 2H); 3.4-3.5(m, 4H); 3.54(t, 2H)

MS - (EI): 143 $[M\cdot]^+$

Example 48

Using a method analogous to that in Example 47, 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (146mg, 0. mmol) in methylene chloride (5ml) was treated with 1-(3-hydroxypropyl)-2-pyrrolidinone (86mg, 0.6mmol), triphenylphosphine (314mg, 1.2mmol) and diethyl azodicarboxylate (209mg, 1.2mmol) and was purified and isolated to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-(2-oxopyrrolidin-1-yl)propoxy)quinazoline** hydrochloride (140mg, 67%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.9-2.0(m, 2H); 2.0-2.1(m, 2H); 2.21(t, 2H); 3.4-3.5(m, 4H); 4.02(s, 3H); 4.20(t, 2H); 7.32(s, 1H); 7.5-7.65(m, 2H); 7.82(d, 1H); 8.15(s, 1H); 8.87(s, 1H)

MS - ESI: 490 $[MH]^+$

Elemental analysis:	Found	C 49.9	H 4.4	N 10.5
$C_{22}H_{22}N_4O_3BrF$ 0.2H_2O 0.95HCl	Requires	C 50.1	H 4.5	N 10.6%

The starting material was prepared as follows:

A solution of 7-benzyloxy-4-chloro-6-methoxyquinazoline (8.35g, 27.8mmol), (prepared as described for the starting material in Example 1), and 4-bromo-2-fluoroaniline (5.65g, 29.7mmol) in 2-propanol (200ml) was heated at reflux for 4 hours. The resulting precipitate was collected by filtration, washed with 2-propanol and then ether and dried under vacuum to give 7-benzyloxy-4-(4-bromo-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (9.46g, 78%).

^{1}H NMR Spectrum: ($DMSOd_6$; CD_3COOD) 4.0(s, 3H); 5.37(s, 2H); 7.35-7.5(m, 4H); 7.52-7.62(m, 4H); 7.8(d, 1H); 8.14(9s, 1H); 8.79(s, 1H)

MS - ESI: 456 $[MH]^+$

Elemental analysis:	Found	C 54.0	H 3.7	N 8.7
$C_{22}H_{17}N_3O_2BrF$ 0.9HCl	Requires	C 54.2	H 3.7	N 8.6%

A solution of 7-benzyloxy-4-(4-bromo-2-fluoroanilino)-6-methoxyquinazoline hydrochloride (9.4g, 19.1mmol) in TFA (90ml) was heated at reflux for 50 minutes. The mixture was allowed to cool and was poured on to ice. The resulting precipitate was collected by filtration and dissolved in methanol (70ml). The solution was adjusted to pH9-10 with concentrated aqueous ammonia solution. The mixture was concentrated to half initial volume by evaporation. The resulting precipitate was collected by filtration, washed with water and then ether, and dried under vacuum to give 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (5.66g, 82%).

^{1}H NMR Spectrum: ($DMSOd_6$; CD_3COOD) 3.95(s, 3H); 7.09(s, 1H); 7.48(s, 1H); 7.54(t, 1H); 7.64(d, 1H); 7.79(s, 1H); 8.31(s, 1H)

MS - ESI: 366 $[MH]^+$

Elemental analysis:	Found	C 49.5	H 3.1	N 11.3
$C_{15}H_{11}N_3O_2BrF$	Requires	C 49.5	H 3.0	N 11.5%

Example 49

Methanesulphonyl chloride (32mg, 0.275mmol) was added dropwise to a mixture of 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-methylaminopropoxy)quinazoline (109mg, 0.25mmol) and triethylamine (30mg, 0.3mmol) in methylene chloride (3ml) cooled at 0°C. The solution was stirred for 2 hours at 0°C and the volatiles were removed by evaporation. The residue was partitioned between ethyl acetate and water, the organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The solid was triturated with ether and collected by filtration. The solid was dissolved in methylene chloride containing methanol (0.5ml) and 3M hydrogen chloride in ethyl acetate (0.3ml) was added. The suspension was diluted with ethyl acetate and concentrated by evaporation. The resulting solid product was collected by filtration, washed with ether and dried under vacuum to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-([N-methyl-N-methylsulphonyl]amino)propoxy)quinazoline** hydrochloride (85mg, 61%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.1-2.2(m, 2H); 2.82(s, 3H); 2.89(s, 3H); 3.29(t, 2H); 4.02(s, 3H); 4.27(t, 2H); 7.35(s, 1H); 7.55-7.65(m, 2H); 7.79(d, 1H); 8.12(s, 1H); 8.88(s, 1H)

MS - (EI): 512 $[M.]^+$

Elemental analysis:	Found	C 43.5	H 4.2	N 10.0
$C_{20}H_{22}N_4O_4BrFS\ 0.6H_2O\ 0.75HCl$	Requires	C 43.5	H 4.4	N 10.2%

The starting material was prepared as follows:

Diethyl azodicarboxylate (522mg, 3mmol) was added dropwise to a suspension of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (364mg, 1mmol), (prepared as described for the starting material in Example 48), triphenylphosphine (786mg, 3mmol) and 3-methylamino-1-propanol (178mg, 2mmol), (J.Am.Chem.Soc., 1954, 76, 2789), in methylene chloride (4ml) under nitrogen. The mixture was stirred for 1 hour at ambient temperature, neutral alumina (~ 20g) was added to the reaction mixture and the solvent was removed by evaporation. The powder was poured onto a column of neutral alumina and was eluted with a mixture of methylene chloride/methanol (95/5 followed by 90/10 and 80/20). The purified product was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(3-methylaminopropoxy)quinazoline (220mg, 50%).

Example 50

A solution of diethyl azodicarboxylate (209mg, 1.2mmol) in methylene chloride (1ml) and then (S)-1-(3-hydroxypropyl)-pyrrolidine-2-carboxamide (97mg, 0.56mmol) was added dropwise to a suspension of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (146mg, 0.4mmol), (prepared as described for the starting material in Example 48), and triphenylphosphine (314mg, 1.2mmol) in methylene chloride (4ml) under nitrogen. The mixture was stirred for 1 hour at ambient temperature and further triphenylphosphine (109mg, 0.4mmol) and (S)-1-(3-hydroxypropyl)-pyrrolidine-2-carboxamide (40mg, 0.23mmol) were added followed by the dropwise addition of diethyl azodicarboxylate (70mg, 0.4mmol). The mixture was stirred for 30 minutes at ambient temperature and further (S)-1-(3-hydroxypropyl)-pyrrolidine-2-carboxamide (34mg, 0.2mmol) was added. The mixture was then stirred for 2 hours at ambient temperature and the mixture was purified by pouring directly onto a column of silica and eluting with methylene chloride/ethyl acetate/methanol (60/35/5). The purified product was triturated with ether, collected by filtration, washed with ether and dried under vacuum. The solid was

dissolved in methylene chloride and 3M hydrogen chloride in ethyl acetate (0.4ml) was added. The resulting precipitate was collected by filtration, washed with ethyl acetate and dried under vacuum to give **(S)-4-(4-bromo-2-fluoroanilino)-7-(3-(2-carbamoylpyrrolidin-1-yl)propoxy)-6-methoxyquinazoline** hydrochloride (110mg, 47%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD; 60°C) 1.9-2.0(m, 2H); 2.0-2.1(m, 2H); 2.15-2.25(m, 2H); 3.2-3.3(m, 1H); 3.3-3.5(m, 2H); 3.7-3.8(m, 1H); 4.02(s, 3H); 4.15-4.2(m, 1H); 4.3-4.4(m, 2H); 7.4(s, 1H); 7.5-7.6(m, 2H); 7.75(d, 1H); 8.2(s, 1H); 8.83(s, 1H)

MS - (EI): 518 $[M.]^+$

Elemental analysis:	Found	C 46.0	H 4.9	N 11.2
$C_{23}H_{25}N_5O_3BrF$ $0.8H_2O$ 1.9HCl	Requires	C 45.9	H 4.8	N 11.6%

The starting material was prepared as follows:

3-Bromo-1-propanol (584mg, 4.2mmol) was added to a mixture of (S)-pyrrolidine-2-carboxamide (399mg, 3.5mmol) and potassium carbonate (966mg, 7mmol) in acetonitrile (10ml). The mixture was heated at reflux for 5 hours and the mixture was stirred for 18 hours at ambient temperature. The insolubles were removed by filtration and the solvent was removed from the filtrate by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/methanol (9/1 followed by 8/2) to give (S)-1-(3-hydroxypropyl)-pyrrolidine-2-carboxamide (365mg, 60%).

MS - (EI): 173 $[M.]^+$

Example 51

Methoxyacetyl chloride (34mg, 0.31mmol) was added to a solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylaminoethoxy)quinazoline (113mg, 0.3mmol), (prepared as described for the starting material in Example 60), and triethylamine (33mg, 0.33mmol) in methylene chloride (3ml). The mixture was stirred for 18 hours at ambient temperature and was then partitioned between ethyl acetate and brine. The organic layer was separated, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by chromatography on silica eluting with methylene chloride/acetonitrile/methanol (6/3/1). The purified solid product was triturated with methylene chloride and ether, collected by filtration, washed with ether and dried under vacuum. The solid was dissolved in a mixture of

methylene chloride/methanol (1/1) and 2M hydrogen chloride in ethyl acetate (0.5ml) was added. The mixture was diluted with ether and and the resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-([N-methyl-N-methoxyacetyl]amino)ethoxy)quinazoline** hydrochloride (62mg, 42%).

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD; 80°C) 2.9-3.2(br s, 3H); 3.35(s, 3H); 3.8-3.9(br s, 2H); 4.05(s, 3H); 4.0-4.3(m, 2H); 4.4(t, 2H); 7.4(s, 1H); 7.45(d, 1H); 7.6-7.7(m, 2H); 8.1(s, 1H); 8.8(s, 1H)

MS - ESI: 449 $[MH]^+$

Elemental analysis:	Found	C 48.8	H 4.6	N 10.7
$C_{21}H_{22}N_4O_4ClF$ 0.9H_2O 1.35HCl	Requires	C 49.0	H 4.9	N 10.9%

Example 52

Diethyl azodicarboxylate (400mg, 2.3mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (250mg, 0.78mmol), (prepared as described for the starting material in Example 2), triphenylphosphine (615mg, 2.3mmol) and 4-(2-hydroxyethyl)-3-morpholinone (170mg, 1.17mmol), (EP 580402A2), in methylene chloride (5ml) under nitrogen. The mixture was stirred for 4 hours at ambient temperature, methylene chloride (5ml) was added and stirring was continued for a further 18 hours at ambient temperature. THF (5ml), 4-(2-hydroxyethyl)-3-morpholinone (113mg, 0.78mmol), triphenylphosphine (204mg, 0.78mmol) were added and diethyl azodicarboxylate (136mg, 0.78mmol) was then added dropwise. The mixture was stirred for 5 minutes at ambient temperature, and was purified by pouring directly onto a silica column, eluting with methylene chloride/ethyl acetate/methanol (5/4/1). The purified solid was dissolved in methylene chloride and 2M methanolic hydrogen chloride (0.5ml) was added. The mixture was concentrated by evaporation and then diluted with ether. The resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(3-oxomorpholino)ethoxy)quinazoline** hydrochloride (150mg, 39%).

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD) 3.6(t, 2H); 3.8-3.9(m, 4H); 4.05(s, 3H); 4.1(s, 3H); 4.4(t, 2H); 7.3(s, 1H); 7.45(d, 1H); 7.65(t, 1H); 7.7(d, 1H); 8.1(s, 1H); 8.9(s, 1H)

MS - ESI: 469 $[MNa]^+$

Elemental analysis:	Found	C 51.6	H 4.4	N 11.8
$C_{21}H_{20}N_4O_4ClF\ 0.35H_2O\ 0.95HCl$	Requires	C 51.7	H 4.5	N 11.5%

Example 53

Diethyl azodicarboxylate (209mg, 1.2mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (128mg, 0.4mmol), (prepared as described for the starting material in Example 2), triphenylphosphine (314mg, 1.2mmol) and 2-(2-morpholinoethoxy)ethanol (97mg, 0.56mmol) in methylene chloride (4ml) under nitrogen. The mixture was stirred for 1 hour at ambient temperature, triphenylphosphine (105mg, 0.4mmol), 2-(2-morpholinoethoxy)ethanol (49mg, 0.28mmol) and diethyl azodicarboxylate (70mg, 0.4mmol) were added. The mixture was stirred for 1 hour at ambient temperature and was purified by pouring directly onto a silica column eluting with methylene chloride/acetonitrile/methanol (6/3/1). The purified product was triturated with ether, collected by filtration and dissolved in methylene chloride. 2M Ethereal hydrogen chloride (0.5ml) was added and the resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-morpholinoethoxy)ethoxy)quinazoline** hydrochloride (100mg, 45%).

^1H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.1-3.2(m, 2H); 3.3-3.5(m, 5H); 3.7-3.8(m, 2H); 3.9-4.0(m, 5H); 4.02(s, 3H); 4.4(br s, 2H); 7.46(s, 1H); 7.48(d, 1H); 7.6(t, 1H); 7.7(d, 1H); 8.25(s, 1H); 8.89(s, 1H)

MS - ESI: 477 $[MH]^+$

Elemental analysis:	Found	C 48.8	H 5.6	N 9.9
$C_{23}H_{26}N_4O_4ClF\ 1H_2O\ 1.95HCl$	Requires	C 48.8	H 5.3	N 9.9%

The starting material was prepared as follows:

2-(2-Chloroethoxy)ethanol (1.25g, 10mmol) was added to a mixture of morpholine (2.58g, 30mmol) and potassium carbonate (5.5g, 40mmol) in acetonitrile (50ml). The mixture was heated at reflux for 6 hours and then stirred for 18 hours at ambient temperature. The insolubles were removed by filtration and the volatiles were removed from the filtrate by evaporation. The residue was purified by column chromatography eluting with methylene

chloride/methanol (95/5 followed by 90/10 and then 80/20) to give 2-(2-morpholinoethoxy)ethanol (600mg, 34%).

^{1}H NMR Spectrum: ($CDCl_3$) 2.5(br s, 4H); 2.59(t, 2H); 3.6-3.85(m, 10H)

MS - (EI): 175 $[M.]^+$

Example 54

Diethyl azodicarboxylate (209mg, 1.2mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (128mg, 0.4mmol), (prepared as described for the starting material in Example 2), triphenylphosphine (314mg, 1.2mmol) and (S)-1-(3-hydroxypropyl)-pyrrolidine-2-carboxamide (97mg, 0.56mmol), (prepared as described for the starting material in Example 50), in methylene chloride (4ml). The mixture was stirred for 2 hours at ambient temperature, and further triphenylphosphine (105mg, 0.4mmol) and (S)-1-(3-hydroxypropyl)-pyrrolidine-2-carboxamide (49mg, 0.28mmol) were added followed by the dropwise addition of diethyl azodicarboxylate (70mg, 0.4mmol). The mixture was stirred for 1 hour at ambient temperature, and was purified by pouring directly onto a silica column eluting with methylene chloride/acetonitrile/methanol (6/3/1 followed by 60/25/15). The purified oil was triturated with ether, collected by filtration, washed with ether and dried under vacuum. The solid was dissolved in methylene chloride and 2M ethereal hydrogen chloride (0.5ml) was added. The mixture was diluted with ether and the resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **(S)-7-(3-(2-carbamoylpyrrolidin-1-yl)propoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline** hydrochloride (70mg, 32%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.8-2.0(m, 2H); 2.05-2.15(m, 2H); 2.2-2.3(m, 2H); 3.1-3.5(m, 2H); 3.7-3.8(m, 1H); 4.02(s, 3H); 4.05-4.2(m, 2H); 4.3(m, 2H); 7.04(s, 1H); 7.45(d, 1H); 7.65(t, 1H); 7.7(d, 1H); 8.22(s, 1H); 8.88(s, 1H)

MS - ESI: 474 $[MH]^+$

Elemental analysis:	Found	C 49.4	H 12.4	N 5.3
$C_{23}H_{25}N_5O_3ClF$ 1.5H_2O 1.55HCl	Requires	C 49.5	H 12.6	N 5.3%

Example 55

Diethyl azodicarboxylate (209mg, 1.2mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (128mg, 0.4mmol), (prepared as described for the starting material in Example 2), cis-3-(2,6-dimethylmorpholino)-1-propanol (97mg, 0.56mmol) and triphenylphosphine (314mg, 1.2mmol) in methylene chloride (4ml) under nitrogen. The mixture was stirred for 1 hour at ambient temperature and the solvent was removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (95/5 followed by 90/10). The purified product was dissolved in methylene chloride and 2M ethereal hydrogen chloride (1ml) was added. The solution was diluted with ether and left to stand. The resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-7-(3-(2,6-dimethylmorpholino)propoxy)-6-methoxyquinazoline** hydrochloride (130mg, 59%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.17(d, 6H); 2.3-2.4(m, 2H); 2.7(t, 2H); 3.25-3.35(m, 2H); 3.55(d, 2H); 3.9-4.0(m, 2H); 4.03(s, 3H); 4.35(t, 2H); 7.43(s, 1H); 7.45(d, 1H); 7.63(t, 1H); 7.70(d, 1H); 8.25(s, 1H); 8.88(s, 1H)

MS - ESI: 475 $[MH]^+$

Elemental analysis:	Found	C 51.7	H 6.0	N 9.7
$C_{24}H_{28}N_4O_3ClF$ 0.6H_2O 1.95HCl	Requires	C 51.8	H 5.6	N 10.0%

The starting material was prepared as follows:

3-Chloro-1-propanol (1.04g, 11mmol) followed by potassium carbonate (2.07g, 15mmol) was added to a solution of 2,6-dimethylmorpholine (1.15g, 10mmol), (supplied by Aldrich Chemical Company Limited as a mixture of isomers), in acetonitrile (15ml). The mixture was heated at reflux overnight and allowed to cool, the insolubles were removed by filtration and the volatiles were removed from the filtrate by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/acetonitrile/methanol (60/35/5 followed by 60/30/10) to give cis-3-(2,6-dimethylmorpholino)-1-propanol (500mg).

^{1}H NMR Spectrum: ($CDCl_3$) 1.16(d, 6H); 1.7-1.8(m, 4H); 2.61(t, 2H); 2.91(d, 2H); 3.6-3.7(m, 2H); 3.81(t, 2H)

MS - ESI: 173 $[M.]^+$

Example 56

A solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(trifluoromethylsulphonyloxy)quinazoline (180mg, 0.4mmol), (prepared as described for the starting material in Example 11), in anhydrous THF (2ml) and benzene (2ml) was purged of oxygen and placed under nitrogen. Tetrakis(triphenylphosphine)palladium(0) (23mg, 0.02mmol) followed by a solution of sodium triisopropylsilylthiolate (102mg, 0.48mmol), (Tetrahedron Lett. 1994, 35, 3221), in THF (2ml) was added and the mixture was heated at reflux for 2 hours. The mixture was allowed to cool to ambient temperature and 4-(3-chloropropyl)morpholine (98mg, 0.6mmol), (J. Am. Chem. Soc. 1945, 67, 736), DMF (2ml) and tetrabutylammonium fluoride (0.5ml of a 1M solution in THF, 0.5mmol) were added sequentially. The mixture was stirred for 1 hour at ambient temperature, the volatiles were removed by evaporation and the residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified on neutral alumina eluting with methylene chloride/acetone (90/10 followed by 80/20). The purified product was triturated in a mixture of ether and hexane, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-morpholinopropylthio)quinazoline** hydrochloride (65mg, 30%).

^1H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.1-2.2(m, 2H); 3.1-3.2(m, 2H); 3.22(t, 2H); 3.3-3.4(m, 2H); 3.47(d, 2H); 3.74(t, 2H); 4.0(d, 2H); 4.08(s, 3H); 7.48(d, 1H); 7.64(t, 2H); 7.68(d, 1H); 7.86(s, 1H); 8.19(s, 1H); 8.91(s, 1H)

MS - ESI: 463 $[MH]^+$

Elemental analysis:	Found	C 47.6	H 5.16	N 47.6
$C_{22}H_{24}N_4O_2ClFS\ 1.2H_2O\ 1.85HCl$	Requires	C 47.8	H 5.16	N 47.8%

Example 57

3-Chloroperbenzoic acid (188mg, 1.05mmol) was added in portions to a solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethylthio)quinazoline (275mg, 0.7mmol), (prepared as described in Example 11), in methylene chloride (6ml). The mixture

was stirred for 30 minutes at ambient temperature, diluted with methylene chloride (20ml), washed with aqueous sodium hydrogen carbonate solution and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/acetone (8/2 followed by 7/3 and 6/4). The purified product was dissolved in methylene chloride and 3M ethereal hydrogen chloride (0.5ml) was added. The mixture was diluted with ether and the resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methoxyethylsulphinyl)quinazoline** hydrochloride (110mg 38%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.05(td, 1H); 3.24(s, 3H); 3.5-3.6(m, 1H); 3.7-3.8(m, 2H); 4.1(s, 3H); 7.5(d, 1H); 7.65(t, 1H); 7.75(d, 1H); 8.2(s, 1H); 8.4(s, 1H); 9.0(s, 1H)

MS - ESI: 410 $[MH]^+$

Elemental analysis:	Found	C 47.9	H 4.2	N 9.3
$C_{18}H_{17}N_3O_3ClFS$ 0.5H_2O 0.85HCl	Requires	C 48.0	H 4.2	N 9.3%

Example 58

Diethyl azodicarboxylate (218mg, 1.25mmol) was added dropwise to a solution of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (159mg, 0.5mmol), (prepared as described for the starting material in Example 2), 4-hydroxy-1-methylpiperidine (115mg, 1mmol) and triphenylphosphine (328mg, 1.25mmol) in methylene chloride (5ml) cooled at 5°C under nitrogen. The mixture was stirred for 1 hour at ambient temperature, the solvent was removed by evaporation and the residue was partitioned between 2M hydrochloric acid and ether. The aqueous layer was separated, adjusted to pH9 with aqueous sodium hydrogen carbonate solution and extracted with methylene chloride. The methylene chloride layer was washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified on neutral alumina eluting with methylene chloride/methanol (97/3). The purified product was triturated with ether, collected by filtration and dried to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-yloxy)quinazoline** (180mg, 79%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.9-2.0(m, 1H); 2.05-2.15(m, 2H); 2.35-2.45(m, 1H); 2.85 and 2.90(2s, 3H); 3.05-3.25(m, 2H); 3.45(m, 1H); 3.6(d, 1H); 4.1 and 4.12(2s, 3H); 4.8-4.9(m, 0.5 H); 5-5.05(m, 0.5H); 7.4-7.7(m, 4H); 8.2(d, 1H); 8.9(s, 1H)

MS - ESI: 417 $[MH]^+$

Example 59

Methanesulphonyl chloride (35µl, 0.46mmol) was added dropwise to a solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-methylaminopropoxy)quinazoline (170mg, 0.43mmol) and triethylamine (67µl, 0.48mmol) in methylene chloride (3ml). The mixture was stirred for 5 hours at ambient temperature, the volatiles was removed by evaporation and the residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with water and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/acetonitrile/methanol (70/28/2). The purified product was dissolved in a mixture of methylene chloride/methanol (1/1) and 2M ethereal hydrogen chloride (1ml) was added. The volatiles were removed by evaporation and the residue was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-([N-methyl-N-methylsulphonyl]amino)propoxy)quinazoline** hydrochloride (133mg, 61%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.1-2.2(m, 2H); 2.82(s, 3H); 2.89(s, 3H); 3.3(t, 2H); 4.02(s, 3H); 4.27(t, 2H); 7.36(s, 1H); 7.46(d, 1H); 7.6-7.7(m, 2H); 8.14(s, 1H); 8.88(s, 1H)

MS - ESI: 469 $[MH]^+$

Elemental analysis:	Found	C 48.1	H 4.7	N 10.8
$C_{20}H_{22}N_4O_4ClFS$ 0.9HCl	Requires	C 47.9	H 4.6	N 11.2%

The starting material was prepared as follows:

A solution of di-tert-butyl dicarbonate (4.9g, 22mmol) in THF (12ml) was added dropwise to a solution of 3-methylamino-1-propanol (2g, 22mmol), (J.Am.Chem.Soc., 1954, 76, 2789), in a mixture of THF (12ml) and water (12ml). The mixture was stirred for 18 hours at ambient temperature, the THF was removed by evaporation. The aqueous residue was extracted with ether. The extracts were combined, washed with 0.1M hydrochloric acid, and then brine, dried ($MgSO_4$) and the solvent removed by evaporation to give 3-([N-(tert-butylcarbonyl)-N-methyl]amino)-1-propanol (3.95g, 95%).

^{1}H NMR Spectrum: ($CDCl_3$) 1.46(s, 9H); 1.6-1.8(m, 2H); 2.83(s, 3H); 3.3-3.4(br s, 2H); 3.5-3.6(br s, 2H)

MS - (EI): 190 $[MH]^+$

Diethyl azodicarboxylate (2.4ml, 15mmol) was added dropwise to a solution of 3-([N-(tert-butylcarbonyl)-N-methyl]amino)-1-propanol (1.77g, 9.4mmol), 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (2g, 6.26mmol), (prepared as described for the starting material in Example 2), and triphenylphosphine (4.1g, 15mmol) in methylene chloride (50ml) under nitrogen. The mixture was stirred for 1 hour at ambient temperature, and further 3-([N-(tert-butylcarbonyl)-N-methyl]amino)-1-propanol (236mg, 1.2mmol), triphenylphosphine (820mg, 3.1mmol) and diethyl azodicarboxylate (492µl, 3.1mmol) were added. The solution was stirred for 1 hour at ambient temperature and concentrated by evaporation. The residue was purified on column chromatography eluting with acetonitrile. The purified product was triturated with ether, collected by filtration and repurified by column chromatography eluting with methylene chloride/methanol (97/3) to give 7-(3-([N-(tert-butylcarbonyl)-N-methyl]amino)propoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (2.2g, 72%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.3(s, 9H); 2.0-2.1(m, 2H); 2.8-2.9(br s, 3H); 3.4-3.5(m, 2H); 4.0(s, 3H); 4.25(t, 2H); 7.3(s, 1H); 7.45(d, 1H); 7.6-7.7(m, 2H); 8.08(s, 1H); 8.88(s, 1H)

MS - (EI): 491 $[MH]^+$

Elemental analysis:	Found	C 58.6	H 5.8	N 11.3	
$C_{24}H_{28}N_4O_4ClF$	Requires	C 58.7	H 5.7	N 11.4%	

A solution of 7-(3-([N-(tert-butylcarbonyl)-N-methyl]amino)propoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (2.1g, 4.3mmol) in a mixture of methylene chloride (6ml) and TFA (5ml) was stirred at ambient temperature for 1 hour. Toluene was added and the volatiles were removed by evaporation. The residue was dissolved in water and the solution was adjusted to pH7-8 with saturated aqueous sodium hydrogen carbonate solution. The resulting precipitate was separated by centrifugation and decanting the filtrate and the solid product was thoroughly washed with water. The solid was recrystallised from methylene chloride/methanol, the product collected by filtration, washed with water, and then

ether and dried under vacuum over phosphorus pentoxide to give 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-methylaminopropoxy)quinazoline (1.4g, 83%).
^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.2-2.3(m, 2H); 2.65(s, 3H); 3.1-3.2(m, 2H); 4.05(s, 3H); 4.32(t, 2H); 7.37(s, 1H); 7.48(d, 1H); 7.64(t, 1H); 7.67(d, 1H); 8.11(s, 1H); 8.9(s, 1H)
MS - (EI): 391 $[MH]^+$

Example 60

Triethylamine (44µl, 0.32mmol) and then a solution of 2-bromoethyl methyl ether (40mg, 0.29mmol) in acetone (0.5ml) was added dropwise to a solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylaminoethoxy)quinazoline (100mg, 0.26mmol) in acetone (2.5ml) heated at 50°C under nitrogen. The mixture was stirred for 7 hours at 50°C, the mixture was allowed to cool and partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the volatiles removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (92/8). The purified product was dissolved in methylene chloride, insolubles were removed by filtration and 2.2M ethereal hydrogen chloride (0.5ml) was added to the filtrate. The volatiles were removed by evaporation and the residue was triturated with ether, collected by filtration and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-([N-(2-methoxyethyl)-N-methyl]amino)ethoxy)quinazoline** hydrochloride (22mg, 16%).
^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.0(s, 3H); 3.35(s, 3H); 3.4-3.6(m, 2H); 3.65-3.85(m, 4H); 4.03(s, 3H); 4.64(t, 2H); 7.45(s, 1H); 7.47(d, 1H); 7.63(t, 1H); 7.69(d, 1H); 8.23(s, 1H); 8.9(s, 1H)
MS - ESI: 435 $[MH]^+$

Elemental analysis:	Found	C 48.9	H 5.3	N 10.4
$C_{21}H_{24}N_4O_3ClF$ 0.8H_2O 1.85HCl	Requires	C 48.8	H 5.3	N 10.8%

The starting material was prepared as follows:

Diethyl azodicarboxylate (3.13g, 24mmol) was added dropwise to a suspension of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (2.56mg, 8mmol), (prepared as

described for the starting material in Example 2), 2-([N-(tert-butylcarbonyl)-N-methyl]amino)ethanol (2.1g, 1.2mmol), (Synth. Commun. 1993, 23, 2443), and triphenylphosphine (6.3g, 24mmol) in methylene chloride (50ml) under nitrogen. The mixture was stirred for 1.5 hours at ambient temperature and further 2-([N-(tert-butylcarbonyl)-N-methyl]amino)ethanol (0.21g, 1.2mmol), triphenylphosphine (630mg, 2.4mmol) and diethyl azodicarboxylate (0.31g, 2.4mmol) were added. The mixture was stirred for 1 hour; the mixture was purified by pouring it directly onto a silica column and eluting with methylene chloride/ether/methanol (60/30/10) to give 7-(2-([N-(tert-butylcarbonyl)-N-methyl]amino)ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (3.8g, 99%).

A solution of 7-(2-([N-(tert-butylcarbonyl)-N-methyl]amino)ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (2.38g, 5mmol) in methylene chloride (5ml) and TFA (10ml) was stirred at ambient temperature for 1 hour. Toluene was added and the volatiles were removed by evaporation. The residue was partitioned between 2M hydrochloric acid and ethyl acetate. The aqueous layer was adjusted to pH8 with sodium hydrogen carbonate and extracted with ethyl acetate. The organic extracts were combined, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation to give 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylaminoethoxy)quinazoline (700mg, 37%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.75(s, 3H); 3.5-3.6(m, 2H); 4.05(s, 3H); 4.5(t, 2H); 7.4(s, 1H); 7.4-7.5(m, 1H); 7.65(t, 1H); 7.7(d, 1H); 8.15(s, 1H); 8.8(s, 1H)

Example 61

Dimethylcarbamyl chloride (38µl, 0.42mmol) was added to a solution of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylaminoethoxy)quinazoline (150mg, 0.4mmol), (prepared as described for the starting material in Example 60), and triethylamine (61µl, 0.44mmol) in methylene chloride (4ml). The mixture was stirred for 2.5 hours at ambient temperature, the resulting precipitate was collected by filtration and washed with ether. The solid was purified by column chromatography, eluting with methylene chloride/methanol (92/8). The purified product was dissolved in methylene chloride/methanol (1/1), 2.9M ethereal hydrogen chloride (1ml) was added and the volatiles were removed by evaporation. The residue was triturated with ether and the solid product collected by filtration, washed with

ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(N',N',N-trimethylureido)ethoxy)quinazoline** hydrochloride (80mg, 41%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.73(s, 6H); 2.91(s, 3H); 3.59(t, 2H); 4.0(s, 3H); 4.34(t, 2H); 7.36(s, 1H); 7.5(d, 1H); 7.63(t, 1H); 7.68(d, 1H); 8.1(s, 1H); 8.9(s, 1H)

MS - (EI): 447 $[M.]^+$

Elemental analysis:	Found	C 51.2	H 5.1	N 13.9
$C_{21}H_{23}N_5O_3ClF$ $0.5H_2O$ 1HCl Requires		C 51.1	H 5.1	N 14.2%

Example 62

7-(2-Bromoethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (150mg, 0.35mmol) and 1-acetylpiperazine (135mg, 1mmol) were heated together at 140°C for 10 minutes. The mixture was allowed to cool and was dissolved in a mixture of methylene chloride/ethyl acetate. The solution was washed with water, and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (9/1). The purified product was dissolved in methylene chloride and 2.9M ethereal hydrogen chloride was added. The precipitate was collected by filtration, washed with ether and dried under vacuum to give **7-(2-(4-acetylpiperazin-1-yl)ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline** hydrochloride (152mg, 79%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD; 50°C) 2.07(s, 3H); 3.3-3.7(br s, 8H); 3.75(t, 2H); 4.05(s, 3H); 4.65(t, 2H); 7.45(br s, 2H); 7.6-7.7(m, 2H); 8.15(s, 1H); 8.9(s, 1H)

MS - (EI): 473 $[M.]^+$

Elemental analysis:	Found	C 49.8	H 5.0	N 12.5
$C_{23}H_{25}N_5O_3ClF$ $0.5H_2O$ 1.9HCl	Requires	C 50.0	H 5.1	N 12.7%

The starting material was prepared as follows:

1,2-Dibromoethane (5.4ml, 62mmol) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (5g, 15.6mmol), (prepared as described for the starting material in Example 2), and potassium carbonate (8.6g, 62mmol) in DMF (50ml) and the mixture stirred for 18 hours at ambient temperature. Water was added and the resulting precipitate was collected by filtration. The solid was purified by chromatography on

neutral alumina eluting with methylene chloride/methanol (95/5). The semi-purified product was repurified by chromatography on silica eluting with methylene chloride/methanol (97/3). The purified product was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give 7-(2-bromoethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (3.58g, 54%).

^{1}H NMR Spectrum: ($DMSOd_6$) 3.28(s, 3H); 3.96(s, 3H); 4.48(t, 2H); 4.85(t, 2H); 7.21(s, 1H); 7.34(d, 1H); 7.5-7.6(m, 2H); 7.80(s, 1H); 8.36(s, 1H); 9.55(s, 1H)

Example 63

A mixture of 7-(2-(2-bromoethoxy)ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (150mg, 0.32mmol) in 1-methylpiperazine (2ml) was heated at 100°C for 1 hour. The mixture was allowed to cool and was partitioned between ethyl acetate and water. The organic layer was separated and washed with water and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/methanol (85/15 followed by 80/20). The purified solid product was dissolved in methylene chloride/methanol (1/1) and 2.9M ethereal hydrogen chloride was added. The volatiles were removed by evaporation and the solid was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-[4-methylpiperazin-1-yl]ethoxy)ethoxy)quinazoline** hydrochloride (54mg, 28%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD; 50°C) 2.9(s, 3H); 3.5-3.8(m, 10H); 3.95(br s, 4H); 4.03(s, 3H); 4.4(m, 2H); 7.40-7.45(m, 1H); 7.42(s, 1H); 7.55-7.65(m, 2H); 8.15(s, 1H); 8.8(s, 1H)

MS - ESI: 490 $[MH]^+$

Elemental analysis:	Found	C 46.0	H 5.6	N 10.9
$C_{24}H_{29}N_5O_3ClF$ 1.5H_2O 2.9HCl	Requires	C 46.3	H 5.6	N 11.2%

The starting material was prepared as follows:

2-Bromoethyl ether (1.57ml, 12mmol) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (1g, 3.1mmol), (prepared as described for the starting material in Example 2), and potassium carbonate (1.73g, 12mmol) in DMF (10ml).

The mixture was stirred for 18 hours at ambient temperature and was partitioned between ethyl acetate and water. The organic layer was separated, washed with water and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/acetonitrile/methanol (60/38/2). The purified product was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give 7-(2-(2-bromoethoxy)ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (763mg, 52%).

^{1}H NMR Spectrum: ($CDCl_3$) 3.5(t, 2H); 3.9(t, 2H); 3.95(t, 2H); 4.03(s, 3H); 4.35(t, 2H); 7.03(s, 1H); 7.2-7.4(m, 4H); 8.55(t, 1H); 8.7(s, 1H)

MS - ESI: 472 $[MH]^+$

Example 64

A solution of 7-(2-(2-bromoethoxy)ethoxy)-4-(4-chloro-2-fluoroanilino)-6-methoxyquinazoline (150mg, 0.32mmol), (prepared as described for the starting material in Example 63), in pyrrolidine (2ml) was heated at 80°C for 5 hours. The mixture was allowed to cool and was partitioned between ethyl acetate and water. The organic layer was washed with water and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/methanol/triethylamine (80/20/0 followed by 80/20/1). The purified product was dissolved in methylene chloride and 2.9M ethereal hydrogen chloride (1ml) was added. The volatiles were removed by evaporation, the residue was triturated with ether, collected by filtration and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-pyrrolidin-1-ylethoxy)ethoxy)quinazoline** hydrochloride (35mg, 20%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.8-1.9(m, 2H); 1.95-2.1(m, 2H); 3.05-3.15(m, 2H); 3.45(t, 2H); 3.55-3.65(m, 2H); 3.8-3.85(m, 2H); 3.95-4.0(m, 2H); 4.01(s, 3H); 4.4(br s, 2H); 7.39(s, 1H); 7.48(d, 1H); 7.65(t, 1H); 7.7(d, 1H); 8.11(s, 1H); 8.89(s, 1H)

MS - ESI: 461 $[MH]^+$

Elemental analysis: Found C 49.4 H 5.4 N 10.1

$C_{23}H_{26}N_4O_3ClF$ $1.2H_2O$ 2HCl Requires C 49.7 H 5.5 N 10.1%

Example 65

1-(2-(2-Bromoethyl)ethoxy)-2-pyrrolidinone (272mg, 1.1mmol) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (250mg, 0.78mmol), (prepared as described for the starting material in Example 2), and potassium carbonate (324mg, 2.3mmol) in DMF (5ml) and the mixture stirred for 4 hours at ambient temperature. The mixture was partitioned between ethyl acetate and water, the organic layer was separated, washed with water and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography on alumina eluting with methylene chloride/acetonitrile/methanol (60/37/3). The semi-purified product was repurified by column chromatography on silica eluting with methylene chloride/methanol (95/5). The purified product was dissolved in methylene chloride and 2.9M ethereal hydrogen chloride (1ml) was added. The volatiles were removed by evaporation, the residue was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-(2-[2-oxopyrrolidin-1-yl]ethoxy)ethoxy)quinazoline** hydrochloride (63mg, 16%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.85-1.95(m, 2H); 2.2(t, 2H); 3.35-3.45(m, 4H); 3.65(t, 2H); 3.9(br s, 2H); 4.02(s, 3H); 4.35(br s, 2H); 7.35(s, 1H); 7.45(d, 1H); 7.65(t, 1H); 7.7(d, 1H); 8.15(s, 1H); 8.88(s, 1H)

MS - ESI: 475 $[MH]^+$

Elemental analysis:	Found	C 53.2	H 5.0	N 11.1
$C_{23}H_{24}N_4O_4ClF$ 0.6H_2O 0.85HCl	Requires	C 53.5	H 5.1	N 10.8%

The starting material was prepared as follows:

A solution of 2-pyrrolidinone (1.5g, 17.6mmol) in anhydrous toluene (8ml) was added dropwise to a suspension of sodium hydride (741mg, 18mmol, prewashed with pentane) in anhydrous toluene (60ml) and the mixture was stirred at 100°C for 1.5 hours. The mixture was allowed to cool to ambient temperature and tetrabutylammonium bromide (57mg, 0.176mmol) was added followed by 2-bromoethyl ether (8ml, 35mmol). The mixture was stirred for 21 hours at ambient temperature, the insolubles were removed by filtration and the solid was washed with ether. The volatiles were removed from the filtrate by evaporation and the residue was purified by column chromatography on silica eluting with methylene

chloride/acetonitrile/methanol (60/38/2) to give 1-(2-(2-bromoethyl)ethoxy)2-pyrrolidinone (971mg, 23%).

^{1}H NMR Spectrum: ($CDCl_3$) 2.0-2.1(m, 2H); 2.4(t, 2H); 3.4-3.5(m, 4H); 3.52(t, 2H); 3.65(t, 1H); 3.78(t, 2H)

MS - (EI): 237 $[M]^+$

Example 66

Diethyl azodicarboxylate (325µl, 2mmol) was added dropwise to a mixture of (E)-4-morpholinobut-2-en-1-ol (151mg, 0.96mmol), (J. Med. Chem. 1972, 15, 110-112), 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (220mg, 0.688mol), (prepared as described for the starting material in Example 2), and triphenylphosphine (541mg, 2mmol) in methylene chloride (4ml). The mixture was stirred for 30 minutes at ambient temperature, and further (E)-4-morpholinobut-2-en-1-ol (10mg, 0.06mmol), triphenylphosphine (36mg, 0.137mol) and diethyl azodicarboxylate (22µl, 0.14mmol) were added. The mixture was stirred for 20 minutes and the volatiles were removed by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/methanol (92/8). The purified solid was dissolved in methylene chloride and 2M ethereal hydrogen chloride (3ml) was added. The volatiles were removed by evaporation and the solid was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **(E)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-morpholinobut-2-en-1-yloxy)quinazoline** hydrochloride (165mg, 45%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.1-3.15(m, 2H); 3.35-3.45(m, 2H); 3.75(t, 2H); 3.9(d, 2H); 4.0(d, 2H); 4.03(s, 3H); 4.95(d, 2H); 6.05(td, 1H); 6.3(td, 1H); 7.45(s, 1H); 7.47(d, 1H); 7.62(t, 1H); 7.7(d, 1H); 8.25(s, 1H); 8.88(s, 1H)

MS - ESI: 459 $[MH]^+$

Elemental analysis:	Found	C 50.3	H 5.3	N 10.1
$C_{23}H_{24}N_4O_3ClF$ 1.4H_2O 1.8HCl	Requires	C 50.2	H 5.2	N 10.2%

Example 67

Diethyl azodicarboxylate (368µl, 2.34mmol) was added dropwise to a mixture of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (284mg, 0.78mmol), (prepared

as described for the starting material in Example 48), triphenylphosphine (613mg, 2.34mmol) and 4-(2-hydroxyethyl)-3-morpholinone (170mg, 1.17mmol), (EP580402A2), in methylene chloride (10ml) under nitrogen. The mixture was stirred for 2.5 hours at ambient temperature, the insolubles were removed by filtration. The filtrate was purified by pouring it directly on to a column of silica and eluting with methylene chloride/ethyl acetate/methanol (60/35/5). The purified product was triturated with ether and collected by filtration. The solid was dissolved in methylene chloride containing a few drops of methanol and 3.8M ethereal hydrogen chloride (0.5ml) was added. The volatiles were removed by evaporation and the residue was triturated with ether, collected by filtration and dried under vacuum to give of **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(3-oxomorpholino)ethoxy)quinazoline** hydrochloride (108mg, 26%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.56(t, 2H); 3.8-3.9(m, 4H); 4.06(s, 3H); 4.06(s, 2H); 4.4(t, 2H); 7.35(s, 1H); 7.5-7.6(m, 2H); 7.8(d, 1H); 8.13(s, 1H); 8.87(s, 1H)

MS - ESI: 491 $[MH]^+$

Elemental analysis:	Found	C 47.1	H 4.1	N 10.5
$C_{21}H_{20}N_4O_4BrF\ 0.3H_2O\ 0.95HCl$	Requires	C 47.5	H 4.1	N 10.5%

Example 68

Diethyl azodicarboxylate (283µl, 1.8mmol) was added dropwise to a mixture of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (218mg, 0.6mmol), (prepared as described for the starting material in Example 48), triphenylphosphine (95µl, 0.84mmol) and 1-(2-hydroxyethyl)-2-pyrrolidinone (95µl, 0.84 mmol) in methylene chloride (8ml) under nitrogen. The mixture was stirred for 4 hours at ambient temperature, and then purified by pouring it directly on to a column of silica and eluting with methylene chloride/acetonitrile/methanol (60/32.5/7.5). The purified product was triturated with ether and collected by filtration. The solid was dissolved in methylene chloride/methanol (1/1) and 2M ethereal hydrogen chloride (1ml) was added. The volatiles were removed by evaporation, the residue was triturated with ether, collected by filtration and dried under vacuum to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(2-oxopyrrolidin-1-yl)ethoxy)quinazoline** hydrochloride (182mg, 60%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.9-2.0(m, 2H); 2.24(t, 2H); 3.53(t, 2H); 3.7(t, 2H); 4.01(s, 3H); 4.34(t, 2H); 7.36(s, 1H); 7.5-7.6(m, 2H); 7.75(d, 1H); 8.16(s, 1H); 8.87(s, 1H)

MS - ESI: 477 $[MH]^+$

Elemental analysis:	Found	C 50.2	H 4.3	N 10.9
$C_{21}H_{20}N_4O_3BrF$ 0.8HCl	Requires	C 50.0	H 4.2	N 11.1%

Example 69

Diethyl azodicarboxylate (236µl, 1.5mmol) was added dropwise to a mixture of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (182mg, 0.5mmol), (prepared as described for the starting material in Example 48), triphenylphosphine (393mg, 1.5mmol) and 2-(2-methoxyethoxy)ethanol (84µl, 0.7mmol) in methylene chloride (7ml) under nitrogen. The mixture was stirred for 4 hours at ambient temperature, the reaction mixture was purified by pouring it directly on to a column of silica and eluting with ethyl acetate/petroleum ether (9/1 followed by 10/0). The purified product was triturated with ether and collected by filtration. The solid was dissolved in methylene chloride/methanol and 2M ethereal hydrogen chloride (1ml) was added. The mixture was concentrated by evaporation and the precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(2-methoxyethoxy)ethoxy)quinazoline** hydrochloride (84mg, 34%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 3.26(s, 3H); 3.47(m, 2H); 3.64(m, 2H); 3.85(m, 2H); 4.02(s, 3H); 4.35(m, 2H); 7.35(s, 1H); 7.5-7.7(m, 2H); 7.82(d, 1H); 8.12(s, 1H); 8.87(s, 1H)

MS - ESI: 468 $[MH]^+$

Elemental analysis:	Found	C 47.5	H 4.4	N 8.7
$C_{20}H_{21}N_3O_4BrF$ 0.65H_2O 0.65HCl	Requires	C 47.9	H 4.6	N 8.3%

Example 70

Diethyl azodicarboxylate (567µl, 3.6mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (383mg, 1.2mmol), (prepared as described for the starting material in Example 2), 4-(3-hydroxypropyl)-3,5-dioxomorpholine

(291mg, 1.68mmol) and triphenylphosphine (944mg, 3.6mmol) in methylene chloride (10ml) under nitrogen. The mixture was stirred at ambient temperature for 6 hours and the insolubles were removed by filtration. The filtrate was purified by pouring it directly on to a column of silica and eluting with methylene chloride/acetonitrile/methanol (60/34/6 followed by 60/24/16 and 60/16/24). The semi-purified product was repurified by column chromatography eluting with methylene chloride/acetonitrile/methanol (5/4/1). The purified product was dissolved in methylene chloride/methanol, 2M ethereal hydrogen chloride (1ml) was added and the volatiles were removed by evaporation. The residue was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-7-(3-(3,5-dioxomorpholino)propoxy)-6-methoxyquinazoline** hydrochloride (56mg, 10%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.0-2.1(m, 2H); 3.35(t, 2H); 3.98(s, 2H); 4.01(s, 3H); 4.24(t, 2H); 7.33(s, 1H); 7.45(d, 1H); 7.62(t, 1H); 7.68(d, 1H); 8.13(s, 1H); 8.87(s, 1H)

MS - ESI: 475 $[MH]^+$

Elemental analysis:	Found	C 48.9	H 4.4
$C_{22}H_{20}N_4O_5ClF$ 1.4H_2O 1HCl	Requires	C 49.2	H 4.5

The starting material was prepared as follows:

A solution of diglycolic anhydride (2.32g, 20mmol) in 3-amino-1-propanol (6ml) was refluxed at 180°C for 3 hours. The volatiles were removed by evaporation and the residue was purified by column chromatography on silica eluting with methylene chloride/methanol (8/2) to give 4-(3-hydroxypropyl)-3,5-dioxomorpholine (3.46g, 99%).

^{1}H NMR Spectrum: ($CDCl_3$) 2.75-2.8(m, 2H); 3.1(br s, 1H); 3.45-3.5(m, 2H); 3.75(t, 2H); 4.04(s, 2H)

MS - (EI): 174 $[MH]^+$

Example 71

Diethyl azodicarboxylate (472µl, 3mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (319.5mg, 1mmol), (prepared as described for the starting material in Example 2), triphenylphosphine (786mg, 3mmol) and 4-(2-hydroxyethyl)-3,5-dioxomorpholine (223mg, 1.4mmol) in methylene chloride (10ml)

under nitrogen. The mixture was stirred at ambient temperature for 4.5 hours and the insolubles were removed by filtration. The solvent was removed from the filtrate by evaporation and the residue was purified by column chromatography eluting with methylene chloride/acetonitrile/methanol (85/12.5/2.5). The purified product was dissolved in methylene chloride, 2M ethereal hydrogen chloride (1ml) was added and the mixture was diluted with ether. The resulting precipitate was collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-7-(2-(3,5-dioxomorpholino)ethoxy)-6-methoxyquinazoline** hydrochloride (97mg, 20%).

^{1}H NMR ($DMSOd_6$; CF_3COOD) 4.0(s, 3H); 4.19(d, 2H); 4.39(t, 2H); 4.45(s, 4H); 7.35(s, 1H); 7.45(d, 1H); 7.67(t, 1H); 7.69(d, 1H); 8.12(s, 1H); 8.87(s, 1H)

MS - ESI: 461 $[MH]^+$

Elemental analysis:	Found	C 49.9	H 3.9	N 11.1
$C_{21}H_{18}N_4O_5ClF\ 0.5H_2O\ 0.9HCl$	Requires	C 50.2	H 4.0	N 11.1%

The starting material was prepared as follows:

Ethanolamine (2.44g, 40mmol) was added dropwise to a solution of diglycolic anhydride (2.32g, 20mmol) in pyridine (10ml). The mixture was stirred for 5 minutes at ambient temperature and then heated at reflux for 2 hours. The volatiles were removed by evaporation and the residue was heated at 180°C for 2 hours. The reaction mixture was allowed to cool and was purified by column chromatography eluting with methylene chloride/methanol (9/1) to give 4-(2-hydroxyethyl)-3,5-dioxomorpholine (400mg, 12.5%)

^{1}H NMR Spectrum: ($CDCl_3$) 1.6(br s, 1H); 3.8(t, 2H); 4.05(t, 2H); 4.4(s, 4H)

MS - EI: 160 $[MH]^+$

Example 72

Diethyl azodicarboxylate (378µl, 2.4mmol) was added dropwise to a mixture of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (292mg, 0.8mmol), (prepared as described for the starting material in Example 48), triphenylphosphine (629mg, 2.4mmol) and 2-(2-morpholinoethoxy)ethanol (196mg, 1.12mmol), (prepared as described for the starting material in Example 53), in methylene chloride (10ml) under nitrogen. The mixture was stirred for 3.5 hours at ambient temperature and the mixture was purified by pouring it

directly on to a column of silica and eluting with methylene chloride/acetonitrile/methanol (6/3/1). The purified product was dissolved in methylene chloride/methanol and the insolubles were removed by filtration. 2M Ethereal hydrogen chloride (1ml) was added to the filtrate and the volatiles were removed by evaporation. The residue was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(2-(2-morpholinoethoxy)ethoxy)quinazoline** hydrochloride (232mg, 49%).

^{1}H NMR Spectrum: (DMSOd_6; CF_3COOD) 3.1-3.2(m, 2H); 3.35-3.4(br s, 2H); 3.45(d, 2H); 3.75(t, 2H); 3.9-4.0(m,6H); 4.02(s, 3H); 4.4(br s, 2H); 7.45(s, 1H); 7.5-7.6(m, 2H); 7.8(m, 1H); 8.22(s, 1H); 8.87(s, 1H)

MS - ESI: 523 $[MH]^+$

Elemental analysis:	Found	C 46.3	H 4.9	N 9.2
$C_{23}H_{26}N_4O_4BrF$ 0.5H_2O 1.8HCl	Requires	C 46.3	H 4.9	N 9.4%

Example 73

Diethyl azodicarboxylate (220µl, 1.4mmol) was added dropwise to a mixture of 4-(4-bromo-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (170mg, 0.46mmol), (prepared as described for the starting material in Example48), triphenylphosphine (367mg, 1.4mmol) and 3-(1,1-dioxothiomorpholino)-1-propanol (135mg, 0.7mmol) in methylene chloride (4ml) under nitrogen. The mixture was stirred for 1 hour at ambient temperature and further triphenylphosphine (61mg, 0.23mmol), 3-(1,1-dioxothiomorpholino)-1-propanol (30mg, 0.23mmol) and diethyl azodicarboxylate (37µl, 0.23mmol) were added. The mixture was stirred for 1 hour at ambient temperature and the mixture was purified by pouring it on to a column of silica and eluting with methylene chloride/methanol (95/5). The purified product was dissolved in methylene chloride/methanol, 2.2M ethereal hydrogen chloride (1ml) was added and the volatiles were removed by evaporation. The residue was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-bromo-2-fluoroanilino)-7-(3-(1,1-dioxothiomorpholino)propoxy)-6-methoxyquinazoline** hydrochloride (138mg, 47%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.3-2.4(m, 2H); 3.5(t, 2H); 3.7-3.8(br s, 4H); 3.85(br s, 4H); 4.03(s, 3H); 4.35(t, 2H); 7.4(s, 1H); 7.5-7.6(m, 2H); 7.8(d, 1H); 8.21(s, 1H); 8.88(s, 1H)

MS - ESI: 539 $[MH]^+$

Elemental analysis:	Found	C 42.1	H 4.6	N 8.6
$C_{22}H_{24}N_4O_4BrFS$ 1.1H_2O 1.85HCl	Requires	C 42.2	H 4.5	N 8.9%

The starting material was prepared as follows:

A mixture of 3-amino-1-propanol (650µl, 8.4mmol) and vinyl sulphone (1g, 8.4mmol) was heated at 110°C for 45 minutes. The mixture was allowed to cool and was purified by column chromatography eluting with methylene chloride/methanol (95/5) to give 3-(1,1-dioxothiomorpholino)-1-propanol (800mg, 90%).

^{1}H NMR Spectrum: ($CDCl_3$) 1.7-1.8(m, 2H); 2.73(t, 2H); 3.06(br s, 8H); 3.25(s, 1H); 3.78(t, 2H)

MS - ESI: 194 $[MH]^+$

Example 74

A solution of 2-methoxyethylsulphonyl chloride (42mg, 0.26mmol), (J. Amer. Chem. Soc. 1992, 114, 1743-1749), in acetonitrile (1ml) was added to a mixture of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylaminoethoxy)quinazoline (94mg, 0.25mmol), (prepared as described for the starting material in Example 60), and triethylamine (80µl, 0.5mmol) in acetonitrile (15ml). The mixture was stirred for 10 minutes at ambient temperature, the volatiles were removed by evaporation and the residue was partitioned between methylene chloride and water. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (97/3). The purified product was dissolved in methylene chloride (5ml) and 2.2M ethereal hydrogen chloride (2ml) was added and the volatiles were removed by evaporation. The residue was triturated with ether, collected by filtration, washed with ether and dried under vacuum to give **4-(4-chloro-2-fluoroanilino)-7-(2-([N-methyl-N-(2-methoxyethylsulphonyl)]amino)ethoxy)-6-methoxyquinazoline** hydrochloride (86mg, 64%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.96(s, 3H); 3.28(s, 3H); 3.47(t, 2H); 3.6-3.7(m, 4H); 4.02(s, 3H); 4.37(t, 2H); 7.37(s, 1H); 7.46(d, 1H); 7.64(t, 1H); 7.7(d, 1H); 8.15(s, 1H); 8.88(s, 1H)

MS - ESI 499 $[MH]^+$

Elemental analysis:	Found	C 47.2	H 4.9	N 10.2
$C_{21}H_{24}N_4O_5ClFS$ 1HCl	Requires	C 47.1	H 4.7	N 10.5%

Example 75

Using a method analogous to that in Example 74, a mixture of 4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-methylaminoethoxy)quinazoline (102mg, 0.27mmol), (prepared as described for the starting material in Example 60), and triethylamine (0.1ml, 0.72mmol) in acetonitrile (17ml) was treated with 3-morpholinopropylsulphonyl chloride (75mg, 0.28mmol), (WO 930181), to give, after purification and hydrochloride salt formation, **4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(2-([N-methyl-N-(3-morpholinopropylsulphonyl)]amino)ethoxy)quinazoline** hydrochloride (96mg, 54%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 2.1-2.2(m, 2H); 3.0(s, 3H); 3.05-3.15(m, 2H); 3.2-3.3(m, 2H); 3.3-3.4(m, 2H); 3.45(d, 2H); 3.65-3.8(m, 4H); 3.95(d, 2H); 4.03(s, 3H); 4.39(t, 2H); 7.42(s, 1H); 7.45(d, 1H); 7.65(t, 1H); 7.7(d, 1H); 8.2(s, 1H); 8.9(s, 1H)

MS - ESI: 568 $[MH]^+$

Example 76

Diethyl azodicarboxylate (0.18ml, 1.14mmol) was added dropwise to a mixture of 4-(4-chloro-2-fluoroanilino)-7-hydroxy-6-methoxyquinazoline (111mg, 0.35mmol), (prepared as described for the starting material in Example 2), triphenylphosphine (312mg, 1.19mmol) and (S)-1-(3-hydroxypropyl)-2-(N,N-dimethylcarbamoyl)pyrrolidine (84mg, 0.42mmol) in methylene chloride (10ml) cooled at 0°C under nitrogen. The mixture was stirred for 15 minutes at 0°C, the mixture was allowed to warm to ambient temperature and was then stirred for 22 hours. Further (S)-1-(3-hydroxypropyl)-2-(N,N-dimethylcarbamoyl)pyrrolidine (10mg, 0.05mmol), triphenylphosphine (35mg, 0.13mmol) and diethyl azodicarboxylate (20µl, 0.13mmol) were added and the mixture was stirred for a further 2 hours. The mixture was partitioned between water and methylene chloride and the aqueous phase was adjusted to pH2

with 2M hydrochloric acid. The aqueous layer was separated, adjusted to pH9 with sodium hydrogen carbonate and was extracted with methylene chloride. The combined organic extracts were washed with water and then brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography on silica eluting with methylene chloride/methanol (85/15 followed by 75/25 and 60/40). The purified product was dissolved in methylene chloride (5ml) and methanol (1ml), 3.9M ethereal hydrogen chloride (0.5ml) was added and the mixture was diluted with ether. The resulting precipitate was collected by filtration, washed with ether, and dried under vacuum to give **(S)-4-(4-chloro-2-fluoroanilino)-7-(3-(2-(N,N-dimethylcarbamoyl)pyrrolidin-1-yl)propoxy)-6-methoxyquinazoline** hydrochloride (86mg, 32%).

^{1}H NMR Spectrum: ($DMSOd_6$; CF_3COOD) 1.8-1.95(m, 2H); 2.1-2.3(m, 4H); 2.92(s, 3H); 3.0(s, 3H); 3.2-3.45(m, 3H); 3.75-3.85(m, 1H); 4.0(s, 3H); 4.32(t, 2H); 4.75(t, 1H); 7.4(s, 1H); 7.45(d, 1H); 7.65(t, 1H); 7.7(d, 1H); 8.25(s, 1H); 8.9(s, 1H)

MS - ESI: 502 $[MH]^+$

Elemental analysis:	Found	C 50.2	H 5.5	N 11.6
$C_{25}H_{29}N_5O_3ClF$ 1H_2O 2HCl	Requires	C 50.6	H 5.6	N 11.8%

The starting material was prepared as follows:

A mixture of (S)-2-(N,N-dimethylcarbamoyl)pyrrolidine (426mg, 3mmol), (Chem. pharm. Bull. 1973, 21, 2112-2116), 3-bromo-1-propanol (0.41ml, 4.5mmol) and potassium carbonate (829mg, 6mmol) in acetonitrile (6ml) was heated at reflux for 8 hours. The mixture was allowed to cool and was partitioned between methylene chloride and water. The organic layer was separated, washed with brine, dried ($MgSO_4$) and the solvent removed by evaporation. The residue was purified by column chromatography, eluting with methylene chloride/methanol (a gradient from 90/10 to 60/40) to give (S)-1-(3-hydroxypropyl)-2-(N,N-dimethylcarbamoyl)pyrrolidine (290mg, 48%).

^{1}H NMR Spectrum: ($CDCl_3$; CD_3COOD) 1.8-2.1(m, 4H); 2.2-2.3(m, 1H); 2.6-2.7(m,1H); 3.0(s, 3H); 3.10(s, 3H); 3.4-3.6(m, 3H); 3.75-3.85(m, 3H); 5.05(m, 1H)

MS - ESI: 223 $[MNa]^+$

Example 77

The following illustrate representative pharmaceutical dosage forms containing the compound of formula I, or a pharmaceutically acceptable salt thereof (hereafter compound X), for therapeutic or prophylactic use in humans:

(a)	Tablet I	mg/tablet
	Compound X	100
	Lactose Ph.Eur	182.75
	Croscarmellose sodium	12.0
	Maize starch paste (5% w/v paste)	2.25
	Magnesium stearate	3.0

(b)	Tablet II	mg/tablet
	Compound X	50
	Lactose Ph.Eur	223.75
	Croscarmellose sodium	6.0
	Maize starch	15.0
	Polyvinylpyrrolidone (5% w/v paste)	2.25
	Magnesium stearate	3.0

(c)	Tablet III	mg/tablet
	Compound X	1.0
	Lactose Ph.Eur	93.25
	Croscarmellose sodium	4.0
	Maize starch paste (5% w/v paste)	0.75
	Magnesium stearate	1.0

(d)	Capsule	mg/capsule
	Compound X	10
	Lactose Ph.Eur	488.5
	Magnesium stearate	1.5

(e) Injection I (50 mg/ml)

Compound X	5.0% w/v
1N Sodium hydroxide solution	15.0% v/v
0.1N Hydrochloric acid (to adjust pH to 7.6)	
Polyethylene glycol 400	4.5% w/v
Water for injection to 100%	

(f) Injection II 10 mg/ml)

Compound X	1.0% w/v
Sodium phosphate BP	3.6% w/v
0.1N Sodium hydroxide solution	15.0% v/v
Water for injection to 100%	

(g) Injection III (1mg/ml,buffered to pH6)

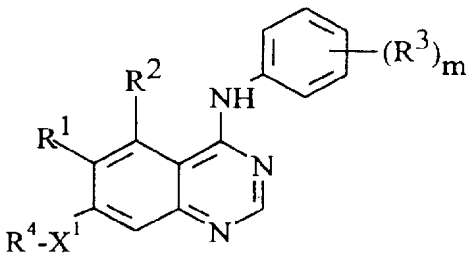
Compound X	0.1% w/v
Sodium phosphate BP	2.26% w/v
Citric acid	0.38% w/v
Polyethylene glycol 400	3.5% w/v
Water for injection to 100%	

Note

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate.

CLAIMS

1. A quinazoline derivative of the formula I:



(I)

[wherein:

m is an integer from 1 to 2;

R^1 represents hydrogen, hydroxy, halogeno, nitro, trifluoromethyl, cyano, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkylthio, or $-NR^5R^6$ (wherein R^5 and R^6, which may be the same or different, each represents hydrogen or C_{1-3}alkyl);

R^2 represents hydrogen, hydroxy, halogeno, methoxy, amino or nitro;

R^3 represents hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}alkoxy, C_{1-3}alkanoyloxy, trifluoromethyl, cyano, amino or nitro;

X^1 represents -O-, $-CH_2-$, -S-, -SO-, $-SO_2-$, $-NR^7CO-$, $-CONR^8-$, $-SO_2NR^9-$, $-NR^{10}SO_2-$ or $-NR^{11}-$ (wherein R^7, R^8, R^9, R^{10} and R^{11} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

R^4 is selected from one of the following thirteen groups:

1) C_{1-5}alkylR^{12} (wherein R^{12} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to C_{1-5}alkyl through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl) or C_{1-5}alkylR^{13} (wherein R^{13} is a group selected from pyrrolidin-1-yl, imidazolidin-1-yl and thiomorpholino, which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-}

$_4$alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

2) C_{2-5}alkenylR^{14} (wherein R^{14} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

3) C_{2-5}alkynylR^{15} (wherein R^{15} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

4) C_{1-5}alkylX^2C_{1-5}alkylX^3R^{16} (wherein X^2 and X^3 which may be the same or different are each -O-, -S-, -SO-, -SO_2-, -NR^{17}CO-, -$CONR^{18}$-, -SO_2NR^{19}-, -$NR^{20}SO_2$- or -NR^{21}- (wherein R^{17}, R^{18}, R^{19}, R^{20} and R^{21} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be -CH_2- when R^4 is C_{1-5}alkylX^2C_{1-5}alkylX^3R^{16};

5) C_{1-5}alkylX^4COR^{22} (wherein X^4 represents -O- or -NR^{23}- (wherein R^{23} represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{22} represents -$NR^{24}R^{25}$ or -OR^{26} (wherein R^{24}, R^{25} and R^{26} which may be the same or different each represents hydrogen, C_{1-4}alkyl or C_{1-3}alkoxyC_{2-3}alkyl));

6) C_{1-5}alkylX^5R^{27} (wherein X^5 represents -O-, -S-, -SO-, -SO_2-, -OCO-, -NR^{28}CO-, -$CONR^{29}$-, -SO_2NR^{30}-, -$NR^{31}SO_2$- or -NR^{32}- (wherein R^{28}, R^{29}, R^{30}, R^{31} and R^{32} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) or X^5 is carbonyl, and R^{27} represents cyclopentyl, cyclohexyl or a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which cyclopentyl, cyclohexyl or heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl or R^{27} is C_{1-3}alkyl with the proviso that when R^{27} is C_{1-3}alkyl, X^5 is -S-, -SO-, -SO_2-, -SO_2NR^{30}- or -$NR^{31}SO_2$- and X^1 is not -CH_2-);

7) C_{1-3}alkoxyC_{2-4}alkyl provided that X^1 is -S-, -SO- or -SO_2-;

8) C_{1-3}alkoxyC_{2-4}alkyl or C_{1-4}alkyl provided that X^1 is -O-;

9) C_{1-5}alkylX^6C_{1-5}alkylR^{33} (wherein X^6 represents -O-, -S-, -SO-, -SO_2-, -NR^{34}CO-, -$CONR^{35}$-, -SO_2NR^{36}-, -$NR^{37}SO_2$- or -NR^{38}- (wherein R^{34}, R^{35}, R^{36}, R^{37} and R^{38} each independently represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and R^{33} represents cyclopentyl, cyclohexyl or a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which cyclopentyl, cyclohexyl or heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

10) R^{39} (wherein R^{39} is a group selected from pyrrolidin-3-yl, piperidin-3-yl and piperidin-4-yl which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, C_{1-4}alkoxy, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl);

11) C_{1-5}alkylR^{40} (wherein R^{40} is piperazin-1-yl which bears at least one substituent selected from C_{1-4}alkanoyl, C_{1-4}alkoxycarbonyl, C_{1-4}hydroxyalkyl and -$CONR^{41}R^{42}$ (wherein R^{41} and R^{42} each independently represents hydrogen or C_{1-4}alkyl);

12) C_{1-5}alkylR^{43} (wherein R^{43} is morpholino which may bear one or two substituents selected from oxo, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl) with the proviso that when R^4 is C_{1-5}alkylR^{43}, X^1 is -S-, -SO-, -SO_2-, -SO_2NR^9- or -$NR^{10}SO_2$-; and

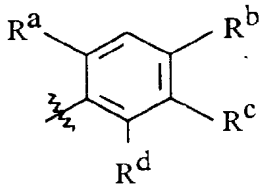
13) C_{1-5}alkylR^{44} (wherein R^{44} is morpholino which bears at least one and optionally two substituents selected from oxo, C_{1-4}alkyl, C_{1-4}hydroxyalkyl, carbamoyl, C_{1-4}alkylcarbamoyl, N,N-di(C_{1-4}alkyl)carbamoyl, C_{1-4}alkanoyl and C_{1-4}alkoxycarbonyl); with the further proviso that when R^4 is selected from group 8) R^1 and/or R^2 is/are nitro or at least one R^3 is C_{1-3}alkanoyloxy;]

and salts thereof.

2. A quinazoline derivative as claimed in claim 1 wherein R^1 represents hydrogen, hydroxy, cyano, nitro, trifluoromethyl, methyl, ethyl, methoxy or ethoxy.

3. A quinazoline derivative as claimed in claim 1 or claim 2 wherein R^2 is hydrogen.

4. A quinazoline derivative as claimed in any one of the preceding claims wherein the phenyl group bearing $(R^3)_m$ is of the formula II:



(II)

wherein:

R^a represents hydrogen, methyl, fluoro or chloro;

R^b represents hydrogen, methyl, methoxy, bromo, fluoro or chloro;

R^c represents hydrogen or hydroxy;

R^d represents hydrogen, fluoro or chloro.

5. A quinazoline derivative as claimed in any one of the preceding claims wherein X^1 represents -O-, -S-, $-NR^7CO-$, $-NR^{10}SO_2-$ or $-NR^{11}-$ (wherein R^7, R^{10} and R^{11} each independently represents hydrogen, C_{1-2}alkyl or C_{1-2}alkoxyethyl).

6. A quinazoline derivative as claimed in any one of the preceding claims wherein R^4 is selected from one of the following eleven groups:

1) C_{1-4}alkylR^{12} (wherein R^{12} is a group selected from 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, pyrrolidin-2-yl, pyrrolidin-3-yl, piperidin-2-yl, piperidin-3-yl, piperidin-4-yl, morpholin-2-yl, morpholin-3-yl and piperazin-2-yl which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-

$_3$alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or C_{2-4}alkylR^{45} (wherein R^{45} is a group selected from imidazolidin-1-yl, pyrrolidin-1-yl and thiomorpholino which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

2) 1-R^{46}prop-1-en-3-yl, 1-R^{46}but-2-en-4-yl, 1-R^{46}but-1-en-3-yl, 1-R^{46}pent-2-en-4-yl or 2-R^{46}pent-3-en-5-yl (wherein R^{46} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to the alkenyl group through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or 1-R^{47}but-2-en-4-yl, 1-R^{47}pent-2-en-4-yl or 2-R^{47}pent-3-en-5-yl (wherein R^{47} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to the alkenyl group through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

3) 1-R^{48}prop-1-yn-3-yl, 1-R^{48}but-2-yn-4-yl, 1-R^{48}but-1-yn-3-yl, 1-R^{48}pent-2-yn-4-yl or 2-R^{48}pent-3-yn-5-yl (wherein R^{48} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to the alkynyl group through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or 1-R^{49}but-2-yn-4-yl, 1-R^{49}pent-2-yn-4-yl or 2-R^{49}pent-3-yn-5-yl (wherein R^{49} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to the alkynyl group through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-}

$_3$hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

4) C_{2-3}alkylX^2C_{1-3}alkylX^3R^{16} (wherein X^2 and X^3 are as defined in claim 1 and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be $-CH_2-$ when R^4 is C_{2-3}alkylX^2C_{1-3}alkylX^3R^{16};

5) C_{2-3}alkylX^4COR^{22} (wherein X^4 is as defined in claim 1 and R^{22} represents $-NR^{24}R^{25}$ or $-OR^{26}$ (wherein R^{24}, R^{25} and R^{26} which may be the same or different each represents hydrogen, C_{1-4}alkyl or C_{1-2}alkoxyethyl));

6) C_{2-3}alkylX^5R^{27} (wherein X^5 is as defined in claim 1 and R^{27} represents a group selected from cyclopentyl, cyclohexyl, pyrrolidinyl and piperidinyl which group is linked to X^5 through a carbon atom and which group may carry one substituent selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, C_{1-2}alkoxy, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl or R^{27} is C_{1-3}alkyl with the proviso that when R^{27} is C_{1-3}alkyl, X^5 is -S-, -SO-, $-SO_2-$, $-SO_2NR^{30}-$ or $-NR^{31}SO_2-$ and X1 is not $-CH_2-$);

7) C_{1-2}alkoxyC_{2-3}alkyl provided that X^1 is -S-, -SO- or $-SO_2-$;

8) C_{2-3}alkylX^6C_{2-3}alkylR^{33} (wherein X^6 is as defined in claim 1 and R^{33} represents a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl, and C_{1-3}alkoxycarbonyl);

9) C_{2-3}alkylR^{40} (wherein R^{40} is piperazin-1-yl which bears at least one substituent selected from acetyl, C_{1-2}alkoxycarbonyl, C_{1-2}hydroxyalkyl and $CONR^{41}R^{42}$ (wherein R^{41} and R^{42} each independently represents hydrogen or C_{1-2}alkyl);

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

11) C_{2-3}alkylR^{44} (wherein R^{44} is morpholino which bears at least one and optionally two substituents selected from oxo, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl).

7. A quinazoline derivative as claimed in claim 6 wherein R^4 is selected from one of the following nine groups:

1) C_{1-3}alkylR^{12} (wherein R^{12} is a group selected from 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, pyrrolidin-2-yl, pyrrolidin-3-yl, piperidin-2-yl, piperidin-3-yl, piperidin-4-yl, morpholin-2-yl, morpholin-3-yl and piperazin-2-yl which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, C_{1-2}alkoxy, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl) or C_{2-3}alkylR^{45} (wherein R^{45} is a group selected from imidazolidin-1-yl, pyrrolidin-1-yl and thiomorpholino which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, C_{1-2}alkoxy, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl);

2) 1-R^{50}but-2-en-4-yl (wherein R^{50} is a group selected from imidazolidin-1-yl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, piperidin-4-yl, pyrrolidin-1-yl, pyrrolidin-3-yl, piperazin-1-yl, morpholino, thiomorpholino and piperidino which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, C_{1-2}alkoxy, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl);

3) 1-R^{51}but-2-yn-4-yl (wherein R^{51} is a group selected from imidazolidin-1-yl, 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, piperidin-4-yl, pyrrolidin-1-yl, pyrrolidin-3-yl, piperazin-1-yl, morpholino, thiomorpholino and piperidino which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-2}alkyl, C_{1-2}hydroxyalkyl, C_{1-2}alkoxy, carbamoyl, C_{1-2}alkylcarbamoyl, N,N-di(C_{1-2}alkyl)carbamoyl, acetyl and C_{1-2}alkoxycarbonyl);

4) C_{2-3}alkylX^2C_{1-3}alkylX^3R^{16} (wherein X^2 and X^3 are as defined in claim 1 and R^{16} represents hydrogen or C_{1-3}alkyl) with the proviso that X^1 cannot be $-CH_2-$ when R^4 is C_{2-3}alkylX^2C_{1-3}alkylX^3R^{16};

5) C_{1-2}alkoxyC_{2-3}alkyl provided that X^1 is -S-, -SO- or -SO_2-;

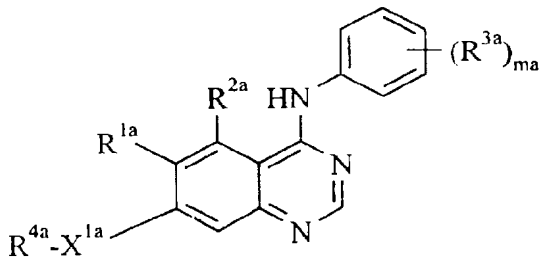
6) 2-(3,3-dimethylureido)ethyl, 3-(3,3-dimethylureido)propyl, 2-(3-methylureido)ethyl, 3-(3-methylureido)propyl, 2-ureidoethyl, 3-ureidopropyl, 2-(N,N-dimethylcarbamoyloxy)ethyl, 3-(N,N-dimethylcarbamoyloxy)propyl, 2-(N-methylcarbamoyloxy)ethyl, 3-(N-methylcarbamoyloxy)propyl, 2-(carbamoyloxy)ethyl, 3-(carbamoyloxy)propyl, 2-(1,3,3-trimethylureido)ethyl, 3-(1,3,3-trimethylureido)propyl, 2-(isopropoxycarbonylamino)ethyl, 3-(isopropoxycarbonylamino)propyl, 2-(isobutoxycarbonylamino)ethyl, 3-(isobutoxycarbonylamino)propyl, 2-(t-butoxycarbonylamino)ethyl or 3-(t-butoxycarbonylamino)propyl;

7) C_{2-3}alkylX^5R^{27} (wherein R^{27} is C_{1-2}alkyl and X^5 is -S-, -SO-, -SO_2-, -SO_2NR^{30}- or -$NR^{31}SO_2$- and with the proviso that X^1 is not -CH_2-);

8) C_{2-3}alkylX^6C_{2-3}alkylR^{33} (wherein X^6 is as defined in claim 1 and R^{33} represents a group selected from morpholino, 2-oxopyrrolidin-1-yl, pyrrolidin-1-yl, piperidino, piperazin-1-yl and 4-methylpiperazin-1-yl); and

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

8. A compound as claimed in claim 1 of the formula Ia:



(Ia)

[wherein:

R^{1a} is hydrogen or methoxy;

R^{2a} is hydrogen;

the phenyl group bearing $(R^{3a})_{ma}$ is the 4-chloro-2-fluorophenyl group or the 4-bromo-2-fluorophenyl group;

X^{1a} is -O-, -S-, $-NR^{5a}CO-$ or $-NR^{6a}SO_2-$ (wherein R^{5a} and R^{6a} each independently represents hydrogen or C_{1-2}alkyl);

R^{4a} is selected from one of the following eleven groups:

1) C_{1-4}alkylR^{7a} (wherein R^{7a} is a group selected from 1,3-dioxolan-2-yl, 1,3-dioxan-2-yl, 1,3-dithiolan-2-yl, 1,3-dithian-2-yl, pyrrolidin-2-yl, pyrrolidin-3-yl, piperidin-2-yl, piperidin-3-yl, piperidin-4-yl, morpholin-2-yl, morpholin-3-yl and piperazin-2-yl which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or C_{2-4}alkylR^{8a} (wherein R^{8a} is a group selected from imidazolidin-1-yl, pyrrolidin-1-yl and thiomorpholino which group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

2) 1-R^{9a}prop-1-en-3-yl, 1-R^{9a}but-2-en-4-yl, 1-R^{9a}but-1-en-3-yl, 1-R^{9a}pent-2-en-4-yl or 2-R^{9a}pent-3-en-5-yl (wherein R^{9a} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to the alkenyl group through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or 1-R^{10a}but-2-en-4-yl, 1-R^{10a}pent-2-en-4-yl or 2-R^{10a}pent-3-en-5-yl (wherein R^{10a} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to the alkenyl group through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

3) 1-R^{11a}prop-1-yn-3-yl, 1-R^{11a}but-2-yn-4-yl, 1-R^{11a}but-1-yn-3-yl, 1-R^{11a}pent-2-yn-4-yl or 2-R^{11a}pent-3-yn-5-yl (wherein R^{11a} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, selected independently from O, S and N, which heterocyclic group is linked to the alkynyl group through a carbon atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl) or 1-R^{12a}but-2-yn-4-yl, 1-R^{12a}pent-2-yn-4-yl or 2-R^{12a}pent-3-yn-5-yl (wherein R^{12a} is a 5 or 6 membered saturated heterocyclic group with one or two heteroatoms, of which one is N and the other is selected independently from O, S and N, which heterocyclic group is linked to the alkynyl group through a nitrogen atom and which heterocyclic group may bear one or two substituents selected from oxo, hydroxy, halogeno, C_{1-3}alkyl, C_{1-3}hydroxyalkyl, C_{1-3}alkoxy, carbamoyl, C_{1-3}alkylcarbamoyl, N,N-di(C_{1-3}alkyl)carbamoyl, C_{2-3}alkanoyl and C_{1-3}alkoxycarbonyl);

4) C_{2-3}alkyl$X^{2a}C_{1-3}$alkyl$X^{3a}R^{13a}$ (wherein X^{2a} and X^{3a} which may be the same or different each represents -O-, -S-, -SO-, -SO_2-, -NR^{14a}CO-, or -NR^{15a}- (wherein R^{14a} and R^{15a} each independently represents hydrogen, C_{1-3}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.

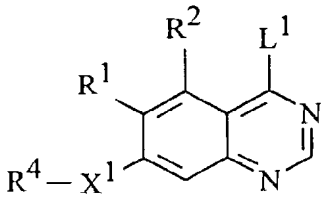
11. A quinazoline derivative as claimed in claim 1 selected from:-
(E)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(4-(pyrrolidin-1-yl)but-2-en-1-yloxy)quinazoline,
4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(3-(methylsulphonyl)propoxy)quinazoline,
(S)-4-(4-chloro-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-3-yl)methoxyquinazoline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

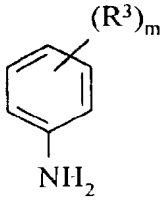
14. A process for the preparation of a quinazoline derivative of formula I or salt thereof (as defined in claim 1) which comprises:-

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



(III)

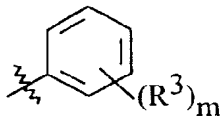
(wherein R^1, R^2, X^1 and R^4 are as defined in claim 1 and L^1 is a displaceable moiety), with a compound of the formula IV:



(IV)

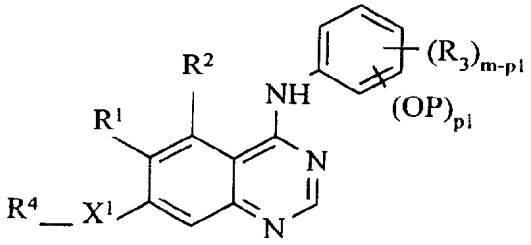
(wherein R^3 and m are as defined in claim 1) whereby to obtain compounds of the formula I and salts thereof;

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



(IIa)

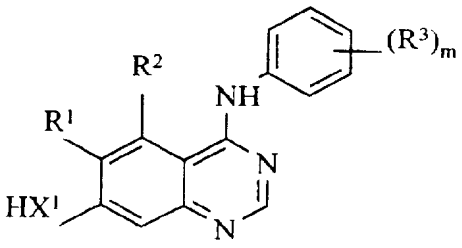
(wherein R^3 and m are as defined in claim 1) represents a phenyl group carrying one or more hydroxy groups, the deprotection of a compound of formula V:



(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:



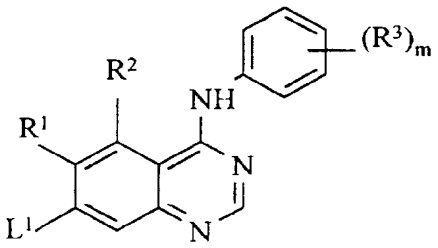
(VI)

(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);

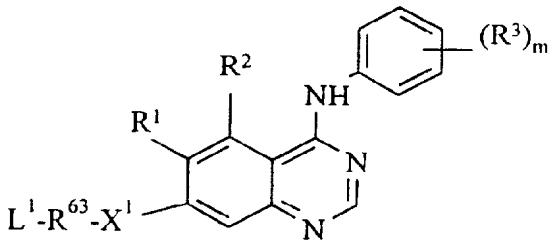
(e) for the preparation of compounds of formula I and salts thereof wherein R^4 is $C_{1-5}alkylR^{53}$, [wherein R^{53} is selected from one of the following three groups:

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

International 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
30 July 2015 (30.07.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/112642 A1

(51) International Patent Classification:
A61K 31/427 (2006.01) *A61K 31/519* (2006.01)
A61K 31/4985 (2006.01)

(21) International Application Number:
PCT/US2015/012315

(22) International Filing Date:
21 January 2015 (21.01.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/929,941 21 January 2014 (21.01.2014) US
61/981,033 17 April 2014 (17.04.2014) US
62/069,155 27 October 2014 (27.10.2014) US

(71) Applicant: **NEUROCRINE BIOSCIENCES, INC.** [US/US]; 12780 El Camino Real, San Diego, California 92130-1102 (US).

(72) Inventor: **GRIGORIADIS, Dimitri, E.**; 10626 Gingerwood Cove, San Diego, California 92130 (US).

(74) Agents: **ROSOK, Mae, Joanne** et al.; Seed Intellectual Property Law Group PLLC, Suite 5400, 701 Fifth Avenue, Seattle, Washington 98104-7064 (US).

(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, 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, 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).

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))*

(54) **Title**: CRF1 RECEPTOR ANTAGONISTS FOR THE TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA

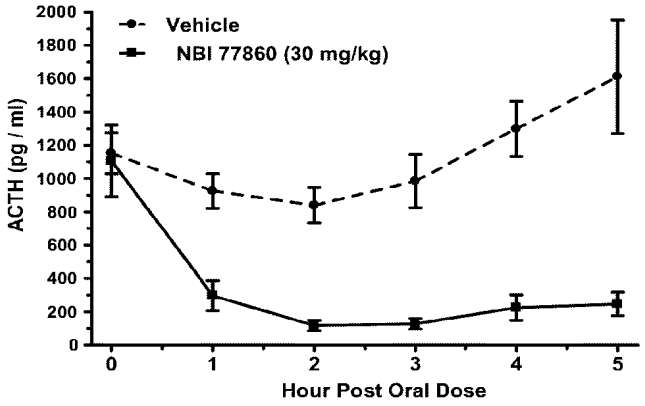


FIG. 2

(57) **Abstract**: CRF_1 receptor antagonists have the potential to directly inhibit ACTH release in patients with CAH and thereby allow normalization of androgen production while using lower, more physiologic doses of hydrocortisone, and thus reducing treatment-associated side effects.

WO 2015/112642 A1

TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA

BACKGROUND

Technical Field

Compositions and methods for treating congenital adrenal hyperplasia are described herein.

Description of the Related Art

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive genetic disorders that result in little or no cortisol biosynthesis. The most frequent form of the disease is 21-hydroxylase deficiency caused by mutations in the *CYP21A2* gene located on chromosome 6p21, which accounts for approximately 95% of CAH cases (*see, e.g.,* Speiser et al., *Int. J. Pediatr. Endocrinol.* 2010:494173 (2010) for a review). These mutations can range from complete loss of enzyme activity required for synthesis of cortisol in the adrenal cortex to a spectrum of partial loss, which results in disease severity that is a direct consequence of a specific mutation. This continuum of 21-hydroxylase deficiency has been broadly classified into salt-wasting and simple-virilizing forms, grouped as classical CAH, and the milder form known as non-classic CAH (NCCAH) or "late-onset" CAH, which is usually diagnosed in late childhood or early adulthood. Non-classic CAH patients are either homozygous or compound heterozygotes, often with a classical CAH allele. These patients have sufficient enzyme activity (> 20-50% of normal) such that they do not have salt-wasting or cortisol deficiency and have normal genitalia at birth, and many remain asymptomatic throughout life (Trapp et al., *Steroids* 77(4):342-46 (2012)). In the less frequent form of the disease, which accounts for 5% of cases, mutation of the 11β-hydroxylase gene *CYP11B1* results in CAH (11β-OH CAH).

Both genetic mutations result in congenital adrenal hyperplasia, cortisol deficiency and excessive adrenocorticotropic hormone (ACTH) production with overproduction of androgens. These patients require lifelong management with

glucocorticoids and the attendant problems associated with such treatment. Accordingly, a significant need exists for treatment regimens to improve the health, well-being, quality of life, and to manage related disorders in patient with CAH.

BRIEF SUMMARY

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

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

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

Embodiment 1. A method for treating Congenital Adrenal Hyperplasia (CAH) by administering to a subject in need thereof a CRF_1 receptor antagonist having a dissociation half-life in excess of 30 minutes.

Embodiment 2. The method of Embodiment 1 wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 40 minutes.

Embodiment 3. The method of Embodiment 1 wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 50 minutes.

Embodiment 4. The method of any one of Embodiments 1-3 wherein the CRF_1 receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

Embodiment 5. The method of any one of Embodiments 1-3 wherein the CRF_1 receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543,

antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

Embodiment 6. The method of any one of Embodiments 1-5 wherein the CRF_1 receptor antagonist is administered at bedtime.

Embodiment 7. The method of any one of Embodiments 1-6 wherein the CRF_1 receptor antagonist is administered at or before the expected circadian release of ACTH.

Embodiment 8. The method of Embodiment 7 wherein the CRF_1 receptor antagonist is administered 3-4 hours before the expected circadian release of ACTH.

Embodiment 9. A method for reducing 17-OHP and ACTH levels in a subject who has Congenital Adrenal Hyperplasia (CAH), said method comprising administering to the subject a CRF_1 receptor antagonist at bedtime.

Embodiment 10. The method of Embodiment 9, wherein the CRF_1 receptor antagonist is administered at or before the expected circadian release of ACTH.

Embodiment 11. The method of Embodiment 9 or Embodiment 10, wherein the CRF_1 receptor antagonist is administered 3-4 hours before the expected circadian release of ACTH.

Embodiment 12. The method of any one of Embodiments 9-11, wherein the CRF_1 receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

Embodiment 13. The method of any one of Embodiments 9-11, wherein the CRF_1 receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

Embodiment 14. A CRF_1 receptor antagonist for use in treating Congenital Adrenal Hyperplasia (CAH), wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 30 minutes.

Embodiment 15. The CRF_1 receptor antagonist of 14, wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 40 minutes.

Embodiment 16. The CRF_1 receptor antagonist of Embodiment 14, wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 50 minutes.

Embodiment 17. The CRF_1 receptor antagonist of any one of Embodiments 14-16, wherein the CRF_1 receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

Embodiment 18. The CRF_1 receptor antagonist of any one of Embodiments 14-16, wherein the CRF_1 receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

Embodiment 19. The CRF_1 receptor antagonist of any one of Embodiments 14-18, wherein the CRF_1 receptor antagonist is suitable for administration at bedtime.

Embodiment 20. The CRF_1 receptor antagonist of any one of Embodiments 14-19, wherein the CRF_1 receptor antagonist is suitable for administration at or before the expected circadian release of ACTH.

Embodiment 21. The CRF_1 receptor antagonist of any one of Embodiments 14-20, wherein the CRF_1 receptor antagonist is suitable for administration 3-4 hours before the expected circadian release of ACTH.

In other embodiments, the methods and uses described above and herein further comprise reducing the amount of a glucocorticoid or mineralocorticoid by at least 10%, 15%, 20%, 30%, 40%, 50%, 60% from the recommended daily dose of GC (such as hydrocortisone (HC), prednisone, prednisolone, dexamethasone, or fludrocortisone) administered to a full grown subject (*e.g.,* a human subject) who has CAH. In other embodiments, the methods and uses described above and herein further comprise reducing the amount of a glucocorticoid or mineralocorticoid by at least 10%, 15%, 20%, 30%, 40%, 50%, 60% from the recommended daily dose of GC (e.g.,

hydrocortisone) or mineralocorticoid (e.g., fludrocortisone) administered to a growing subject (*e.g.,* a human subject) who has CAH.

These and other embodiments will be apparent upon reference to the following detailed description. To this end, various references are set forth herein that describe in more detail certain background information, procedures, compounds and compositions, and are each hereby incorporated by reference in their entirety.

Terms not specifically defined herein should be given the meanings that would be given to them by one of skill in the art in light of the disclosure and the context. As used in the specification, however, unless specified to the contrary, the terms have the meaning indicated. Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment. Thus, the appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

Also, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a non-human animal" may refer to one or more non-human animals, or a plurality of such animals, and reference to "a cell" or "the cell" includes reference to one or more cells and equivalents thereof (*e.g.,* plurality of cells) known to those skilled in the art, and so forth. When steps of a method are described or claimed, and the steps are described as occurring in a particular order, the description of a first step occurring (or being performed) "prior to" (*i.e.,* before) a second step has the same meaning if rewritten to state that the second step occurs (or is performed) "subsequent" to the first step. The term "about" when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range may vary between 1% and 15% of the stated number or numerical range. It should also be noted that the term "or" is generally

employed in its sense including "and/or" unless the content clearly dictates otherwise. The term, "at least one," for example, when referring to at least one compound or to at least one composition, has the same meaning and understanding as the term, "one or more."

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic of steroid synthetic pathways in the adrenal gland. The most common form of CAH is caused by 21-hydroxylase (also called 21-α hydroxylase) deficiency resulting in a decrease of cortisol and increase in androgens such as testosterone and estrogen. A more rare type of classical CAH is 11β-hydroxylase deficiency.

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

Figures 3A-C present graphs illustrating the effect of CRF_1 receptor antagonists, differentiated by their dissociation half-life, on ACTH concentration in adrenalectomized rats.

Figure 4 presents the study design schematic for the clinical trial described in Example 6 that was designed to assess the safety, tolerability and plasma exposure of NBI-77860, as well as the effect of this compound on endogenous levels of HPA (hypothalamic-pituitary-adrenal) axis hormones.

Figure 5 presents the data for mean 17-OHP (upper panel) and ACTH levels (lower panel) throughout the 24-hour postdose period for the clinical trial described in Example 6.

Figure 6 presents the response of a specific individual subject for 17-OHP (upper panel) and ACTH levels (lower panel) following administration of 300 mg and 600 mg NBI-77860 and placebo over time.

DETAILED DESCRIPTION

As described herein, CRF_1 receptor antagonists have been found to directly inhibit ACTH release in patients with CAH and thereby allow normalization of androgen production. Administration of a CRF_1 receptor antagonist permits use of lower, more physiologic doses of hydrocortisone in subjects with CAH and thus reduces treatment-associated side effects.

Newborn screening for CAH is performed by immunoassay to measure 17-OHP levels in heel-stick capillary blood specimens obtained within the first 72 hours of life. The blood sample is analyzed for 17-OHP by commercially available dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA; PerkinElmer, Waltham Massachusetts) (White et al., J. Pediatr. 163:10-12 (2013)).

Second-tier screening tests utilizing biochemical and molecular genetic testing methods, performed between 8 and 14 days of life, are employed by nine states in the United States and strongly recommended by an additional 5 states. The biochemical method includes immunoassay with organic solvent extraction or liquid chromatography followed by tandem mass spectrometry to measure steroid ratios of 17-OHP, androstenedione, and 21-deoxycortisol to cortisol (*see, e.g.,* Speiser et al., *Int. J. Pediatr. Endocrinol.* 2010:494173, 2010). The genetic screen looks for *CYP21A2* mutations that are associated with CAH. While not widely employed in the U.S., the addition of a second screening could potentially improve the sensitivity of the overall screening process, where sensitivity of the first screen alone is approximately 72%.

In absence of results from the newborn screening, female infants with classical CAH are typically identified due to the presence of ambiguous genitalia. Males have normal genitalia at birth and therefore are not diagnosed unless newborn screening is conducted or other medical complications come to attention. Infants who are not initially diagnosed with CAH and suffer from the salt-wasting form of the disease are later diagnosed in the setting of poor weight gain, vomiting, hyperkalemia and hyponatremia within the first few weeks of life.

Treatment of CAH is based on normalization of hormone and steroid levels using a variety of medications from diagnosis in infancy through adulthood.

Glucocorticoids are the current standard treatment in CAH and are used both to correct the endogenous cortisol deficiency and for reducing the elevated ACTH levels from the pituitary, which drives increased androgen production. Unlike the treatment of Addison's disease (adrenal insufficiency), in which cortisol replacement is sufficient, the treatment of CAH must also reduce ACTH production, to control the subsequent androgen excess as well. Thus, the goals of glucocorticoid treatment include cortisol replacement and suppression of ACTH to prevent virilization and menstrual disturbances in women and to inhibit testicular adrenal rest tumors in men. Mineralocorticoid replacement is needed to achieve normal plasma renin activity for maintenance of regular blood pressure, electrolyte balance, and volume status in those patients with the salt-wasting form of CAH.

The regimen of glucocorticoid treatment must support normal physiology and also ensure that sufficient cortisol is available during events that may elicit a strong stress response (*e.g.,* intercurrent illness, exercise, hypotension). Careful monitoring is also necessary to avoid the development of iatrogenic Cushing's syndrome due to glucocorticoid overtreatment in an effort to adequately suppress androgen production, or Addisonian syndrome due to under-treatment. Overtreatment with mineralocorticoids may cause hypertension while under-treatment may lead to low blood pressure, salt loss, fatigue and increased requirements for glucocorticoids. Typical laboratory tests for monitoring treatment efficacy include measurement of plasma concentrations of 17-OHP, androstenedione, testosterone, renin activity, and electrolytes.

Adult patients with CAH have an increased prevalence of risk factors for cardiovascular disease including obesity, hypertension, and insulin resistance (*see, e.g.,* Kim et al., *Semin. Reprod. Med.* 27(4):316-21 (2009)). A study of a large cohort of pediatric and adult CAH patients (n=244) demonstrated that patients are prescribed a variety of glucocorticoid treatment regimens yet frequently suffer from poor hormonal control and the aforementioned adverse outcomes (*see, e.g.,* Finkielstain et al., *J. Clin. Endocrinol Metab.* 97(12):4429-38 (2012)).

Treatment of CAH includes efforts to normalize the cortisol deficiency with glucocorticoids (usually hydrocortisone in children but often more potent agents with narrow therapeutic indices, such as dexamethasone, in adults) and, if necessary for salt-wasting, mineralocorticoids (usually fludrocortisone). The glucocorticoid doses required to achieve sufficient suppression of excess androgens, however, are usually well above the normal physiologic dose used for cortisol replacement alone as in patients with Addison's disease. This increased exposure to glucocorticoids can lead to iatrogenic Cushing's syndrome, increased cardiovascular risk factors, glucose intolerance, and decreased bone mineral density in CAH patients (*see, e.g.,* Elnecave et al., *J. Pediatr. Endocrinol. Metab.* 21:1155-62 (2008); King et al., *J. Clin. Endocrinol. Metab.* 91(3):8656-59 (2006); Migeon et al., *Endocrinol. Metab. Clin. North Am.* 30:193-206 (2001)).

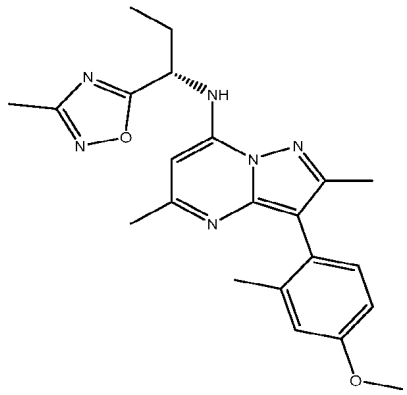
Corticotropin-releasing factor (CRF) was isolated from ovine hypothalami and identified as a 41-amino acid peptide. CRF has been found to produce profound alterations in endocrine, nervous, and immune system function. CRF is believed to be the major physiological regulator of the basal and stress-induced release of adrenocorticotropic hormone ("ACTH"), ß-endorphin, and other pro-opiomelanocortin ("POMC")-derived peptides from the anterior pituitary (*see, e.g.,* Vale et al., *Science 213*:1394-1397, 1981). Secretion of CRF causes release of ACTH from corticotrophs in the anterior pituitary via binding to the CRF_1 receptor, a member of the class B family of G-protein coupled receptors.

Due to the physiological significance of CRF, the development of biologically-active small molecules having significant CRF_1 receptor binding activity and which are capable of antagonizing the CRF_1 receptor remains a desirable goal and has been the subject of ongoing research and development for the treatment of anxiety, depression, irritable bowel syndrome, post-traumatic stress disorder, and substance abuse.

The pituitary hormone ACTH, under the control of hypothalamic corticotropin-releasing factor (CRF), stimulates uptake of cholesterol and drives the synthesis of pregnenolone initiating steroidogenesis in the adrenal gland (*see* Figure 1).

The adrenal cortex is comprised of three zones, which produce distinct classes of hormones many of which are driven by ACTH mobilizing cholesterol through this pathway. Deficiencies in these enzymes as a result of mutation or deletion cause the substrate concentrations to increase. In the most common form of CAH resulting from mutations or deletions in the 21-hydroxylase gene (CYP21A2), potent androgens are produced by the adrenal because of the accumulation of the steroid precursors, progesterone and 17-hydroxyprogesterone (17-OHP). Plasma levels of 17-OHP can reach 10-1000 times the normal concentration in these cases. These increases result in the overproduction of androgens, specifically androstenedione, testosterone, and dihydroxytestosterone causing virilization in females. In addition, 21-hydroxylase deficiency in CAH causes insufficient biosynthesis of glucocorticoids and mineralocorticoids, specifically cortisol and aldosterone. Cortisol is a critical negative feedback regulator of hypothalamic CRF secretion and pituitary ACTH release. The lack of glucocorticoid synthesis and release eliminates the restraint on the hypothalamus and pituitary, which causes ACTH levels to increase. The excessive ACTH stimulation causes hypertrophy of the zona fasciculata and zona reticularis resulting in adrenal hyperplasia.

In an embodiment, a CRF_1 receptor antagonist useful for the treatment of CAH is NBI-77860, 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine (also referred to as "Compound I" herein), and which has the following structure.



Compound I

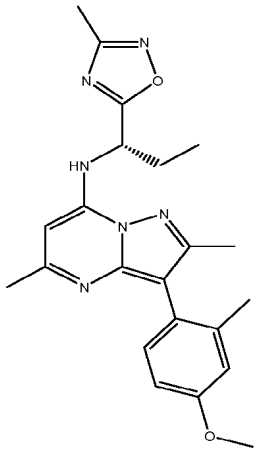
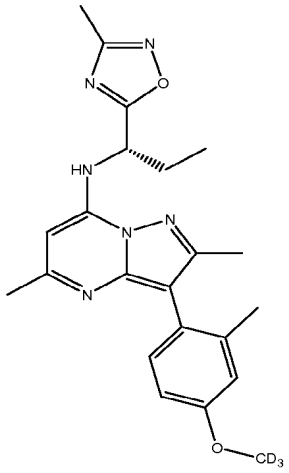
Compound I is a potent CRF_1 antagonist possessing a binding pKi = 8.2 (*see, e.g.,* Tellew et al., *Bioorg. Med. Chem. Lett.* 20:7259, 2010 and Int'l. Patent Appl. Publ. No. WO 2006/044958, both of which references are incorporated by reference herein in their entirety). As described herein, Compound I has potent ACTH lowering effects as shown in adrenalectomized rats.

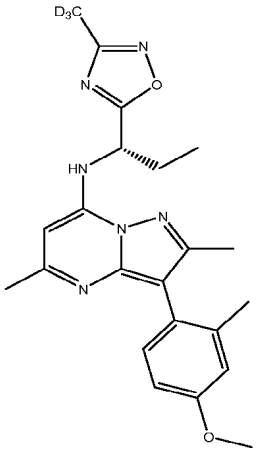
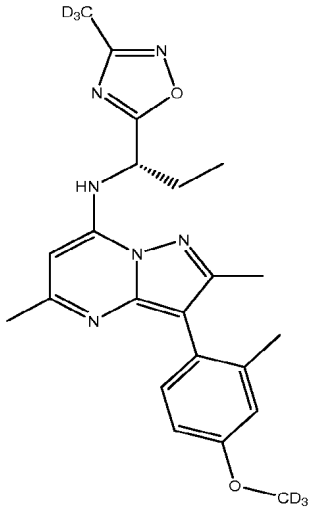
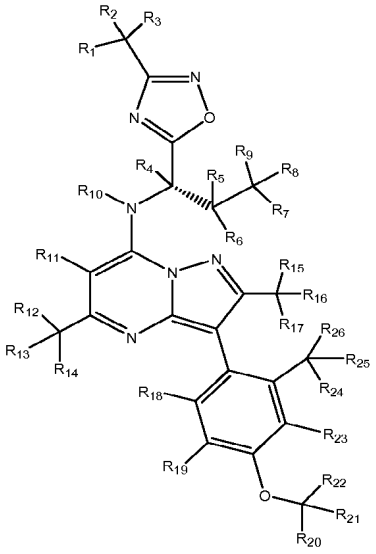
In another embodiment, the CRF_1 receptor antagonist useful for the treatment of CAH is a small molecule antagonist as described in U.S. 6,586,456, US 6,806,282, US 6,531,475, US 6,664,261, US 6,610,678, WO 98/08846, WO 98/11075, WO 99/10350, WO 2000/059888, WO 2006/044821, WO 2006/102194, WO 2006/107784, WO 2006/116412, WO 2006/126718, WO 2007/069565, WO 2007/069671, WO 2008/036541, WO 2008/036579, WO 2008/051533, WO 2008/082003, WO 2008/083070, WO 2008/136377, WO 2009/008552, WO 2009/144632, WO 2010/014280, WO 2010/014687, WO 2010/015628, WO 2010/015655, WO 2010/062718, WO 2010/096426, WO 2011/043387, WO 2011/092293, WO 2011/095450, WO 2011/092290, and WO 2011/043381.

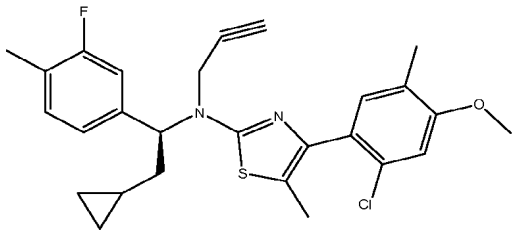
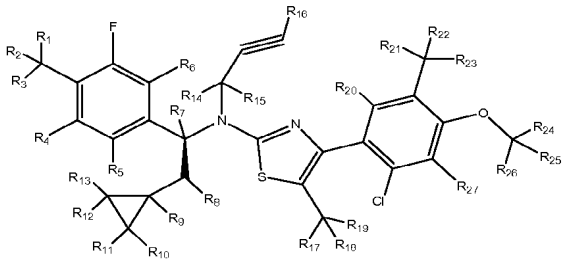
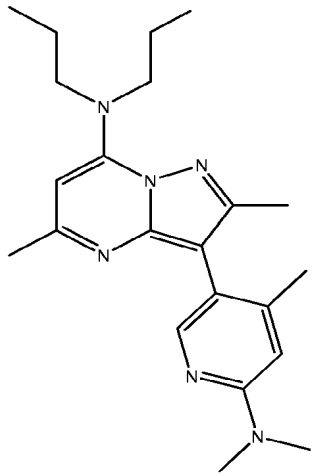
In still another embodiment, the CRF_1 receptor antagonist is NBI-30775, CP-316,311, pexacerfont, emicerfont, SSR-125543 [4-(2-chloro-4-methoxy-5-methylphenyl)-*N*-[(1*S*)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-*N*-(2-propyn-1-yl)-2-thiazolamine], SSR-126374, ONO-2333, NBI-34041, JNJ-19567470, GSK586529, PF-00572778, CP-376395, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), and DMP904.

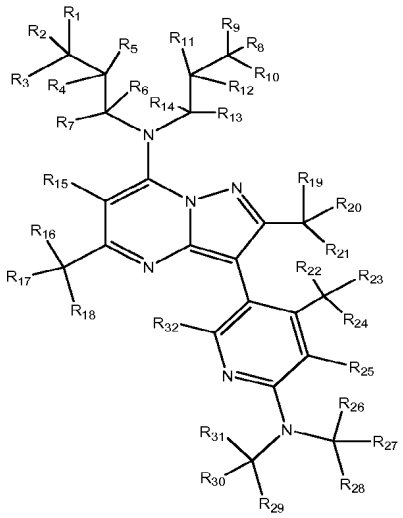
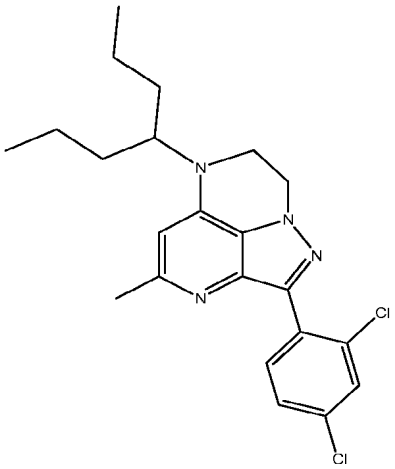
In yet another embodiment, the CRF_1 receptor antagonist has a dissociation half-life ($t_{1/2}$) in excess of 30 minutes, and in another embodiment in excess of 40 minutes, and in another embodiment in excess of 50 minutes. The dissociation half-life of a particular CRF_1 receptor antagonist is determined by the technique disclosed in Example 3. Representative CRF_1 receptor antagonists of these embodiments include Compound I, NBI-30775, NBI-34041, SSR-125543A, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), and DMP904.

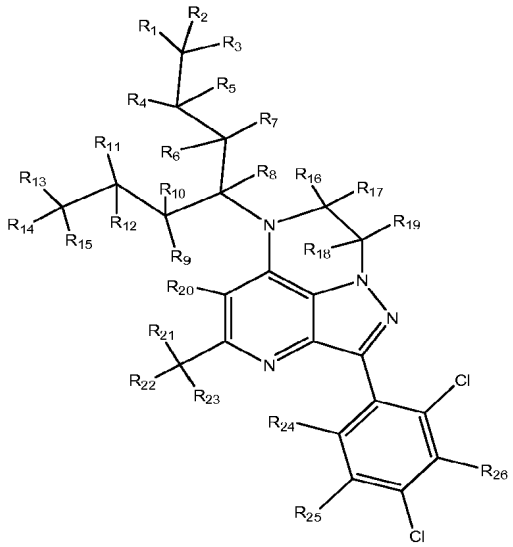
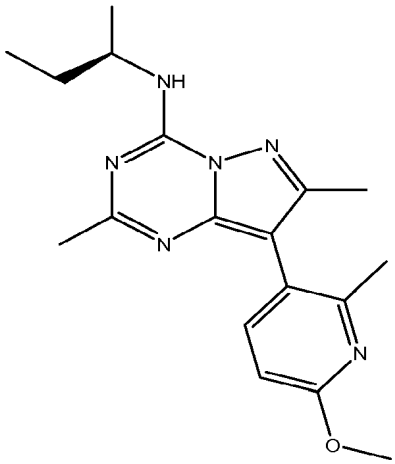
With respect to the compounds described herein, it should also be understood that when a particular position is designated as hydrogen, that such hydrogen (H) may be replaced with deuterium (D). Incorporation of deuterium in place of hydrogen is known to produce significant effects on the physiological and pharmacological activities of the substituted compound. To this end, it should be understood that deuterium replacement of hydrogen means that the abundance of deuterium at that position is substantially greater than the natural abundance of deuterium. Accordingly, in one embodiment representative compounds include the following.

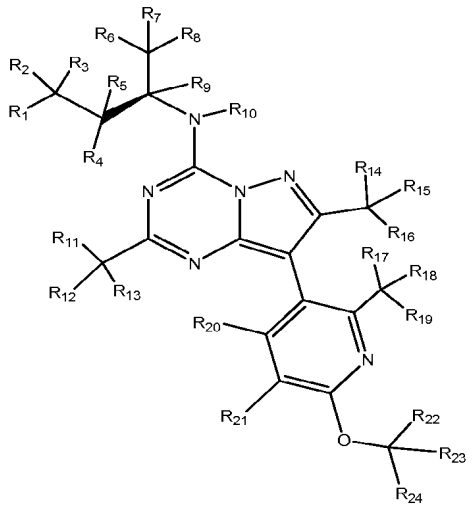
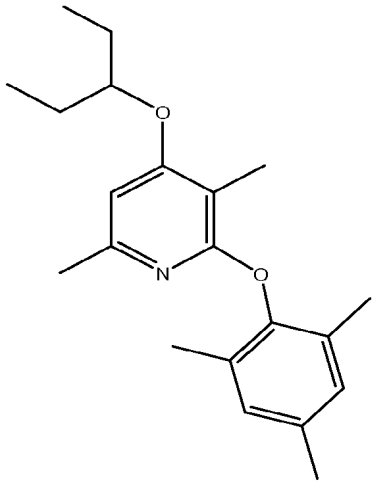
Cpd.	Structure	Chemical Name
NBI-77860		2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine
Deuterated NBI-77860		2,5-dimethyl-3-[2-methyl-4-(d_3-methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine

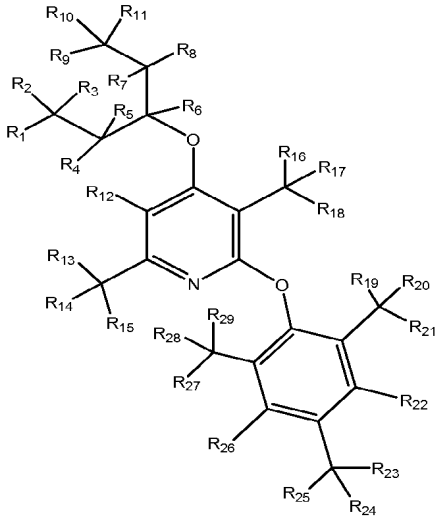
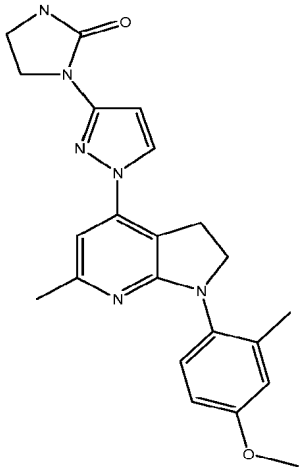
Cpd.	Structure	Chemical Name
Deuterated NBI-77860		2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-d_3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a] pyrimidin-7-amine
Deuterated NBI-77860		2,5-dimethyl-3-[2-methyl-4-(d_3-methyloxy)phenyl]-N-[(1S)-1-(3-d_3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine
Deuterated NBI-77860		

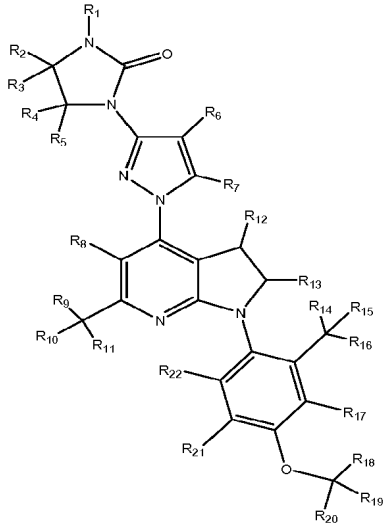
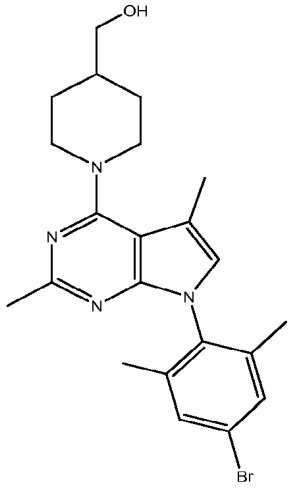
Cpd.	Structure	Chemical Name
	R_1-R_{26} independently H or D	
SSR-125543		4-(2-chloro-4-methoxy-5-methylphenyl)-*N*-[(1*S*)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-*N*-(2-propyn-1-yl)-2-thiazolamine
Deuterated SSR-125543	 R_1-R_{27} independently H or D	
NBI-30775		5-[7-(dipropylamino)-2,5-dimethylpyrazolo[1,5-a]pyrimidin-3-yl]-N,N,4-trimethylpyridin-2-amine

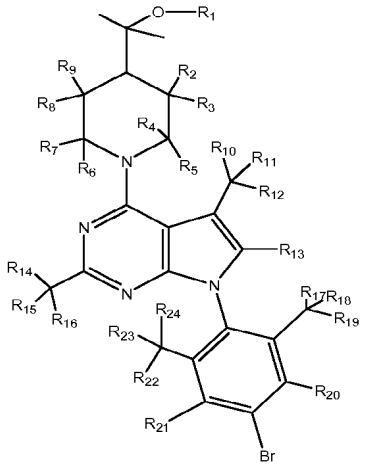
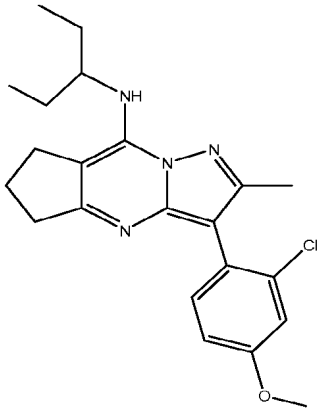
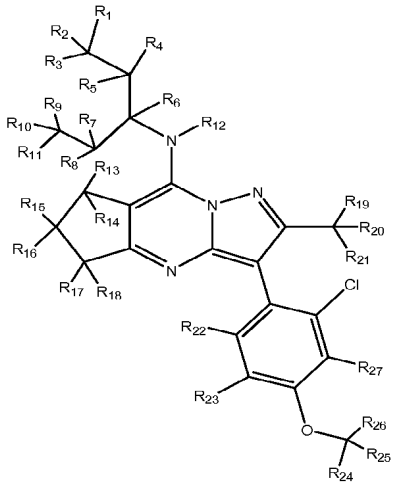
Cpd.	Structure	Chemical Name
Deuterated NBI-30775	 R_1-R_{32} independently H or D	
NBI-34041		3-(2,4-dichlorophenyl)-9-(heptan-4-yl)-6-methyl- 1,2,5,9-tetraazatricyclo[6.3.1.$0^{4,12}$]dodeca- 2,4(12),5,7-tetraene

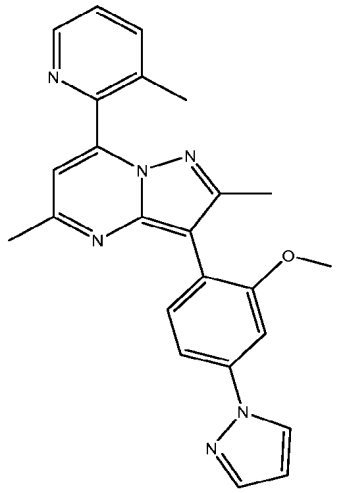
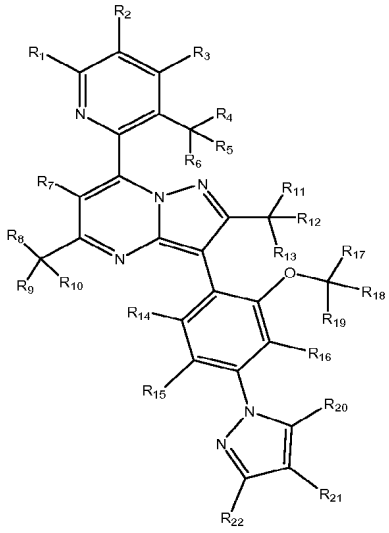
Cpd.	Structure	Chemical Name
Deuterated NBI-34041	 R_1-R_{26} independently H or D	
Pexacerfont		N-[(2R)-butan-2-yl]-8-(6-methoxy-2-methylpyridin-3-yl)-2,7-dimethylpyrazolo[1,5-a][1,3,5]triazin-4-aminc

Cpd.	Structure	Chemical Name
Deuterated Pexacerfont	 R_1-R_{24} independently H or D	
CP-316,311		3,6-dimethyl-4-(pentan-3-yloxy)-2-(2,4,6-trimethylphenoxy)pyridine

Cpd.	Structure	Chemical Name
Deuterated CP-316,311	 R_1-R_{29} independently H or D	
GSK876008		

Cpd.	Structure	Chemical Name
Deuterated GSK876008	 R_1-R_{22} independently H or D	
CRA5626/R3 17573		

Cpd.	Structure	Chemical Name
Deuterated CRA5626/R3 17573	 R_1-R_{24} independently H or D	
ONO2333Ms		10-(2-chloro-4-methoxyphenyl)-11-methyl-N-(pentan-3-yl)-1,8,12-triazatricyclo[7.3.0.0^{3,7}] dodeca- 2,7,9,11-tetraen-2-amine
Deuterated ONO2333	 R_1-R_{27} independently H or D	

Cpd.	Structure	Chemical Name
NBI76169		
Deuterated NBI76169	 R_1-R_{22} independently H or D	

In another embodiment, any of the aforementioned compounds may incorporate stable or radioactive isotopes. Accordingly, also contemplated is use of isotopically-labeled compounds identical to those described herein, wherein one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into these compounds include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine and chlorine, such as, but not limited to

deuterium as discussed above (2H), as well as 3H, 13C, 14C, 15N, 18O, 17O, 31P, 32P, 35S, 18F, and 36Cl, respectively. Certain isotopically-labeled compounds, for example those into which radioactive isotopes such as 3H and 14C are incorporated, are also useful in drug or substrate tissue distribution assays. Tritiated hydrogen (3H) and carbon-14 (14C) isotopes are particularly preferred for their ease of preparation and detectability. Substitution with heavier isotopes such as deuterium (2H) can provide certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dose requirements and, therefore, may be preferred in some circumstances. Isotopically-labeled compounds can generally be prepared by performing procedures routinely practiced in the art.

Effect on Plasma ACTH in Adrenalectomized Rats

Adrenalectomy eliminates circulating corticosterone (the primary glucocorticoid) in rats and removes the negative feedback control of the HPA axis at both the hypothalamic and pituitary (corticotroph cells) levels and thus chronically elevates plasma ACTH (*see, e.g.,* Mims et al., *J. Natl. Med. Assoc.* 69:145-47 (1977)). Intravenous injection of peptide CRF_1 receptor antagonists have been demonstrated to reduce the high plasma ACTH levels in adrenalectomized (ADX) rats (*see, e.g.,* Rivier et al., *J. Med. Chem.* 12:42:3175-82 (1999)). These findings were recapitulated with the small molecule NBI-77860 (Compound I). In adrenalectomized rats, Compound I has potent capability for lowering ACTH. The maximum reduction in ACTH correlated with peak plasma concentrations of NBI-77860; however, the duration of the ACTH-lowering effect exceeded drug plasma exposure. In adrenalectomized rats, a predictable relationship therefore exists between integrated plasma exposure of NBI-77860 and *in vivo* efficacy following oral administration.

The effectiveness of a compound as a CRF receptor antagonist may be determined by various assay methods. CRF antagonists described herein may be capable of inhibiting the specific binding of CRF to its receptor and consequently antagonizing activities associated with CRF. A compound may be assessed for activity as a CRF antagonist by one or more generally accepted assays including the assay

described in the Examples. CRF antagonists useful for the methods described herein include compounds that demonstrate affinity for CRF receptor.

Without wishing to be bound by theory, in the treatment of CAH, CRF receptor antagonists would potentially block the release of ACTH from pituitary corticotrophs, thereby decreasing the production of androgens, and allow a more refined treatment paradigm for replacement of cortisol. Animal and human studies have shown the pharmacologic effect of Compound I (NBI-77860) on ACTH release. Standard biomarker assessments used by endocrinologists when monitoring treatment efficacy may be used for monitoring the effects of this CRF_1 receptor antagonist. Plasma levels of 17-OHP, androstenedione, testosterone, cortisol and ACTH, as well as urinary metabolites of these steroids, are easily measured in both children and adults giving rapid and meaningful data regarding treatment impact.

Pharmaceutical Compositions and Methods of Treatment

The present disclosure further provides for pharmaceutical compositions comprising any one of the CRF antagonist compounds described herein and a pharmaceutically acceptable excipient for use in the methods for treating CAH. A pharmaceutically acceptable excipient is a physiologically and pharmaceutically suitable non-toxic and inactive material or ingredient that does not interfere with the activity of the active ingredient; an excipient also may be called a carrier. The CRF antagonist compounds may be formulated in a pharmaceutical composition for use in treatment or preventive (or prophylactic) treatment (*e.g.,* reducing exacerbation of CAH disease, or occurrence or recurrence of one or more symptoms of the disease). The methods and excipients described herein are exemplary and are in no way limiting. Pharmaceutically acceptable excipients are well known in the pharmaceutical art and described, for example, in Rowe *et al.*, Handbook of Pharmaceutical Excipients: A Comprehensive Guide to Uses, Properties, and Safety, 5th Ed., 2006, and in *Remington: The Science and Practice of Pharmacy* (Gennaro, 21st Ed. Mack Pub. Co., Easton, PA (2005)). Examples of pharmaceutically acceptable excipients include sterile saline and phosphate buffered saline at physiological pH. Preservatives, stabilizers, dyes, buffers,

and the like may be provided in the pharmaceutical composition. In addition, antioxidants and suspending agents may also be used.

Therapeutic and/or prophylactic benefit includes, for example, an improved clinical outcome, both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow or retard (lessen) an undesired physiological change or disorder, or to prevent or slow or retard (lessen) the expansion or severity of such disorder. As discussed herein, beneficial or desired clinical results from treating a subject include, but are not limited to, abatement, lessening, or alleviation of symptoms that result from or are associated the disease, condition, or disorder to be treated; decreased occurrence of symptoms; improved quality of life; longer disease-free status (*i.e.,* decreasing the likelihood or the propensity that a subject will present symptoms on the basis of which a diagnosis of a disease is made); diminishment of extent of disease; stabilized (*i.e.,* not worsening) state of disease; delay or slowing of disease progression; amelioration or palliation of the disease state; and remission (whether partial or total), whether detectable or undetectable; and/or overall survival. "Treatment" can also mean prolonging survival when compared to expected survival if a subject were not receiving treatment. Subjects in need of treatment include those who already have the condition or disorder as well as subjects prone to have or at risk of developing the disease, condition, or disorder, and those in which the disease, condition, or disorder is to be prevented (*i.e.,* decreasing the likelihood of occurrence of the disease, disorder, or condition). A subject may be a human or non-human mammal (e.g., rat, mouse, dog, cat, livestock, zoo animal).

Optimal doses may generally be determined using experimental models and/or clinical trials. The optimal dose may depend upon the body mass, weight, or blood volume of the subject. In general, the amount of a compound described herein, that is present in a dose ranges from about 0.1 mg to about 30 mg per kg weight of the subject. In certain embodiments, a single dose is about 50-1000 mg. The use of the minimum dose that is sufficient to provide effective therapy is usually preferred. Subjects may generally be monitored for therapeutic effectiveness by clinical evaluation and using assays suitable for the condition being treated or

prevented, which assays will be familiar to those having ordinary skill in the art and are described herein. The level of a compound that is administered to a subject may be monitored by determining the level of the compound in a biological fluid, for example, in the blood, blood fraction (*e.g.,* serum), and/or in the urine, and/or other biological sample from the subject. Any method practiced in the art to detect the compound may be used to measure the level of compound during the course of a therapeutic regimen.

The dose of a composition comprising at least one of the compounds described herein for treating CAH or a related disease or disorder may depend upon the subject's condition, that is, stage of the disease, severity of symptoms caused by the disease, general health status, as well as age, gender, and weight, and other factors apparent to a person skilled in the medical art. Similarly, the dose of the compound may be determined according to parameters understood by a person skilled in the medical art.

The pharmaceutical compositions described herein that comprise at least one of the CRF_1 antagonist compounds described herein may be administered to a subject in need by any one of several routes that effectively deliver an effective amount of the compound. Such administrative routes include, for example, oral, parenteral, enteral, rectal, intranasal, buccal, sublingual, intramuscular, and transdermal. Compositions administered by these routes of administration and others are described in greater detail herein.

Administration of the compounds or compositions disclosed includes nighttime administration or administration prior to sleep (*i.e.,* bedtime administration). As used herein, bedtime administration refers to dosing intended to deliver clinically relevant concentrations of the CRF_1 antagonist at or before (such as 2-5 hours before) the expected circadian release of ACTH. Since this ACTH release is typically at 1-2 A.M., and since most orally administered drugs have a Tmax of several hours, dosing at 10 P.M., for example, which is 3-4 hours in advance of the expected circadian release of ACTH is desirable. This same pre-pulse bedtime dosing may be adapted for shift workers (*e.g.,* those who work at night and sleep during the day), in which case

administration will not necessarily occur at nighttime. Administration is therefore dependent upon the expected circadian release of ACTH, and can vary depending upon the individual's (*i.e.,* subject, patient) particular work and sleep patterns. In certain embodiments, a CRF_1 receptor antagonist described herein (for example, NBI-77860 or any one of NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904) is administered between about 2-5 hours prior to (*i.e.,* before, in advance of) the expected circadian release of ACTH. In other embodiments, the CRF_1 receptor antagonist is administered to the subject between about 2-4 hours or 3-5 hours prior to the expected circadian release of ACTH. In a more specific embodiment, CRF_1 receptor antagonist is administered to the subject between about 3-4 hours prior to the expected circadian release of ACTH.

Also provided herein is a method for reducing 17-OHP and/or ACTH levels in a subject (*i.e.,* patient, individual) who has CAH by administering a CRF_1 receptor antagonist. In certain embodiments, the antagonist is administered at or before the expected circadian release of ACTH. In other certain embodiments, the CRF_1 receptor antagonist as administered between about 2-4 hours or between about 3-5 hours prior to the expected circadian release of ACTH. In a more specific embodiment, CRF_1 receptor antagonist is administered to the subject between about 3-4 hours prior to the expected circadian release of ACTH.

The methods described herein that comprise administering a CRF_1 receptor antagonist to a female subject in need thereof in the manner described herein that causes a decrease in level of ACTH and 17-OHP in the subject may result in decreased release of androgens such as testosterone and androstenedione. The dose of glucocorticoids may concomitantly be decreased by a clinically significant amount, which in turn results in decreased side effects.

The amount of glucocorticoids (GC) and mineralocorticoids (MC) for maintenance therapy in patients who have CAH and who are young and growing and for maintenance therapy in patients who are full grown patients is known to a person skilled in the art. For example, guidelines are described in Speiser et al. (*J. Clin.*

Endocrinol. Metab. 95:4133-60 (2010), incorporated by reference in its entirety) and shown in the Tables 1 and 2 therein. Particularly, for young, growing patients who have CAH, persons skilled in the art appreciate that too high dosing with GCs can impede statural growth in the patient. Accordingly, the methods described herein for treating a patient with a CRF_1 receptor antagonist may comprise reducing the dose of a GC in a clinically significant manner.

In certain embodiments, methods for treating CAH by administering a CRF_1 receptor antagonist may further comprise administering a GC at a dose lower than the currently recommended dose of a GC for treating a subject who has CAH. When the subject is a fully grown patient, the dose of a GC, such as the dose of hydrocortisone (HC), prednisone, prednisolone, dexamethasone, or fludrocortisone recommended for maintenance therapy in a fully grown subject may be decreased by about 10%, 15%, 20%, 30%, 40%, 50%, 60% or more from the recommended doses of 15-25 mg/day HC; 5-7.5 mg/day prednisone, 4-6 mg/day prednisolone; 0.25-0.5 mg/day dexamethasone, or 0.05-0.2 mg/day of fludrocortisone. In a subject who is a growing patient with CAH, the recommended dose of GCs, such as HC may be decreased from the total recommended dose of 10-15 mg/m^2 per day and/or the total dose of fludrocortisone of 0.05-0.2 mg/day may each be decreased by about 10%, 15%, 20%, 30%, 40%, 50%, 60% or more in a subject who receives a CRF_1 receptor antagonist as described herein. In an embodiment, the methods described herein comprising administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of ACTH levels relative to placebo. In a specific embodiment, administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of ACTH levels relative to placebo, wherein that reduction is at least 25%. In another embodiment, administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of ACTH levels relative to placebo, wherein that reduction is at least 50%. See Speiser et al., *supra*, for guidelines regarding administering glucocorticoids and mineralocorticoids.

In another embodiment, the methods described herein comprising administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of 17-OHP levels relative to placebo. In another specific embodiment, administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of 17-OHP levels relative to placebo wherein that reduction is at least 25%. In still another embodiment, administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of 17-OHP levels relative to placebo wherein that reduction is at least 50%.

In an embodiment, the methods described herein comprising administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of both ACTH and 17-OHP levels relative to placebo. In a certain embodiment, administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of both ACTH and 17-OHP levels relative to placebo wherein the reduction is at least 25%. In another specific embodiment, administering to a subject in need thereof an effective amount of a CRF_1 antagonist results in a clinically significant reduction of both ACTH and 17-OHP levels relative to placebo wherein the reduction is at least 50%.

The pharmaceutical compositions may be in the form of a solution. Alternatively, they may be in the form of a solid, such as powder, tablets, or the like. A composition comprising any one of the compounds described herein may be formulated for sustained or slow release. Such compositions may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain the compound dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Excipients for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. The amount of active compound contained within a sustained release formulation depends

upon the site of implantation, the rate and expected duration of release, and the nature of the condition to be treated or prevented.

For oral formulations, at least one of the compounds described herein can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders; with disintegrators; with lubricants; and if desired, with diluents, buffering agents, moistening agents, preservatives, coloring agents, and flavoring agents. The compounds may be formulated with a buffering agent to provide for protection of the compound from low pH of the gastric environment and/or an enteric coating. A compound included in the compositions may be formulated for oral delivery with a flavoring agent, *e.g.*, in a liquid, solid or semi-solid formulation and/or with an enteric coating. Oral formulations may be provided as gelatin capsules, which may contain the active compound along with powdered carriers, such as lactose, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similar carriers and diluents may be used to make compressed tablets.

EXAMPLES

EXAMPLE 1

CRF RECEPTOR BINDING ACTIVITY

CRF antagonists as used in the methods described herein may be evaluated for binding activity to the CRF receptor by a standard radioligand binding assay as generally described by Grigoriadis et al. (*see, e.g., Mol. Pharmacol* vol 50, pp 679-686, 1996) and Hoare et al. (*see, e.g., Mol. Pharmacol* 63: 751-765, 2003.) By utilizing radiolabeled CRF ligands, the assay may be used to evaluate the binding activity of the compounds described herein with any CRF receptor subtype.

Briefly, the binding assay involves the displacement of a radiolabeled CRF ligand from the CRF receptor. More specifically, the binding assay is performed in 96-well assay plates using 1-10 μg cell membranes from cells stably transfected with

human CRF receptors. Each well receives about 0.05 mL assay buffer (*e.g.*, Dulbecco's phosphate buffered saline, 10 mM magnesium chloride, 2 mM EGTA) containing compound of interest or a reference ligand (for example, sauvagine, urocortin I, or CRF), 0.05 mL of [^{125}I] tyrosine - sauvagine (final concentration ~150 pM or approximately the K_D as determined by Scatchard analysis) and 0.1 mL of a cell membrane suspension containing the CRF receptor. The mixture is incubated for 2 hours at 22°C followed by separation of the bound and free radioligand by rapid filtration over glass fiber filters. Following three washes, the filters are dried and radioactivity (Auger electrons from ^{125}I) is counted using a scintillation counter. All radioligand binding data may be analyzed using the non-linear least-squares curve-fitting programs Prism (GraphPad Software Inc) or XL*fit* (ID Business Solutions Ltd).

EXAMPLE 2

CRF_1 Receptor Agonist Activity

As reported in Fleck *et al.* (*J. Pharmacology and Experimental Therapeutics*, 341(2):518-531, 2012) (hereinafter "Fleck *et al.*" and incorporated by reference in it's entirely) the activity of previously identified CRF_1 receptor antagonists are presented. Such activity is reported as the kinetically derived affinity (K_i) calculated from the association (k_1) and dissociation (k_{-1}) rate constants by the following equation:

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

Also as reported in Fleck *et al.*, the kinetic K_i of the CRF_1 receptor antagonists listed in Table 1 below have been reported:

Table 1

Representative CRF_1 Receptor Antagonists

Ligand	**k_1 ($10^6 M^{-1}min^{-1}$)**	**Kinetic K_i (nM)**
NBI-27914	9.4 ± 3	25

Ligand	k_1 ($10^6 M^{-1}min^{-1}$)	Kinetic K_i (nM)
CP-316,311	13 ± 2	12
NBI-46200	6.2 ± 2	22
DMP696	7.7 ± 2	9.5
pexacerfont	2.6 ± 0.1	19
NBI-35965	20 ± 2	2.3
ONO-2333Ms	4.4 ± 2.2	15
antalarmin	3.4 ± 0.6	3.9
NBI-34041	8.3 ± 2.0	1.7
DMP904	18 ± 1	0.38
NBI-30775	14 ± 2.0	0.36
SSR125543A	33 ± 5	0.049

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

Table 1 (cont.)

Ligand	k_1 ($10^6 M^{-1}min^{-1}$)	Kinetic K_i (nM)
NBI-77860	0.24 ± 0.05	48 ± 9

EXAMPLE 3

DISSOCIATION HALF-LIFE ($T_{1/2}$) OF CRF_1 RECEPTOR ANTAGONISTS

The dissociation half-life ($t_{1/2}$) of a CRF_1 receptor antagonist as used in the methods described herein is evaluated by the technique described in Fleck *et al.* As described therein, the dissociation rate constant for labeled and unlabeled ligands is

denoted as k_{-1}, while the half-life of drug dissociation from the receptor ($t_{1/2}$), which is equal to the median residence time, is calculated from the dissociation rate constant (k_{-1}) by the following equation:

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

As reported in Fleck *et al.*, the dissociation half-life ($t_{1/2}$) of the CRF_1 receptor antagonists listed in Table 2 below have been reported.

Table 2

Dissociation Half-Life of Representative Compounds

Ligand	**k_{-1} (min^{-1})**	**Dissociation $t_{1/2}$ (min)**
NBI 27914	0.27 ± 0.07	2.6
CP-316,311	0.17 ± 0.04	4.1
NBI-46200	0.13 ± 0.002	5.3
DMP696	0.095 ± 0.02	7.3
pexacerfont	0.049 ± 0.001	14
NBI-35965	0.048 ± 0.005	16
ONO-2333Ms	0.063 ± 0.029	17
antalarmin	0.013 ± 0.002	53
NBI-34041	0.013 ± 0.002	53
DMP904	0.0072 ± 0.002	96
NBI-30775	0.0054 ± 0.0006	130
SSR125543A	0.0016 ± 0.0003	430

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

Table 2 (cont.)

Ligand	k_{-1}, min^{-1}	Dissociation $t_{1/2}$, min
NBI-77860	0.012 ± 0.0003	58

Accordingly, CRF_1 receptor antagonists that have a dissociation half-life ($t_{1/2}$) in excess of 30 minutes include (but are not limited to) antalarim, NBI-34041, DMP904, NBI-30775, SSR125543A, and NBI-77860 (Compound I). These same compounds are also representative of CRF_1 receptor antagonists having a dissociation half-life ($t_{1/2}$) in excess of 40 minutes, and a dissociation half-life ($t_{1/2}$) in excess of 50 minutes.

EXAMPLE 4

LOWERING OF ACTH IN ADRENALECTOMIZED RATS

Compound I (NBI-77860) (*see, e.g.,* Tellew et al., *Bioorg. Med. Chem. Lett.* 2010, 20:7259; WO2006044958) is a potent CRF_1 antagonist possessing a binding pKi of 8.2, a kinetic Ki of 49 nM (Table 1 above), and a Dissociation $t_{1/2}$ of 58 minutes (Table 2 above).

Intravenous injection of peptide CRF_1 receptor antagonists have been demonstrated to reduce the high plasma ACTH levels in adrenalectomized (ADX) rats (*see, e.g.,* Rivier et al., *J. Med. Chem.* 12:42:3175-82 (1999)). These findings were recapitulated with the small molecule NBI-77860. When administered orally to ADX rats (n=6/group), 30 mg/kg single doses of NBI-77860 significantly lowered the plasma ACTH levels for up to 5 hours (*see* Figure 2). The duration of efficacy correlated with peak plasma concentrations, with a time course exceeding drug plasma exposure. In adrenalectomized rats, a predictable relationship exists between integrated plasma exposure of NBI-77860 and *in vivo* efficacy following oral administration.

Fleck *et al.* also report the effects of CRF_1 receptor antagonists differentiated by their dissociation half-life on plasma ACTH levels in adrenalectomized rats; namely, NBI 30775, NBI 34041 and NBI 35965. At the highest

dose (10 mg/kg), all three ligands reduced ACTH acutely (1 hr post-injection, Figure 3A-C). After a longer duration, a clear difference emerged between NBI 35965 and the other two ligands. The ACTH level returned to the vehicle level by 2 hr for NBI 35965 (Fig. 3C), whereas the response was sustained for 4-6 hr for NBI 30775 and NBI 34041 (Fig. 3A and 3B).

EXAMPLE 5

PHARMACODYNAMIC EFFECTS OF COMPOUND I IN HUMANS

In this study, pharmacodynamic effects of NBI-77860 (Compound I) on human subjects were evaluated by observing the effect of single oral doses on HPA axis response produced by metyrapone (0.04 g/kg) with respect to placebo and to a single dose of alprazolam (0.75 mg). Metyrapone blocks cortisol synthesis in the adrenal, thereby mimicking the cortisol deficiency of CAH, and is associated with an acute reflex rise ACTH levels.

The primary analysis was performed by determining the ACTH values between 30 min and 4 h post dose (AUC (30 min to 4 h)). In this analysis, when compared to placebo, the following observations were made. A significant reduction of ACTH AUC (30 min to 4 h) was observed with the alprazolam and the NBI-77860, 400 mg treatments. A non-significant reduction in ACTH AUC (30 min to 4 h) was seen with the NBI-77860, 50 mg treatment. An increase in AUC (30 min to 4 h) was seen with the NBI-77860, 10 mg treatment.

In the analysis performed on the ACTH values between 2 h to 4 h post-dose (AUC (2 h to 4 h)) (the period when exposure reached the C_{max} value), there was a significant reduction of ACTH levels in the NBI-77860, 400 mg and 50 mg treatment periods compared with the placebo treatment period; however, the reduction was not seen with the NBI-77860 at the 10 mg dose.

EXAMPLE 6

CLINICAL STUDY

In this study, NBI-77860 (Compound I) was evaluated in the clinical study entitled "A Phase 1, Single-Blind, Placebo-Controlled, Fixed-Sequence, Single-Dose Study to Evaluate the Safety and Tolerability of NBI-77860 in Adult Females with Congenital Adrenal Hyperplasia" (IND 117,388). The study was a single-blind, placebo-controlled, single center, fixed-sequence, single-dose clinical trial in adult female classical CAH patients. It was designed to assess the safety, tolerability, and plasma exposure of NBI-77860, as well as the effect of this compound on endogenous levels of HPA axis hormones.

A total of 8 female subjects, ages 19 to 58, with a medical diagnosis of classical 21-hydroxylase deficiency CAH were administered single bedtime doses (hs) of NBI-77860 300 mg, 600 mg, and placebo during three separate treatment periods (see study design schematic in Figure 4). The subjects' usual morning dose of concurrent steroidal treatment was delayed until after the 16-hour postdose blood samples were collected (*i.e.*, until approximately 1400h).

The PD endpoints for this study included the HPA axis biomarkers of interest in this patient population; namely, 17-hydroxyprogesterone (17-OHP; as the primary PD endpoint), adrenocorticotropic hormone (ACTH), androstenedione, testosterone, and serum cortisol levels. The initial analysis of PD variables was an examination of the grouped subject data for each biomarker, expressed as a mean percent change from predose levels for the two active dosing conditions relative to the placebo condition. The data for mean 17-OHP and ACTH levels throughout the 24-hour postdose period are provided in Figure 5.

Consistent and clinically meaningful reductions from predose levels of both 17-OHP and ACTH were observed throughout the postdose period following administration of NBI-77860 relative to placebo in these CAH patients. In addition to the group mean data, individual responses were evaluated and treatment "responders" were conservatively defined as those subjects with at least a 50% decrease in 17-OHP and ACTH under active NBI-77860 relative to placebo during the peak morning period.

This responder analysis yielded a sizeable responder rate of 50% in the study (none of these subjects were responders during the initial placebo treatment period). Furthermore, the 300 mg dose yielded nearly identical effects on 17-OHP and ACTH as the 600 mg dose. An example of an individual subject response for these biomarkers is provided in Figure 6.

The various embodiments described above can be combined to provide further embodiments. All U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, including U.S. Provisional Application Serial Nos. 61,929,941, filed January 21, 2014; 61,981,033, filed April 17, 2014; and 62/069,155, filed October 27, 2014, are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

I claim the following:

1. A method for treating Congenital Adrenal Hyperplasia (CAH) by administering to a subject in need thereof a CRF_1 receptor antagonist having a dissociation half-life in excess of 30 minutes.

2. The method of claim 1 wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 40 minutes.

3. The method of claim 1 wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 50 minutes.

4. The method of claim 1 wherein the CRF_1 receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

5. The method of claim 1 wherein the CRF_1 receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

6. The method of any one of claims 1-5 wherein the CRF_1 receptor antagonist is administered at bedtime.

7. The method of any one of claims 1-5 wherein the CRF_1 receptor antagonist is administered at or before the expected circadian release of ACTH.

8. The method of claim 7 wherein the CRF_1 receptor antagonist is administered 3-4 hours before the expected circadian release of ACTH.

9. A method for reducing 17-OHP and ACTH levels in a subject who has Congenital Adrenal Hyperplasia (CAH), said method comprising administering to the subject a CRF_1 receptor antagonist at bedtime.

10. The method of claim 9, wherein the CRF_1 receptor antagonist is administered at or before the expected circadian release of ACTH.

11. The method of claim 9, wherein the CRF_1 receptor antagonist is administered 3-4 hours before the expected circadian release of ACTH.

12. The method of any one of claims 9-11, wherein the CRF_1 receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

13. The method of any one of claims 9-11, wherein the CRF_1 receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

14. A CRF_1 receptor antagonist for use in treating Congenital Adrenal Hyperplasia (CAH), wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 30 minutes.

15. The CRF_1 receptor antagonist of claim 14, wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 40 minutes.

16. The CRF_1 receptor antagonist of claim 14, wherein the CRF_1 receptor antagonist has a dissociation half-life in excess of 50 minutes.

17. The CRF_1 receptor antagonist of claim 14, wherein the CRF_1 receptor antagonist is Compound I (NBI-77860; 2,5-dimethyl-3-[2-methyl-4-(methyloxy)phenyl]-N-[(1S)-1-(3-methyl-1,2,4-oxadiazol-5-yl)propyl]pyrazolo[1,5-a]pyrimidin-7-amine).

18. The CRF_1 receptor antagonist of claim 14, wherein the CRF_1 receptor antagonist is NBI-30775, NBI-34041, SSR-126374, SSR-125543, antalarmin (N-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[3,2-e]pyrimidin-4-amine), or DMP904.

19. The CRF_1 receptor antagonist of any one of claims 14-18, wherein the CRF_1 receptor antagonist is suitable for administration at bedtime.

20. The CRF_1 receptor antagonist of any one of claims 14-18, wherein the CRF_1 receptor antagonist is suitable for administration at or before the expected circadian release of ACTH.

21. The CRF_1 receptor antagonist of any one of claims 14-18, wherein the CRF_1 receptor antagonist is suitable for administration 3-4 hours before the expected circadian release of ACTH.

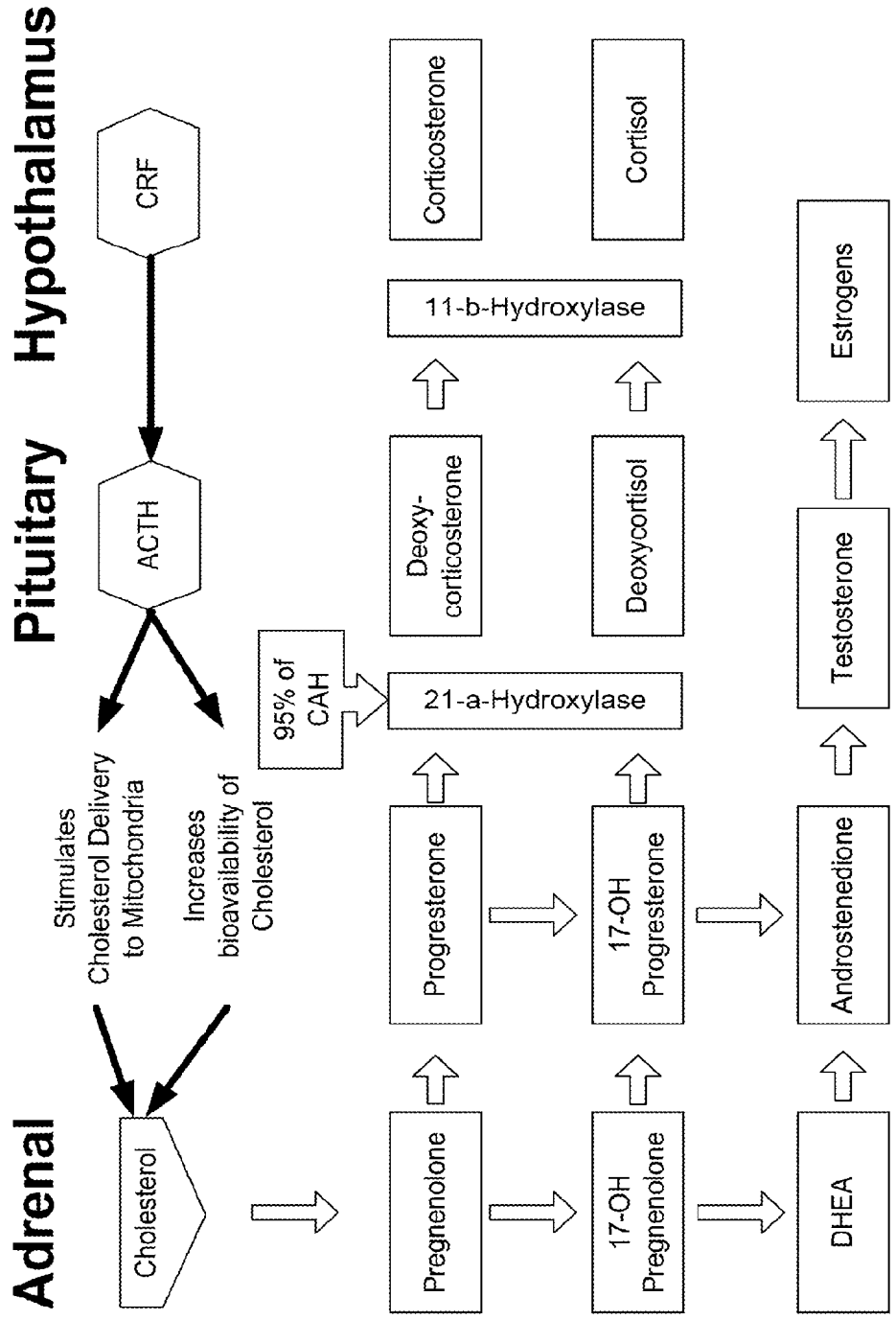

Hypothalamus
Pituitary
Adrenal
CRF
ACTH
Stimulates Cholesterol Delivery to Mitochondria
Increases bioavailability of Cholesterol
Cholesterol
95% of CAH
Pregnenolone
Progresterone
17-OH Pregnenolone
17-OH Progresterone
DHEA
Androstenedione
21-a-Hydroxylase
Deoxy-corticosterone
Deoxycortisol
11-b-Hydroxylase
Corticosterone
Cortisol
Testosterone
Estrogens

FIG. 1

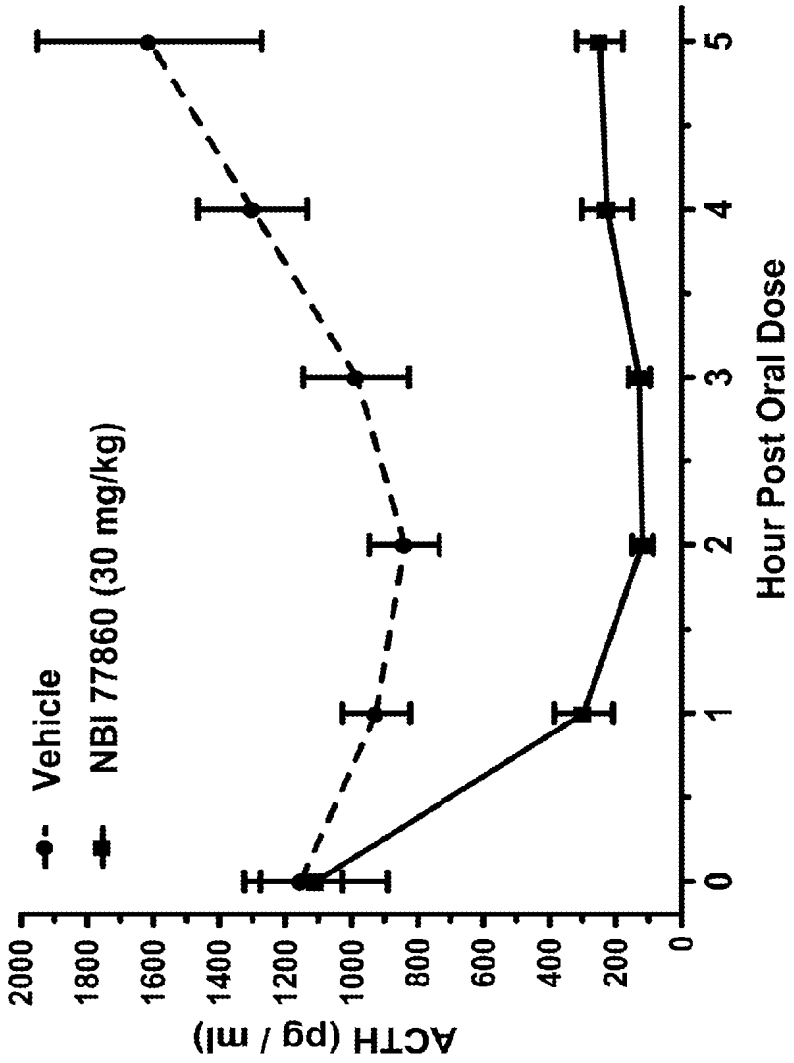

ACTH (pg / ml)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
1
2
3
4
5
Hour Post Oral Dose
Vehicle
NBI 77860 (30 mg/kg)

FIG. 2

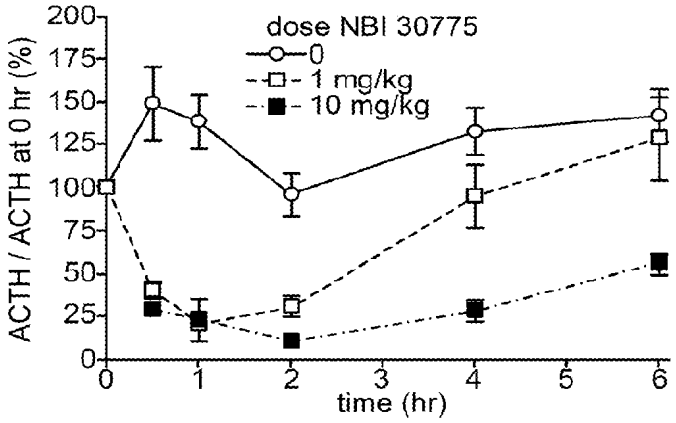


FIG. 3A

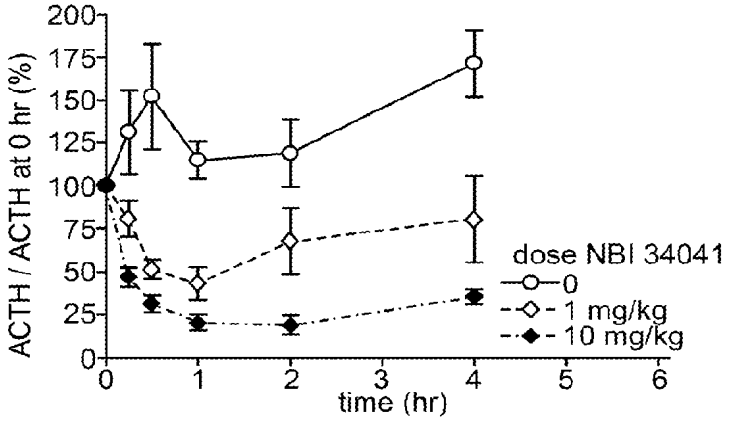


FIG. 3B

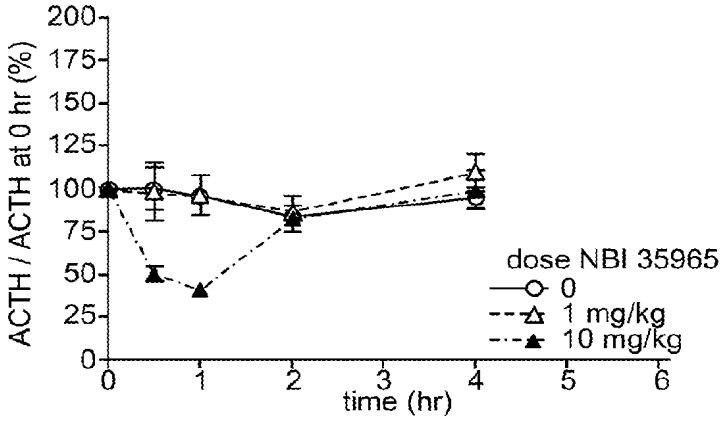


FIG. 3C

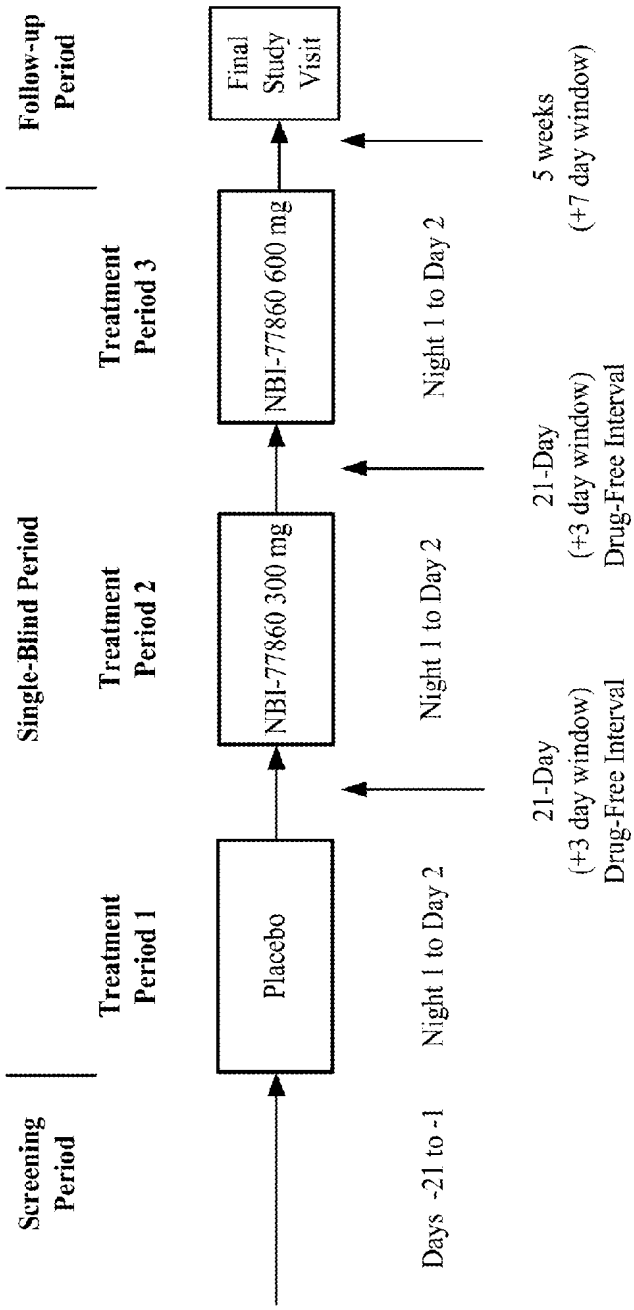
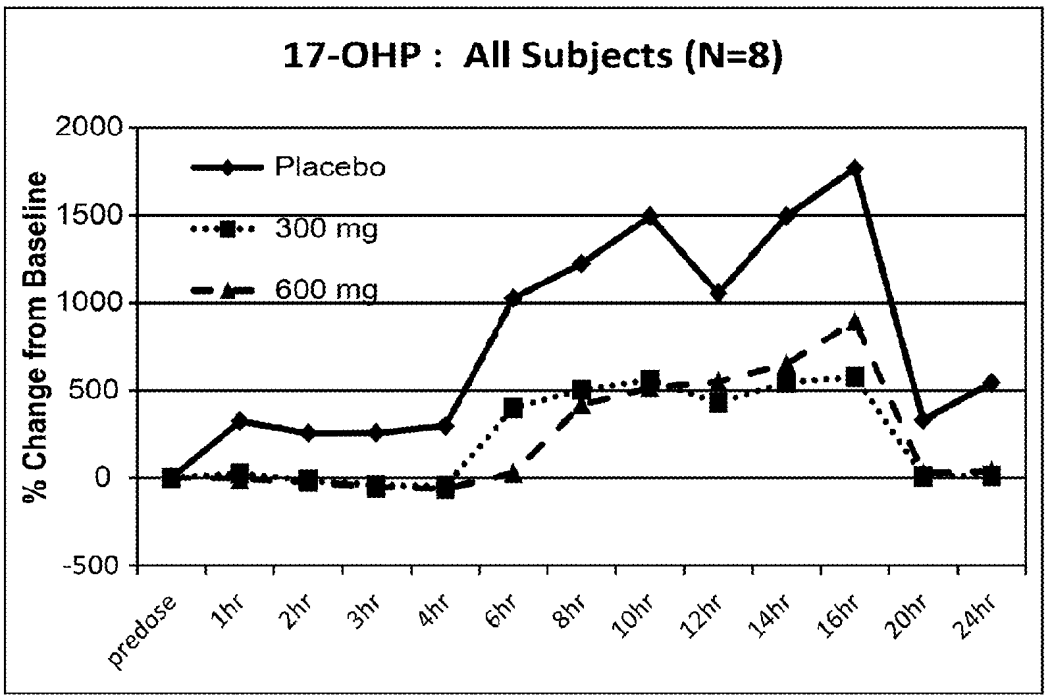

Screening Period
Single-Blind Period
Follow-up Period
Treatment Period 1
Treatment Period 2
Treatment Period 3
Placebo
NBI-77860 300 mg
NBI-77860 600 mg
Final Study Visit
Days -21 to -1
Night 1 to Day 2
Night 1 to Day 2
Night 1 to Day 2
21-Day (+3 day window) Drug-Free Interval
21-Day (+3 day window) Drug-Free Interval
5 weeks (+7 day window)

FIG. 4


17-OHP : All Subjects (N=8)
% Change from Baseline
2000
1500
1000
500
0
-500
Placebo
300 mg
600 mg
predose
1hr
2hr
3hr
4hr
6hr
8hr
10hr
12hr
14hr
16hr
20hr
24hr

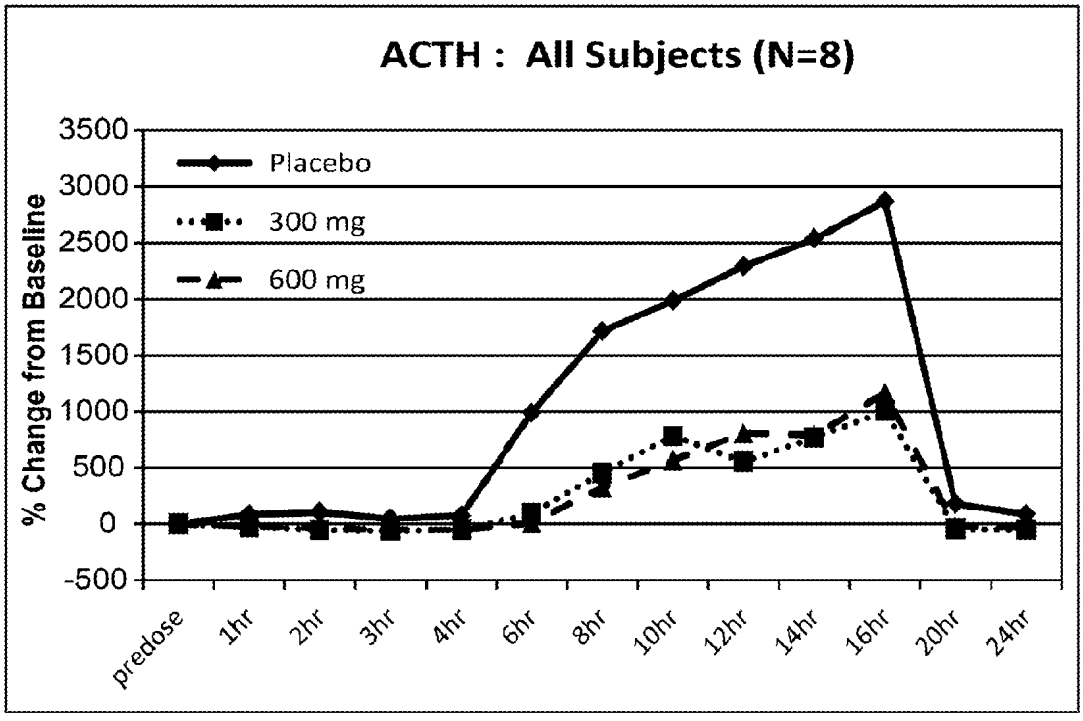

ACTH : All Subjects (N=8)
% Change from Baseline
3500
3000
2500
2000
1500
1000
500
0
-500
Placebo
300 mg
600 mg
predose
1hr
2hr
3hr
4hr
6hr
8hr
10hr
12hr
14hr
16hr
20hr
24hr

FIG. 5

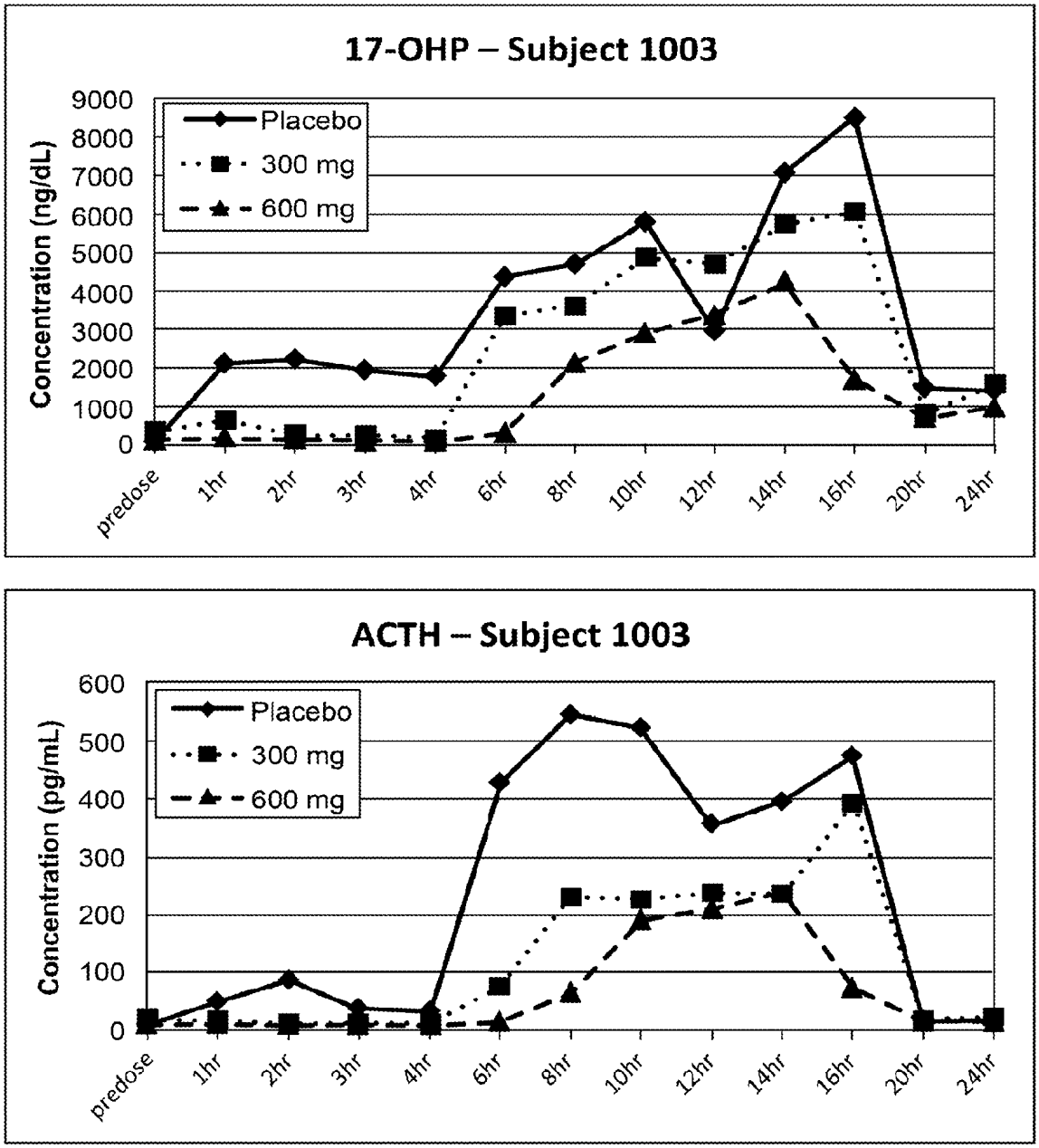

17-OHP – Subject 1003
Concentration (ng/dL)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Placebo
300 mg
600 mg
predose
1hr
2hr
3hr
4hr
6hr
8hr
10hr
12hr
14hr
16hr
20hr
24hr
ACTH – Subject 1003
Concentration (pg/mL)
600
500
400
300
200
100
0
Placebo
300 mg
600 mg
predose
1hr
2hr
3hr
4hr
6hr
8hr
10hr
12hr
14hr
16hr
20hr
24hr

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/012315

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/427 A61K31/4985 A61K31/519
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 8 030 304 B2 (CHEN ZHAOGEN [US] ET AL) 4 October 2011 (2011-10-04) cited in the application	1-3, 14-16
Y	column 2, lines 13-46 column 2, line 56 -----	1-21
X	ZOUMAKIS E ET AL: "Corticotropin-releasing hormone receptor antagonists", EUROPEAN JOURNAL OF ENDOCRINOLOGY, vol. 155, no. Suppl. 1, November 2006 (2006-11), pages S85-S91, XP002738177, ISSN: 0804-4643	1-3,5, 14-16,18
Y	page S86, left-hand column, paragraph 3; table 1 -----	1-21
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"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
8 April 2015	21/04/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Engl, Brigitte

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KAREN J LOECHNER ET AL: "Alternative Strategies for the Treatment of Classical Congenital Adrenal Hyperplasia: Pitfalls and Promises", INTERNATIONAL JOURNAL OF PEDIATRIC ENDOCRINOLOGY, BIOMED CENTRAL LTD, LONDON, UK, vol. 2010, no. 1, 8 June 2010 (2010-06-08) , page 670960, XP021109266, ISSN: 1687-9856, DOI: 10.1186/1687-9856-2010-670960 page 2, left-hand column, paragraph 1 -----	1-21
Y	NEWFIELD ET AL: "ACTH receptor blockade: A novel approach to treat congenital adrenal hyperplasia, or Cushing's disease", MEDICAL HYPOTHESES, EDEN PRESS, PENRITH, US, vol. 74, no. 4, 1 April 2010 (2010-04-01), pages 705-706, XP026975604, ISSN: 0306-9877 [retrieved on 2010-03-18] the whole document -----	1-21
Y	JOHN E TELLEW ET AL: "Discovery of NBI-77860/GSK561679, a potent corticotropin-releasing factor (CRF) receptor antagonist with improved pharmacokinetic properties", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, AMSTERDAM, NL, vol. 20, no. 24, 19 October 2010 (2010-10-19), pages 7259-7264, XP028129093, ISSN: 0960-894X, DOI: 10.1016/J.BMCL.2010.10.095 [retrieved on 2010-10-24] cited in the application page 7264, left-hand column, lines 17-21 -----	1-21
Y	CHEN C ET AL: "NBI 30775 (R121919), an orally active antagonist of the corticotropin-releasing factor (CRF) type-1 receptor for the treatment of anxiety and depression", DRUG DEVELOPMENT RESEARCH 200508 US, vol. 65, no. 4, August 2005 (2005-08), pages 216-226, XP002738174, ISSN: 0272-4391 page 219, left-hand column, line 28 - page 220, left-hand column, line 4 ----- -/--	1-21

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WEBSTER E L ET AL: "In vivo and in vitro characterization of Antalarmin, a nonpeptide corticotropin releasing hormone (CRH) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation", ENDOCRINOLOGY, THE ENDOCRINE SOCIETY, US, vol. 137, no. 12, 1 January 1996 (1996-01-01), pages 5747-5750, XP000651739, ISSN: 0013-7227, DOI: 10.1210/EN.137.12.5747 the whole document -----	1-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/012315

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 8030304 B2	04-10-2011	AR 062886 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 101516887 A	26-08-2009
		CR 10682 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 146388 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

Bibliographic data

Title:	ANTITUMOR AGENT
Pub/Pat no:	JPH0971528A
Pub/Issue Date:	1997-03-18
Inventor(s):	ARAI NARIYUKI[JP]| NISHIZAKI TAIJI[JP]| KIMOTO TETSUO[JP]| KURIMOTO MASASHI[JP]|ARAI NARIYUKI |NISHIZAKI TAIJI |KIMOTO TETSUO |KURIMOTO MASASHI
Applicant(s):	HAYASHIBARA BIOCHEM LAB[JP]
Classification:	A61K35/64AI; A61K31/19AI; A61K36/28AI; A61P35/00AI; C07C59/52AI
Application number:	JP19960177237 1996-06-19
Priority number:	JP19960177237 19960619; JP19950191015 19950705;

Abstract of JPH0971528A

PROBLEM TO BE SOLVED: To obtain a safe antitumor agent exhibiting excellent antitumor action on malignant tumor and causing little side actions. SOLUTION: This agent contains >=0.01wt.% of 3-[4-hydroxy-3,5-bis(3-methyl-2- butenyl)phenyl]-2-propenoic acid of formula (X is a cation) or its salt as an active component. It can be administered by oral or parenteral administration at a daily dose of 10m to 10mg, preferably 10g to 1mg for adult in 1-4 or 1-5 divided doses. The agent exhibits remarkable antitumor action on solid cancers such as colon cancer, rectal cancer, gastric cancer, thyroid cancer, lingual cancer, bladder cancer, chorionic carcinoma, hepatic cancer, uterine cancer, prostate cancer, laryngeal cancer, pulmonary cancer, mammary cancer, malignant melanoma, Kaposi's sarcoma, cerebral tumor, neuroblastoma, ovarian tumor, testicular tumor, osteogenic sarcoma, pancreatic cancer, renal cancer, hypernephroma and hemangioendothelioma and corpuscular malignant tumors such as leukemia and lymphoma.

(19)日本国特許庁（ＪＰ）　(12) **公開特許公報**（Ａ）　(11)特許出願公開番号

特開平9－71528

(43)公開日　平成９年(1997)３月18日

(51)Int.Cl.[6]		識別記号	庁内整理番号	ＦＩ			技術表示箇所
Ａ６１Ｋ	31/19	ＡＤＵ		Ａ６１Ｋ	31/19	ＡＤＵ	
	35/64				35/64		
	35/78				35/78	Ｔ	
// Ｃ０７Ｃ	59/52		9450－4H	Ｃ０７Ｃ	59/52		

審査請求　未請求　請求項の数４　ＦＤ　（全　７　頁）

(21)出願番号　特願平8－177237

(22)出願日　平成８年(1996)６月19日

(31)優先権主張番号　特願平7－191015

(32)優先日　平７(1995)７月５日

(33)優先権主張国　日本（ＪＰ）

(71)出願人　000155908
株式会社林原生物化学研究所
岡山県岡山市下石井１丁目２番３号

(72)発明者　新井　成之
岡山県岡山市奥田１丁目７番10－403号

(72)発明者　西▲ざき▼　泰司
岡山県玉野市八浜町八浜1330番地の22

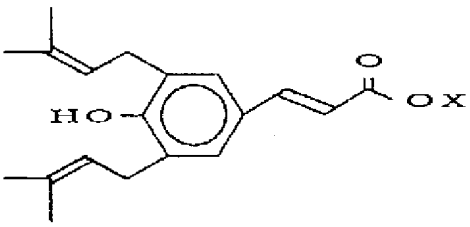
(72)発明者　木本　哲夫
岡山県岡山市山崎311番地の９

(72)発明者　栗本　雅司
岡山県岡山市学南町２丁目７番25号

(54)【発明の名称】　抗腫瘍剤

(57)【要約】
【課題】　著明な抗腫瘍作用を示しつつも、副作用を起こし難い、安全な抗腫瘍剤を提供することを課題とする。
【解決手段】　有効成分として３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸又はその生理学的に許容される塩を含んでなる抗腫瘍剤により解決する。

【特許請求の範囲】
【請求項１】　有効成分として３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸又はその生理学的に許容される塩を含んでなる抗腫瘍剤。
【請求項２】　有効成分として３　［４　ヒドロキシ　３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸又はその生理学的に許容される塩を０．０１重量％以上含んでなる請求項１に記載の抗腫瘍剤。
【請求項３】　３－［４－ヒドロキシ－３，５－ビス（３　メチル　２　ブテニル）フェニル］　２　プロペン酸又はその生理学的に許容される塩がプロポリスから採取されたものである請求項１又は２に記載の抗腫瘍剤。
【請求項４】　生理学的に許容される塩がナトリウム塩、カリウム塩、カルシウム塩、マグネシウム塩又はアンモニウム塩である請求項１、２又は３に記載の抗腫瘍剤。
【発明の詳細な説明】
【０００１】
【発明の属する技術分野】この発明は抗腫瘍剤、とりわけ、有効成分として３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸又はその生理学的に許容される塩を含んでなる抗腫瘍剤に関する。
【０００２】
【従来の技術】悪性腫瘍を治療する現在の医療現場においては、悪性腫瘍の早期発見と外科的切除に主眼が置かれている。悪性腫瘍が外科的に切除し難い部位に発生したり、原発部位以外の部位への転移や浸潤が考えられる場合には、患部に放射線を照射したり、抗腫瘍剤を定期的に投与する治療法が行なわれる。
【０００３】後者の治療法の場合、現在頻用されている抗腫瘍剤は、（ｉ）免疫賦活作用を利用するタイプ、（ｉｉ）代謝拮抗作用を利用するタイプ及び（ｉｉｉ）腫瘍細胞を直接殺傷する作用を利用するタイプに大別することができる。このうち、（ｉｉ）及び（ｉｉｉ）のタイプ、とりわけ、人工的に創成したものは抗腫瘍作用が強い半面、副作用が強く、長期連用の難しいことがある。（ｉ）のタイプは副作用は弱いものの、抗腫瘍作用が充分でないものが多く、その多くは他の薬剤と併用するか、末期の悪性腫瘍患者で、他に有効な治療方法がない場合に適用されることが多い。
【０００４】
【発明が解決しようとする課題】斯かる状況に鑑み、この発明の目的は著明な抗腫瘍作用を示しつつも、副作用を起こし難い、安全な抗腫瘍剤を提供することにある。
【０００５】
【課題を解決するための手段】斯かる課題を解決すべく、本発明者が種々の天然抽出物、とりわけ、プロポリス抽出物中の抗腫瘍性物質について鋭意研究したところ、カルボン酸の一種である３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸及びその塩に著明な抗腫瘍作用のあることが判明した。
【０００６】プロポリス抽出物は、抗菌剤、抗炎症剤、抗腫瘍剤などとして古くから民間伝承薬として用いられている。最近になってプロポリス抽出物の生物作用に近代科学のメスが入るようになり、例えば、松田忍『フーズ・アンド・フード・イングレディエンツ・ジャーナル・オブ・ジャパン』、１９９４年、第１６０号、第６４乃至７３頁には、プロポリス抽出物に含まれるケルセチン、カフェイン酸フェネチルエステル及びクロレダン系ジテルペンに分類される新規化合物がヒトの悪性腫瘍に対して抗腫瘍作用を発揮したことが記載されている。
【０００７】本発明者がプロポリス抽出物中に存在と抗腫瘍作用を確認した３　［４　ヒドロキシ　３，５　ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸は自体公知の化合物であり、例えば、本特許出願人は特開平６－２５６１７７号公報において同化合物をプロポリス抽出物から単離し得ることを開示した。しかしながら、本発明者が知るかぎりにおいて、同化合物の抗腫瘍作用を教示又は示唆する刊行物等は存在せず、それを有効成分として含んでなる抗腫瘍剤はこの発明をもって嚆矢とするものである。
【０００８】
【作用】有効成分として３　［４　ヒドロキシ　３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸又はその生理学的に許容される塩を含んでなるこの発明の抗腫瘍剤は、ヒトに発生する種々の悪性腫瘍に対して著明な抗腫瘍作用を発揮する。実験動物を用いる急性毒性試験の結果に依ると、それら化合物は毒性が極めて低い。
【０００９】以下、この発明の抗腫瘍剤について詳細に説明すると、この発明の抗腫瘍剤で用いる３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸及びその塩は下記の化１に示す構造を有している。化１において、Ｘは生理学的に許容される陽イオンを表わし、斯かる陽イオンとしては、例えば、水素イオン、ナトリウムイオン、カリウムイオン、カルシウムイオン、マグネシウムイオン、アンモニウムイオンなどが挙げられる。
【００１０】
【化１】



【００１１】この発明で用いる３－「４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸及びその塩は天然の給源から採取しても化学合成してもよく、所期の効果を発揮するかぎり、出所・由来は問わない。天然の給源としてはプロポリスや、例えば、カワラヨモギなどのキク科植物の葉茎が挙げられ、例えば、特開平６－２５６１７７号公報、シー・ツェロら『フィトケミストリー』、第２５巻、第１２号、第２，８４１乃至２，８５５頁（１９８６年）及びアイ・オクノら『ケミカル・ファーマシューティカル・ブレティン』、第３６巻、第２号、第７６９乃至７７５頁（１９８８年）には、それら給源から３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸を単離する方法が詳述されている。化学合成する場合には、例えば、特開昭６０－１６３８４１号公報に開示された方法が有利に適用できる。

【００１２】天然の給源から単離する方法について概説すれば、まず、原料のプロポリスやキク科植物の葉茎を破砕し、水若しくはメタノール、エタノール、アセトン、ジエチルエーテル、酢酸エチルなどの有機溶媒又はそれらの混液により抽出し、得られた抽出物を濃縮し、精製する。抽出物の濃縮・精製には類似の化合物を濃縮・精製するための通常の方法を適用すればよく、斯かる方法としては、例えば、塩析、透析、沪過、分液、分別沈澱、結晶化、ゲル沪過クロマトグラフィー、イオン交換クロマトグラフィー、ガスクロマトグラフィー、高速液体クロマトグラフィーなどが挙げられ、これらは必要に応じて適宜組合せて適用される。なお、３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル」－２－プロペン酸の生理学的に許容される塩を得るには、３－［４－ヒドロキシ－３，５－ビス（３　メチル　２　ブテニル）フェニル」　２　プロペン酸に、例えば、水酸化ナトリウム、水酸化カリウム、水酸化カルシウム、水酸化マグネシウム、水酸化アンモニウムなどの水酸化物を作用させればよい。斯かる塩は３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸に比べて水溶性良好であり、製剤化や高用量投与の際の取扱いに優れている。

【００１３】この発明でいう抗腫瘍剤とは、３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸又はその塩単独の形態はもとより、これらと製剤化に際して通常用いられる、例えば、担体、賦形剤、希釈剤、安定剤、さらには、必要に応じて、インターフェロン－α、インターフェロン－β、インターフェロン－γ、インターロイキン２、インターロイキン１２、ＴＮＦ－α、ＴＮＦ－β、シクロフォスファミド、アドリアマイシン、α　ジフルオロメチルオルニチン、メルファラン、５－フルオロウラシル、ドキソルビシン、クロラムブシル、ビンブラスチン、１，３－ビス（２－クロロエチル）－１－ニトロソウレア、シスプラチン、レバミゾール、Ｄ－ペニシラン、金製剤、ＢＣＧ、クレスチン、ピシバニール（ＯＫ　４３２）、丸山ワクチン及びレンチナンを始めとする他の生理活性物質の１種又は２種以上との組成物としての形態をも包含する。

【００１４】さらに、この発明の抗腫瘍剤は投薬単位形態の薬剤としての形態であってもよく、その投薬単位形態の薬剤とは、この発明の抗腫瘍剤における有効成分である３　［４　ヒドロキシ　３，５　ビス（３　メチル－２－ブテニル）フェニル］－２－プロペン酸又はその塩の１回当りの用量又はその整数倍（４倍まで）若しくはその約数（１／４０まで）に相当する量を含有し、投与に適した物理的に分離した一体の剤形にある形態を意味する。斯かる投薬単位形態の薬剤とは、例えば、注射剤、液剤、散剤、顆粒剤、錠剤、カプセル剤、舌下剤、点眼剤、点鼻剤、坐剤などが挙げられる。剤形にも依るが、この発明の抗腫瘍剤は、通常、３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］　２　プロペン酸又はその塩を０．０１重量％以上含有する。

【００１５】この発明の抗腫瘍剤の使用方法について説明すると、この発明の抗腫瘍剤は経口的に投与しても非経口的に投与しても、著明な抗腫瘍作用を発揮する。悪性腫瘍の種類と症状にも依るが、具体的には、患者の症状や投与後の経過を観察しながら、成人当たり約１μｇ乃至１０ｍｇ、望ましくは、約１０μｇ乃至１ｍｇを１乃至４回／日又は１乃至５回／週の用量で経口投与するか、皮内、皮下、筋肉内若しくは静脈内又は経皮的に非経口投与すればよい。

【００１６】この発明の抗腫瘍剤は、ヒトに発生する各種の悪性腫瘍に対して著明な抗腫瘍作用を発揮する。個々の悪性腫瘍としては、例えば、結腸癌、直腸癌、胃癌、甲状腺癌、舌癌、膀胱癌、絨毛癌、肝癌、子宮癌、前立腺癌、喉頭癌、肺癌、乳癌、悪性黒色腫、カポジ肉腫、脳腫瘍、神経芽細胞腫、卵巣腫瘍、睾丸腫瘍、骨肉腫、膵臓癌、腎癌、副腎腫、血管内皮腫などの固形腫瘍及び白血病、リンパ腫などの血球系悪性腫瘍が挙げられる。

【００１７】次の実験例では、齧歯類を用いる動物実験に基づきこの発明の抗腫瘍剤の有効性と毒性を説明するが、齧歯類における後記悪性腫瘍がヒトにおける悪性腫

瘍の信頼できるモデルであることはすでに知られている。

【００１８】

【実験例１】

〈３－［４－ヒドロキシ－３，５－ビス（３－メチル－２　ブテニル）フェニル］　２　プロペン酸及びその塩の調製〉

【００１９】

【実験例１－１】

〈３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸の調製〉特開平６　２５６１７７号公報に記載された方法にしたがって、プラジル産プロポリスから３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸（以下、「化合物１」と言う。）を調製した。

【００２０】すなわち、プラジル産プロポリス塊１５３．３重量部を破砕し、酢酸エチルを加えて抽出後、メタノールを加え、生じた沈澱部を遠心分離により除去する一方、エバポレータにより上清部を濃縮し、エタノールを加えた。新たに生じた沈澱部を遠心分離により除去し、残る上清部を上記と同様に濃縮後、メタノールを加えて抽出液６３．９重量部を得た。

【００２１】この抽出液を固形物換算で３５重量部とり、片山化学工業製『シリカゲル６０Ｇ６５０』のカラムに負荷し、濃度勾配下、ヘキサンと酢酸エチルの混液を通液した。そして、ヘキサンと酢酸エチルの濃度比が５９：４１乃至５７：４３の範囲で溶出した画分を採取し、これをファルマシア製『セファデックスＬＨ－２０』のカラムに負荷し、メタノールをＳＶ０．１６で通液し、ゲル容量の１．１倍付近で溶出した画分を採取し、濃縮したところ、結晶が析出した。この結晶をヘキサンで洗浄後、乾燥したところ、結晶性固状物が０．２８重量部得られた。

【００２２】この結晶性固状物の一部を採り、常法にしたがって元素分析法、質量分析法、紫外線分光法、赤外線分光法及び核磁気共鳴分光法により分析し、分析結果をアイ・オクノら『ケミカル・ファーマシューティカル・ブレティン』、第３６巻、第２号、第７６９乃至７７５頁（１９８８年）に報告されている３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸の分析結果と比較したところ、いずれも良好な一致を示した。斯くして、上記操作によりプロポリスから得られた結晶状の化合物は３－［４－ヒドロキシ　３，５　ビス（３　メチル　２　ブテニル）フェニル］－２－プロペン酸と同定された。

【００２３】

【実験例１－２】

〈３－［４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル］－２－プロペン酸塩の調製〉実験例１　１の方法で調製した化合物１を約１０重量％になるようにエタノールに溶解し、等モルの水酸化ナトリウム、水酸化カルシウム、水酸化マグネシウム又は水酸化カリウムを加えた後、遠心濃縮機により濃縮・乾燥して固状物を得た。実験例１－１の方法により調製した化合物１及び本例において調製したそれらナトリウム塩（以下、「化合物２」と言う。）、カルシウム塩（以下、「化合物３」と言う。）、マグネシウム塩（以下、「化合物４」と言う。）及びカリウム塩（以下、「化合物５」と言う。）を適宜濃度の水溶液とし、常法により滅菌沪過した後、次に述べる動物実験に供した。

【００２４】

【実験例２】

〈担癌マウスにおける抗腫瘍作用〉８週齢の雄ＢＡＬＢ／ｃマウスの腹腔内にＭｅｔｈ　Ａ腹水腫瘍細胞を約1×10^{6}個／匹注射移植した。翌日よりマウスを１群１０匹とし、毎週２回、化合物１乃至５のいずれかを０．０００５μｇ、０．００５μｇ、０．０５μｇ又は０．５μｇ含む生理食塩水０．２ｍｌを腹腔内に注射投与し、４０日間観察を続けた。対照群には、同じ投与スケジュールで薬剤無含有の生理食塩水を０．２ｍｌ／回腹腔内注射投与した。

【００２５】上記の条件における投与群の平均生存日数（Ｔ）と対照群の平均生存日数（Ｃ）に基づき、下記の数１により延命率（Ｔ／Ｃ　％）を算出した。結果を表１に示す。

【００２６】

【数１】

$$\text{延命率（Ｔ／Ｃ％）}=\frac{\text{投与群の平均生存日数}}{\text{対照群の平均生存日数}}\times 100$$

【００２７】

【表１】

化合物	投与量（μg/0.2ml/匹）	平均生存日数	４０日後生存匹数	延命率（T/C%）
1	0.0005	33.5±0.6	3	119.2
	0.005	35.0±0.9	5	124.6
	0.05	37.3±0.4	7	132.7*
	0.5	39.8±0.1	9	141.6*
2	0.0005	32.3±0.8	2	114.9
	0.005	36.5±0.5	6	129.9
	0.05	38.1±0.1	8	135.6*
	0.5	40.0 <	10	142.3*
3	0.0005	31.1±0.6	2	110.7
	0.005	35.9±0.3	6	127.8
	0.05	38.9±0.2	8	138.4*
	0.5	39.0±0.2	9	138.8*
4	0.0005	34.9±0.3	3	124.2
	0.005	35.7±0.4	5	127.0
	0.05	37.5±0.2	7	133.5*
	0.5	40.0 <	10	142.3*
5	0.0005	32.6±0.6	2	116.0
	0.005	36.1±0.5	6	128.5
	0.05	38.2±0.4	8	135.9*
	0.5	39.2±0.3	9	139.5*
対照	−	28.1±0.2	0	100

註：(*)危険率５％以下で、対照に対して統計学的に有意差あり。

【００２８】表１の結果から明らかなように、化合物１乃至５はいずれも０．０５μｇ／匹／回以上の用量で同系腫瘍担癌マウスに対して著明な延命効果を発揮した。

【００２９】

【実験例３】

〈腫瘍増殖抑制による抗腫瘍作用〉実験例２の結果より、ヌードマウスにおける化合物１乃至５の最少有効用量を０．０５μｇ／匹／回前後と推定し、ヒトの悪性腫瘍を移植したヌードマウスに同用量の化合物１乃至５を投与したときの抗腫瘍作用について試験した。

【００３０】すなわち、８週齢の雄ＢＡＬＢ／ｃヌードマウスの背部皮下に表２に示すヒトの悪性腫瘍細胞を５×10^7 個／匹注射移植した。その後、着生の確認された個体を１群７匹とし、週２回、化合物２を０．０５μｇ含む生理食塩水０．２ｍｌを背部皮下に注射投与し、さらに２５日間観察を続けた。対照群には、同じ投与スケジュールで薬剤無含有の生理食塩水を０．２ｍｌ／回投与した。

【００３１】移植から２７日目に、腫瘍湿重量を『人癌とヌードマウス』、１９８２年、医歯薬出版発行、第３２１乃至３２３頁に記載されている方法に準じて測定するとともに、３５日目の生存率（％）を計算した。結果を表２に示す。

【００３２】

【表２】

腫瘍細胞	平均腫瘍湿重量（ｇ）	判定時生存率（％）
HLC-1細胞（肺癌）	0.124±0.06（1.25±0.81）	100（71.4）
HGC-27細胞（胃癌）	0.35 ±0.09（1.86±0.64）	100（85.7）
Hep G2細胞（肝癌）	0.18 ±0.02（2.06±0.85）	100（42.8）
HeLa細胞（子宮癌）	0.54 ±0.06（1.36±0.61）	100（100）
RPMI-4788細胞（大腸癌）	0.21 ±0.03（2.95±1.36）	100（71.4）
WiDr細胞（結腸癌）	0.36 ±0.02（1.83±0.85）	100（85.7）
KB細胞（鼻咽腔癌）	0.54 ±0.03（2.12±0.96）	100（42.8）
HEp-2細胞（喉頭癌）	0.81 ±0.65（2.16±1.31）	100（42.8）
G-361細胞（悪性黒色腫）	0.36 ±0.02（1.96±0.98）	100（57.1）
HL-60細胞（白血病）	0.18 ±0.03（1.43±0.08）	100（100）
U-937細胞（リンパ腫）	0.25 ±0.02（0.99±0.81）	100（85.7）

註：括弧内の数字は、対照系における平均腫瘍湿重量及び判定時生存率を示す。

【００３３】表２の結果から明らかなように、化合物２は比較的低用量を投与したにも拘らず、ヒトの肺癌、胃癌、肝癌、子宮癌、大腸癌、結腸癌、鼻咽腔癌、喉頭癌、悪性黒色腫、白血病、リンパ腫などのヒトの悪性腫瘍に対して著明な抗腫瘍作用を発揮し、移植後３５日目におけるマウスの生存率も高かった。なお、データは示していないものの、化合物１及び化合物３乃至５について同様の試験をしたところ、ほぼ同様の結果が得られた。

【００３４】

【実験例４】

〈急性毒性試験〉体重１０乃至２０ｇのｄｄｙマウス１０匹を１群とし、その腹腔内に実験例１－１及び１－２の方法により得た適宜濃度の化合物１乃至５と５重量％アラビアガムを含む生理食塩水を注射投与するか、胃ゾンデにより経口投与し、その後７日間観察して死亡数を調べ、Ｖａｎ　ｄｅｒ　Ｖａｅｒｄｅｎの面積法によりＬＤ５０値を求めた。

【００３５】その結果、化合物１乃至５のＬＤ５０値はいずれの投与経路によっても２００ｍｇ／ｋｇ以上と著しく高く、これは、３－［４－ヒドロキシ－３，５－ビス（３　メチル　２　ブテニル）フェニル］　２　プロペン酸及びその塩が、通常、成人において数μｇ／回以上の用量で有効であることを考え併せれば、この発明の抗腫瘍剤が極めて安全性の高いことを裏付けていると言える。

【００３６】以下、実施例によりこの発明を具体的に説明する。

【００３７】

【実施例１】

〈錠剤〉常法により、１錠当たり実験例１－１及び１－２の方法により得た化合物１乃至５のいずれかを０．１ｍｇ、乳糖７９．９ｍｇ、コーンスターチ６２．５ｍｇ及び蔗糖脂肪酸エステル７．５ｍｇ含有する５種類の錠剤を調製した。なお、錠剤が胃溶性剤の場合には、５重量％のヒドロキシプロピルメチルセルロースでコーティングした後、糖衣を施し、腸溶性剤の場合には、１０重量％のヒドロキシプロピルメチルセルロースでコーティングした後、糖衣を施した。

【００３８】本例の錠剤は、いずれも胃癌、肺癌、肝癌、子宮癌、乳癌、大腸癌、結腸癌及び悪性黒色腫を始めとするヒトの悪性腫瘍の治療剤として有用である。

【００３９】

【実施例２】

〈カプセル剤〉常法により、１錠当たり実験例１－１及び１－２の方法により得た化合物１乃至５のいずれかを０．２ｍｇ、乳糖１４６．８ｍｇ及び蔗糖脂肪酸エステル３．０ｍｇ含有する５種類のカプセル剤を調製した。

【００４０】本例のカプセル剤は、いずれも胃癌、肺癌、肝癌、子宮癌、乳癌、大腸癌、結腸癌及び悪性黒色腫を始めとするヒトの悪性腫瘍の治療剤として有用である。

【００４１】

【実施例３】

〈注射剤〉常法により、重炭酸ナトリウム５ｇに実験例１－１及び１－２の方法により得た化合物１乃至５のいずれかを５ｍｇ混和し、滅菌したガラス容器に０．１ｇずつ充填して５種類の注射剤を調製した。

【００４２】本例の注射剤は、いずれも用時溶解型の注射剤であり、胃癌、肺癌、肝癌、子宮癌、乳癌、大腸癌、結腸癌及び悪性黒色腫を始めとするヒトの悪性腫瘍の治療剤として有用である。

【００４３】

【実施例４】

〈外用剤〉常法により、実験例１－１及び１－２の方法により得た化合物１乃至５のいずれかを有効成分として少量の流動パラフィンに研和した後、ワセリンを加えて有効成分を５ｍｇ／ｇ含有する５種類の外用剤を調製した。

【００４４】本例の外用剤は、いずれも皮膚癌、乳癌及びリンパ腫を始めとするヒトの悪性腫瘍の治療剤として有用である。

【００４５】

【実施例５】

〈散剤〉常法により、１回用量当たり実験例１－１及び１　２の方法により得た化合物１乃至５のいずれかを０．３ｍｇ及び重炭酸ナトリウム４９９．７ｍｇを含有する５種類の散剤を調製した。

【００４６】本例の散剤は、いずれも胃癌、肺癌、肝臓癌、子宮癌、乳癌、大腸癌、結腸癌及び悪性黒色腫を始めとするヒトの悪性腫瘍の治療剤として有用である。

【００４７】

【実施例６】

〈坐剤〉常法により、１回用量当たり実験例１－１及び１－２の方法により得た化合物１乃至５のいずれかを０．３ｍｇ、ポリエチレングリコール＃１０００を１，２８０ｍｇ、ポリエチレングリコール＃４０００を３１９．７ｍｇ含有する５種類の坐剤を調製した。

【００４８】本例の坐剤は、いずれも大腸癌及び結腸癌を始めとするヒトの悪性腫瘍の治療剤として有用である。

【００４９】

【発明の効果】以上説明のごとく、この発明は３－「４－ヒドロキシ－３，５－ビス（３－メチル－２－ブテニル）フェニル」－２－プロペン酸及びその生理学的に許容される陽イオンとの塩がヒトの悪性腫瘍に対して著明な抗腫瘍作用を発揮するという独自の知見に基づくものである。これら化合物は自然界の生物から単離された物質であり、人工的に創成されたものと比べて安全性が高い。したがって、有効成分としてこれら化合物を含んでなるこの発明の抗腫瘍剤は重篤な副作用を惹起することなく、ヒトの悪性腫瘍の治療に安全に用いることができる実益がある。

【００５０】斯くも顕著な作用効果を発揮するこの発明は、斯界に貢献すること誠に多大な、意義のある発明であるといえる。

(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-$Y^1(CH_2)_0R^{11}$ or -NHCH(R^{12})COOH;[wherein Y^1 is a direct single bond, -O-, -C(O)-, -N(R^{13})C(O)- or -C(O)N(R^{13})- (wherein R^{13} is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{2-3}alkyl, aminoC_{2-3}alkyl or hydroxyC_{2-3}alkyl); e is O or an integer from 1 to 4 inclusive.

COLCHINOL DERIVATIVES AS ANGIOGENESIS INHIBITORS

The present invention relates to vascular damaging agents, to the use of compounds of the invention in the manufacture of medicaments for use in the production of antiangiogenic effects in warm-blooded animals such as humans, to processes for the preparation of such compounds, to pharmaceutical compositions containing such compounds as active ingredient, to methods for the treatment of disease states associated with angiogenesis and to the use of such compounds as medicaments.

Normal angiogenesis plays an important role in a variety of processes including embryonic development, wound healing and several components of female reproductive function. Undesirable or pathological angiogenesis has been associated with disease states including diabetic retinopathy, psoriasis, cancer, rheumatoid arthritis, atheroma, Kaposi's sarcoma and haemangioma (Fan et al, 1995, Trends Pharmacol. Sci. 16: 57-66; Folkman, 1995, Nature Medicine 1: 27-31). Formation of new vasculature by angiogenesis is a key pathological feature of several diseases (J. Folkman, New England Journal of Medicine 333, 1757-1763 (1995)). For example, for a solid tumour to grow it must develop its own blood supply upon which it depends critically for the provision of oxygen and nutrients; if this blood supply is mechanically shut off the tumour undergoes necrotic death. Neovascularisation is also a clinical feature of skin lesions in psoriasis, of the invasive pannus in the joints of rheumatoid arthritis patients and of atherosclerotic plaques. Retinal neovascularisation is pathological in macular degeneration and in diabetic retinopathy.

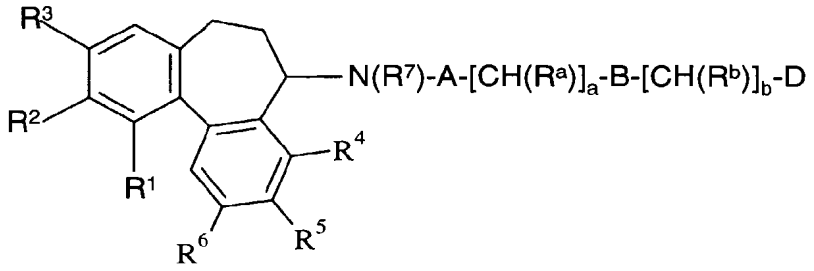
Reversal of neovascularisation by damaging the newly-formed vascular endothelium is expected to have a beneficial therapeutic effect. The present invention is based on the discovery of tricyclic compounds that surprisingly specifically damage newly formed vasculature without affecting the normal, established vascular endothelium of the host species, a property of value in the treatment of disease states associated with angiogenesis such as cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation, endometriosis, dysfunctional uterine bleeding and ocular diseases with retinal vessel proliferation.

Compounds of the present invention are colchinol derivatives. Colchinol derivatives for example *N*-acetyl-colchinol are known. Anti-tumour effects have been noted on animal

models (see for example - Jnl. Natl. Cancer Inst. 1952, 13, 379-392). However, the effect studied was that of gross damage (haemorrhage, softening and necrosis) and there is no suggestion of treatment of inappropriate angiogenesis by destruction of neovasculature.

It is believed, though this is not limiting on the invention, that the use of compounds of the invention damages newly-formed vasculature, for example the vasculature of tumours, thus effectively reversing the process of angiogenesis as compared to known anti-angiogenic agents which tend to be less effective once the vasculature has formed.

According to one aspect of the present invention there is provided a compound of the formula (I):



(I)

wherein:

R^1, R^2 and **R^3** are each independently hydroxy, phosphoryloxy ($-OPO_3H_2$), C_{1-4}alkoxy or an in vivo hydrolysable ester of hydroxy, with the proviso that at least 2 of R^1, R^2 and R^3 are C_{1-4}alkoxy;

A is - CO-, -C(O)O-, $-CON(R^8)-$, $-SO_2-$ or $-SO_2N(R^8)-$ (wherein **R^8** is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl or hydroxyC_{1-3}alkyl);

a is an integer from 1 to 4 inclusive;

R^a and **R^b** are independently selected from hydrogen, hydroxy and amino;

B is -O-, -CO-, $-N(R^9)CO-$, $-CON(R^9)-$, -C(O)O-, $-N(R^9)-$, $-N(R^9)C(O)O-$, $-N(R^9)CON(R^{10})-$, $-N(R^9)SO_2-$, $-SO_2N(R^9)-$ or a direct single bond (wherein **R^9** and **R^{10}** are independently selected from hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl and hydroxyC_{1-3}alkyl);

b is 0 or an integer from 1 to 4 inclusive, (provided that when b is 0, B is a single direct bond);

D is carboxy, sulpho, tetrazolyl, imidazolyl, phosphoryloxy, hydroxy, amino,

N-(C_{1-4}alkyl)amino, N,N-di(C_{1-3}alkyl)amino or of the formula $-Y^1-(CH_2)_cR^{11}$ or $-NHCH(R^{12})COOH$; [wherein $\mathbf{Y^1}$ is a direct single bond, -O-, -C(O)-, $-N(R^{13})-$, $-N(R^{13})C(O)-$ or $-C(O)N(R^{13})-$ (wherein $\mathbf{R^{13}}$ is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{2-3}alkyl, aminoC_{2-3}alkyl or hydroxyC_{2-3}alkyl); **c** is 0 or an integer from 1 to 4 inclusive; $\mathbf{R^{11}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, or a 5-6-membered unsaturated or partially unsaturated heteroaryl group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently form O, S and N, which heterocyclic group or heteroaryl group may bear 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-(C_{1-4}alkyl)carbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, aminoC_{1-4}alkyl, N-C_{1-4}alkylaminoC_{1-4}alkyl, di-N,N-(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein $\mathbf{R^{14}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl);

$\mathbf{R^{12}}$ is an amino acid side chain;

$\mathbf{R^5}$ is C_{1-4}alkoxy;

$\mathbf{R^4}$ and $\mathbf{R^6}$ are each independently selected from:
hydrogen, fluoro, nitro, amino, N-C_{1-4}alkylamino, N,N-di-(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy and C_{1-4}alkyl;

$\mathbf{R^7}$ is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl or hydroxyC_{1-3}alkyl;
or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

In another aspect, the invention relates to a compound of the formula (I) as hereinabove defined or to a pharmaceutically-acceptable salt thereof.

In this specification the generic term "alkyl" includes both straight-chain and branched-chain alkyl groups. However references to individual alkyl groups such as "propyl" are specific for the straight-chain version only and references to individual branched-chain

alkyl groups such as "isopropyl" are specific for the branched-chain version only. An analogous convention applies to other generic terms.

R^{12} is an amino acid side chain. This includes side chains from natural and non-natural amino acids and includes the possibility of R^{12} joining to the NH group so as to form a ring as in the amino acid proline. It includes α-amino acids β-amino acids and γ-amino acids. In addition, the amino acids may be L-isomers or D-isomers, but preferably L-isomers. Preferred amino acids include glycine, alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tryptophan, serine, threonine, cysteine, tyrosine, asparaginine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, β-alanine and ornithine. More preferred amino acids include glutamic acid, serine, threonine, arginine, glycine, alanine, β-alanine and lysine. Especially preferred amino acids include glutamic acid, serine, threonine, arginine, alanine and β-alanine. Specific values for R^{12} include hydrogen, C_{1-4}alkyl, C_{1-4}alkylthioC_{1-4}alkyl, hydroxyC_{1-4}alkyl, thioC_{1-4}alkyl, phenylC_{1-4}alkyl (optionally substituted by hydroxy), guanidinoC_{1-4}alkyl, carboxyC_{1-4}alkyl, carbamoylC_{1-4}alkyl, aminoC_{1-4}alkyl and imidazolyl C_{1-4}alkyl and R^{12} forming a pyrrolidinyl ring with the NH group. Preferred values for R^{12} include hydrogen, C_{1-4}alkyl, C_{1-4}alkylthioC_{1-4}alkyl, hydroxyC_{1-4}alkyl, thioC_{1-4}alkyl, guanidinoC_{1-4}alkyl, carboxyC_{1-4}alkyl, carbamoylC_{1-4}alkyl and aminoC_{1-4}alkyl.

It is to be understood that, insofar as certain of the compounds of Formula (I) defined above may exist in optically active or racemic forms by virtue of one or more asymmetric carbon atoms, the invention includes in its definition any such optically active or racemic form which possesses vascular damaging activity. The synthesis of optically active forms may be carried out by standard techniques of organic chemistry well known in the art, for example by synthesis from optically active starting materials or by resolution of a racemic form. Similarly, the above-mentioned activity may be evaluated using the standard laboratory techniques referred to hereinafter.

Suitable values for the generic radicals referred to above include those set out below. Within the present invention it is to be understood that a compound of the formula (I) or a salt thereof may exhibit the phenomenon of tautomerism and that the formulae drawings within this specification can represent only one of the possible tautomeric forms. It is to be understood that the invention encompasses any tautomeric form which has vascular damaging

activity and is not to be limited merely to any one tautomeric form utilised within the formulae drawings.

It is also to be understood that certain compounds of the formula (I) and salts thereof can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms which have vascular damaging activity.

The present invention relates to the compounds of formula (I) as hereinbefore defined as well as to the salts thereof. Salts for use in pharmaceutical compositions will be pharmaceutically acceptable salts, but other salts may be useful in the production of the compounds of formula (I) and their pharmaceutically acceptable salts. Pharmaceutically acceptable salts of the invention may, for example, include acid addition salts of the compounds of formula (I) as hereinbefore defined which are sufficiently basic to form such salts. Such acid addition salts include for example salts with inorganic or organic acids affording pharmaceutically acceptable anions such as with hydrogen halides (especially hydrochloric or hydrobromic acid of which hydrochloric acid is particularly preferred) or with sulphuric or phosphoric acid, or with trifluoroacetic, citric or maleic acid. Suitable salts include hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates and tartrates. In addition where the compounds of formula (I) are sufficiently acidic, pharmaceutically acceptable salts may be formed with an inorganic or organic base which affords a pharmaceutically acceptable cation. Such salts with inorganic or organic bases include for example an alkali metal salt, such as a sodium or potassium salt, an alkaline earth metal salt such as a calcium or magnesium salt, an ammonium salt or for example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

Various forms of prodrugs are known in the art. For examples of such prodrug derivatives, see:

a) Design of Prodrugs, edited by H. Bundgaard, (Elsevier, 1985) and Methods in Enzymology, Vol. 42, p. 309-396, edited by K. Widder, *et al.* (Academic Press, 1985);

b) A Textbook of Drug Design and Development, edited by Krogsgaard-Larsen and H. Bundgaard, Chapter 5 "Design and Application of Prodrugs", by H. Bundgaard p. 113-191 (1991);

c) H. Bundgaard, Advanced Drug Delivery Reviews, 8, 1-38 (1992);

d) H. Bundgaard, *et al.*, Journal of Pharmaceutical Sciences, 77, 285 (1988); and

e) N. Kakeya, *et al.*, Chem. Pharm. Bull., 32, 692 (1984).

Examples of such pro-drugs may be used to form in-vivo-cleavable esters of a compound of the Formula (I). An in-vivo-cleavable ester of a compound of the Formula (I) containing a carboxy group is, for example, a pharmaceutically-acceptable ester which is cleaved in the human or animal body to produce the parent acid. Suitable pharmaceutically-acceptable esters for carboxy include C_{1-6}alkoxymethyl esters, for example methoxymethyl; C_{1-6}alkanoyloxymethyl esters, for example pivaloyloxymethyl; phthalidyl esters; C_{3-8}cycloalkoxycarbonyloxy C_{1-6}alkyl esters, for example 1-cyclohexylcarbonyloxyethyl; 1,3-dioxolan-2-ylmethyl esters, for example 5-methyl-1,3-dioxolan-2-ylmethyl; and C_{1-6}alkoxycarbonyloxyethyl esters, for example 1-methoxycarbonyloxyethyl; and may be formed at any carboxy group in the compounds of this invention.

Suitable values for R^1, R^2, R^3 R^4, R^5, R^6, R^7, R^8, R^9, R^{10} or R^{13} or for various substituents on D or R^{14} include:

for halogeno	fluoro, chloro, bromo and iodo;
for C_{1-4}alkyl:	methyl, ethyl, propyl, isopropyl and tert-butyl;
for N-C_{1-4}alkylamino:	methylamino, ethylamino, propylamino, isopropylamino and butylamino;
for N,N-di-[C_{1-4}alkyl]amino:	dimethylamino, diethylamino, N-ethyl-N-methylamino and diisopropylamino;
for C_{2-4}alkanoyl:	acetyl and propionyl;
for C_{1-4}alkoxy:	methoxy and ethoxy;
for cyanoC_{1-4}alkyl:	cyanomethyl and 2-cyanoethyl;
for N-C_{1-4}alkylcarbamoyl:	N-methylcarbamoyl, N-ethylcarbamoyl and N-propylcarbamoyl;
for N,N-di-[(C_{1-4}))alkyl]carbamoyl:	N,N-dimethylcarbamoyl, N-ethyl-N-methylcarbamoyl and N,N-diethylcarbamoyl;
for C_{1-4}alkylsulphonylalkyl:	methylsulphonylmethyl and ethylsulphonylmethyl;
for hydroxyC_{1-4}alkyl:	hydroxymethyl, 2-hydroxyethyl, 1-hydroxyethyl and 3-hydroxypropyl as appropriate;

for C_{1-4}alkoxyC_{1-4}alkyl:	methoxymethyl, ethoxymethyl, 1-methoxyethyl, 2-methoxyethyl, 2-ethoxyethyl and 3-methoxypropyl as appropriate;
for aminoC_{1-4}alkyl	aminomethyl, 2-aminoethyl, 1-aminoethyl and 3-aminopropyl as appropriate;
for N-C_{1-4}alkylaminoC_{1-4}alkyl:	methylaminomethyl, ethylaminomethyl, 1-methylaminoethyl, 2-methylaminoethyl, 2-ethylaminoethyl and 3-methylaminopropyl as appropriate;
for N,N-di-[C_{1-4}alkyl]aminoC_{1-4}alkyl:	dimethylaminomethyl, diethylaminomethyl, 1-dimethylaminoethyl, 2-dimethylaminoethyl and 3-dimethylaminopropyl as appropriate:
for carboxyC_{1-4}alkyl:	carboxymethyl, 1-carboxyethyl, 2-carboxyethyl, 3-carboxypropyl and 4-carboxybutyl;
for carbamoylC_{1-4}alkyl:	carbamoylmethyl, 1-carbamoylethyl, 2-carbamoylethyl and 3-carbamoylpropyl;
for C_{1-4}alkoxyC_{1-4}alkyl	methoxymethyl, ethoxyethyl, methoxyethyl, and methoxypropyl.

Carbamoyl refers to —$CONH_2$.

Piperazino refers to piperazin-1-yl.

Examples of 5- or 6-membered saturated heterocyclic groups include pyrrolidinyl, imidazolidinyl, pyrazolidinyl, piperidyl, piperazinyl and morpholinyl.

Examples of 5- or 6-membered unsaturated or partially unsaturated heteroaryl groups include: imidazolyl, imidazolinyl pyridyl pyrrolyl, furanyl, triazolyl, pyrazinyl, pyrazolinyl, pyrimidinyl, pyridazinyl, isoxazolyl, oxazolyl, isothiazolyl, thiazolyl and thienyl.

Preferably at least 2 of R^1, R^2, and R^3 are methoxy.

Preferably R^1 , R^2, and R^3 are all C_{1-4}alkoxy.

Most preferably R^1, R^2, and R^3 are all methoxy.

Preferably R^8 is hydrogen, methyl, ethyl, 2-methoxyethyl, 2-aminoethyl or 2-hydroxyethyl.

More preferably R^8 is hydrogen, 2-aminoethyl or 2-hydroxyethyl and most preferably R^8 is hydrogen.

Preferably A is -CO-, -C(O)O- or -CON(R^8)-. Most preferably A is -C(O)O-.

Preferably a is 1, 2 or 3 and most preferably a is 2 or 3.

Preferably R^a, and R^b are hydrogen.

Preferably B is -N(R^9)CO-, -CON(R^9), -C(O)O-, -N(R^9)-, -N(R^9)C(O)O-, N(R^9)CON(R^{10})- or a single direct bond.

More preferably B is -CO-, -N(R^9)CO- or a single direct bond.

Yet more preferably B is -CO- or a single direct bond.

Most preferably B is -CO-. In another aspect B is a single direct bond.

Preferably R^9, and R^{10} are independently selected from hydrogen, methyl, ethyl, 2-methoxyethyl, 2-aminoethyl and 2-hydroxyethyl.

More preferably R^9 and R^{10} are independently selected from hydrogen, 2-aminoethyl and 2-hydroxyethyl.

Most preferably R^9, and R^{10} are hydrogen.

Preferably b is 0, 1 or 2, more preferably b is 0 or 1 and most preferably b is 0.

Preferably R^{11} is a 5 or 6 membered saturated heterocyclic ring containing 1 or 2 ring heteroatoms selected from N and O.

More preferably R^{11} is a 6 membered saturated heterocyclic ring containing 1 or 2 ring heteroatoms selected from N and O.

Further preferably R^{11} contains at least 1 ring nitrogen atom.

Further preferably R^{11} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents mentioned above for R^{11}.

Further preferably R^{11} is linked via a ring nitrogen atom.

Most preferably R^{11} is piperazino or morpholino, each ring being optionally substituted by 1 or 2 of the substituents mentioned hereinabove for R^{11}.

The saturated heterocyclic ring may be substituted on ring carbon or ring nitrogen atoms, providing this does not result in quaternisation.

Preferred substituents for the saturated heterocyclic ring in R^{11} include C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl.

More preferred substituents for the saturated heterocyclic ring in R^{11} include C_{1-3}alkyl, C_{2-3}alkanoyl, carbamoyl and hydroxyC_{2-3}alkyl.

Yet more preferred substituents for the saturated heterocyclic ring in R^{11} include methyl, acetyl, carbamoyl and 2-hydroxyethyl.

The most preferred substituents for the saturated heterocyclic ring include methyl, acetyl and carbamoyl.

Preferably the saturated heterocyclic ring in R^{11} is unsubstituted or substituted by 1 substituent.

When the saturated heterocyclic ring in R^{11} is morpholino, preferably it is unsubstituted. When the saturated heterocyclic ring in R^{11} is piperazino, preferably it is unsubstituted or substituted by 1 substituent on a ring nitrogen atom.

Preferably Y^1 is -CONH - or -NHCO -.

Preferably c is 0, 1 or 2.

More preferably c is 0.

Preferred values for R^{11} include morpholino, 4-methylpiperazin-1-yl and 4-acetylpiperazin-1-yl.

Preferably R^{14} is morpholino or piperazin-1-yl, each optionally substituted by 1 or 2 substituents selected from C_{1-3}alkyl, hydroxyC_{2-3}alkyl, C_{1-3}alkoxy and C_{1-3}alkoxy C_{1-3}alkyl.

More preferably R^{14} is morpholino, or piperazin-1-yl unsubstituted or substituted by methyl.

Preferably D is carboxy, phosphoryloxy, hydroxy, amino, N-C_{1-4} alkylamino, N,N-di(C_{1-4} alkyl)amino or of the formula $-Y^1(CH_2)_cR^{11}$ wherein Y^1, c and R^{11} are as hereinabove defined.

More preferably D is carboxy phosphoryloxy, hydroxy, amino or of the formula $-Y^1-(CH_2)_cR^{11}$ wherein Y^1,c and R^{11} are as hereinabove defined.

More preferably D is phosphoryloxy, amino or of the formula $-Y^1-(CH_2)_cR^{11}$ wherein Y^1, c and R^{11} are as hereinabove defined.

Yet more preferably D is phosphoyloxy, amino or of the formula $-Y^1-(CH_2)_cR^{11}$ wherein Y^1 and c are as hereinabove defined and R^{11} is morpholino, imidazolyl, or piperazinyl, which heterocyclic group may bear one or more substituents as defined above.

Yet more preferably D is phosphoyloxy, amino or of the formula $-Y^1-(CH_2)_cR^{11}$ wherein Y^1 and c are as hereinabove defined and R^{11} is morpholino, imidazolyl, 4-methylpiperazin-l-yl or 4-acetylpiperazin-1-yl.

Yet even more preferably D is phosphoyloxy, amino or of the formula $-Y^1-(CH_2)_cR^{11}$ wherein Y^1 is a direct single bond and c is 0 and R^{11} is morpholino, imidazol-1-yl, 4-methylpiperazin-1-yl or 4-acetylpiperazin-1-yl.

Preferably R^5 is methoxy.

Preferably R^4 and R^6 are independently selected from hydrogen, hydroxy, C_{1-3} alkoxy, and C_{1-3}alkyl.

More preferably at least one of R^4 and R^6 is hydrogen.

Most preferably R^4 and R^6 are both hydrogen.

Preferably R^7 is hydrogen or methyl. Most preferably R^7 is hydrogen.

A preferred class of compound is of the formula (I) wherein:

R^1 , R^2, and R^3 are all C_{1-4}alkoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, C_{1-3} alkoxy, and C_{1-3}alkyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 1, 2 or 3;

B is -CO-, -NHCO-, -CONH, -C(O)O-, -NH-, -NHC(O)O-, NHCONH- or a single direct bond;

b is 0, 1 or 2;

D is carboxy, sulpho, phosphoryloxy, hydroxy, amino, N-C_{1-4} alkylamino, N,N-di(C_{1-4} alkyl)amino or of the formula $-Y^1(CH_2)_cR^{11}$ (wherein $\mathbf{Y^1}$ is -NHC(O)- or -C(O)NH-; **c** is 1 or 2; $\mathbf{R^{11}}$ is a 5-6-membered saturated heterocyclic group (linked via nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O and N, which heterocyclic group may bear 1 or 2 substituents selected from:

C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

R^7 is hydrogen;

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

Another preferred class of compound is of the formula (I) wherein:

R^1 , R^2, and R^3 are all methoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, methoxy and methyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

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

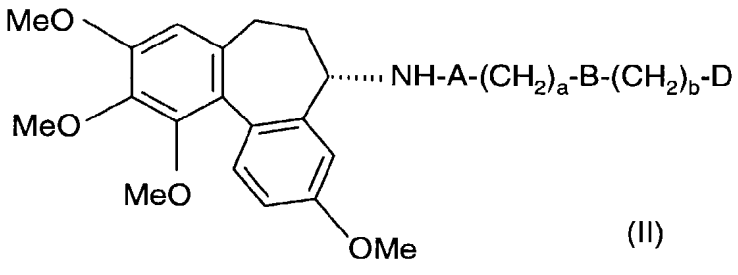
b is 0 or 1;

D is carboxy, phosphoryloxy, hydroxy, amino, N-C_{1-4} alkylamino, N,N-di(C_{1-4} alkyl)amino or of the formula -$Y^1(CH_2)_cR^{11}$ (wherein $\mathbf{Y^1}$ is -NHC(O)- or -C(O)NH-; **c** is 1 or 2; $\mathbf{R^{11}}$ is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 or 2 substituents selected from: C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

R^7 is hydrogen;

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

Another preferred class of compounds is that of the formula (II):



(II)

wherein a, b, A, B and D are as hereinabove defined;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Another preferred class of compounds is that of the formula (II) wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

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

b is 0 or 1;

D is carboxy, phosphoryloxy, hydroxy, amino, N-C_{1-4} alkylamino, N,N-di(C_{1-4} alkyl)amino or of the formula $-Y^1(CH_2)_cR^{11}$ (wherein $\mathbf{Y^1}$ is -NHC(O)- or -C(O)NH-; **c** is 1 or 2; $\mathbf{R^{11}}$ is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 or 2 substituents selected from: C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Another preferred class of compounds is that of the formula (II) wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

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

b is 0 or 1;

D is phosphoryloxy, carboxy, amino or imidazolyl;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Another preferred class of compounds is that of the formula (II) wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

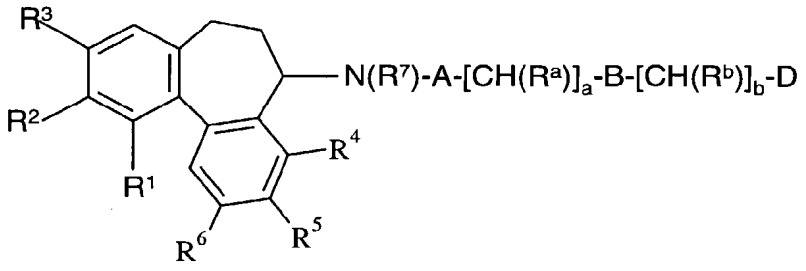
B is -CO-, -NHCO- or a single direct bond;

b is 0 or 1;

D is phosphoryloxy amino or imidazolyl;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

A further preferred class of compounds of the invention is that of a compound of formula (III)::



(III)

wherein:

R^1, R^2 and **R^3** are each independently hydroxy, phosphoryloxy ($-OPO_3H_2$), C_{1-4}alkoxy or an in vivo hydrolysable ester of hydroxy, with the proviso that at least 2 of R^1, R^2 and R^3 are C_{1-4}alkoxy;

A is - CO-, -C(O)O-, $-CON(R^8)$-, $-SO_2$- or $-SO_2N(R^8)$- (wherein **R^8** is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{2-3}alkyl, aminoC_{2-3}alkyl or hydroxyC_{2-3}alkyl);

a is an integer from 1 to 4 inclusive;

R^a and **R^b** are independently selected from hydrogen, hydroxy and amino;

B is -O-, -CO-, $-N(R^9)CO$-, $-CON(R^9)$ -, -C(O)O-, $-N(R^9)$ -, - $N(R^9)C(O)O$-, $-N(R^9)CON(R^{10})$-, $-N(R^9)SO_2$-, $-SO_2N(R^9)$- or a direct single bond (wherein **R^9** and **R^{10}** are independently selected from hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{2-3}alkyl, aminoC_{2-3}alkyl and hydroxyC_{2-3}alkyl);

b is 0 or an integer from 1 to 4 inclusive;

D is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O and N, which heterocyclic group may bear 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-(C_{1-4}alkyl)carbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, aminoC_{1-4}alkyl, N-C_{1-4}alkylaminoC_{1-4}alkyl, di-N,N-(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein **R^{14}** is a 5-6-membered saturated heterocyclic group (linked via carbon or

nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from: oxo, hydroxy, halogeno, C_{1-4}alkyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl);

R^5 is C_{1-4}alkoxy;

R^4 and **R^6** are each independently selected from: hydrogen, halogeno, nitro, amino, N-C_{1-4}alkylamino, N,N-di-(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy and C_{1-4}alkyl;

R^7 is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl or hydroxyC_{1-3}alkyl;

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

Another further preferred class of compound is of the formula (III) wherein:

R^1 , R^2, and R^3 are all C_{1-4}alkoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, C_{1-3} alkoxy, and C_{1-3}alkyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 1, 2 or 3;

B is -CO-, -NHCO-, -CONH, -C(O)O-, -NH-, -NHC(O)O-, NHCONH- or a single direct bond;

b is 0, 1 or 2;

D is piperazinyl or morpholinyl or piperidinyl, each ring being optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl;

R^7 is hydrogen;

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

Another further preferred class of compound is of the formula (III) wherein:

R^1 , R^2, and R^3 are all methoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, methoxy and methyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

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

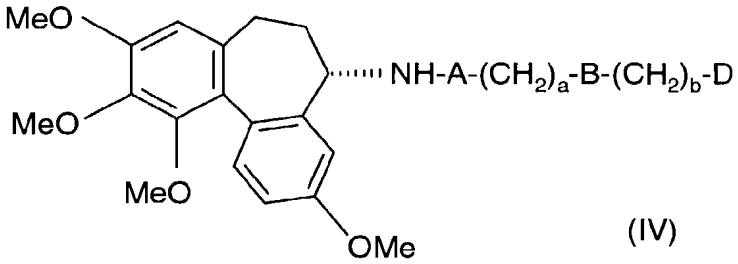
b is 0 or 1;

D is piperazino or morpholino, each ring being optionally substituted by 1 or 2 substituents selected from methyl, ethyl, acetyl, propionyl, carbamoyl and 2-hydroxyethyl;

R^7 is hydrogen;

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

In another aspect the invention relates to a compound of the formula (IV):



(IV)

wherein a, b, A, B and D are as hereinabove defined for formula (III);

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

Another preferred class of compounds is that of the formula (IV) wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

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

b is 0 or 1;

D is piperazino or morpholino, each ring being optionally substituted by 1 or 2 substituents selected from methyl, ethyl, acetyl, propionyl, carbamoyl and 2-hydroxyethyl;

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

Another preferred class of compounds is that of the formula (IV) wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

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

b is 0 or 1;

D is morpholino, 4-methylpiperazin-1-yl or 4-acetylpiperazin-1-yl;

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

Another preferred class of compounds is that of the formula (IV) wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

B is -CO- or a single direct bond;

b is 0;

D is morpholino, 4-methylpiperazin-1-yl or 4-acetylpiperazin-1-yl;

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

Particular compounds of the present invention include:

N-[(5S) -3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-2-[2-aminoacetylamino]acetamide;

4-oxo-4-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]amino]butyl disodium phosphate;

N-{N-[2-(imidazol-1-yl)ethyl]carbamoyl}-5(S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine; and

2-{N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamoyloxy}ethyl disodium phosphate;

and pharmaceutically-acceptable salts, solvates or pro-drugs thereof.

Further particular compounds of the present invention include:

2-morpholinoethyl *N*-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5*H*-dibenzo [a,c]cyclohepten-5-yl]carbamate;

3-(1-methylpiperazin-4-yl)propyl N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo [a,c]cyclohepten-5-yl] carbamate;

N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-2-[2-aminoacetylamino]acetamide;

2-(1-acetylpiperazin-4-yl)ethyl-N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl] carbamate;

N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-4-(1-methylpiperazin-4-yl)-4-oxobutan-l-amide;

4-oxo-4-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]amino]butyl disodium phosphate;

N-{N-[2-(imidazol-1-yl)ethyl]carbamoyl}-5(S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine;

3-(1-acetylpiperazin-4-yl) propyl-N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamate;

N-l-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamoyloxy]ethyl disodiumphosphate;

4-morpholino-4-oxobutyl-N-[(5S)-3,9,10, 11-tetramethoxy-6,7-dihydro-5H-dibenzo [a-c]cyclohepten-5-yl]carbamate; and

4-(1-methylpiperazin-4-yl)-4-oxobutyl-N-[(5S)-3,9,10, 11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cylcohepten-5-yl]carbamate;

and pharmaceutically-acceptable salts, , solvates or pro-drugs thereof.

Synthesis of Compounds of the Formula (I)

Compounds of Formula (I) may be prepared by a number of processes as generally described hereinbelow and more specifically in the Examples hereinafter. Processes for the preparation of novel compounds of formula (I), are provided as a further feature of the invention and are as described hereinafter. Necessary starting materials may be obtained by standard procedures of organic chemistry. The preparation of such starting materials is described within the accompanying non-limiting Examples. Alternatively necessary starting materials are obtainable by analogous procedures to those illustrated which are within the ordinary skill of an organic chemist.

Thus according to another aspect of the invention, a compound of the Formula (I) may be formed by deprotecting a compound of the formula (I) wherein at least 1 functional group is protected. For example, amino, hydroxy, carboxy or phosphoryloxy groups may be protected during the reaction sequence used to prepare a compound of the formula (I).

Protecting groups may in general be chosen from any of the groups described in the literature or known to the skilled chemist as appropriate for the protection of the group in question, and may be introduced by conventional methods.

Protecting groups may be removed by any convenient method as described in the literature or known to the skilled chemist as appropriate for the removal of the protecting

group in question, such methods being chosen so as to effect removal of the protecting group with minimum disturbance of groups elsewhere in the molecule.

A suitable protecting group for a hydroxy group is, for example, an arylmethyl group (especially benzyl), a triC_{1-4}alkysilyl group (especially trimethysilyl or tert-butyldimethylsilyl), an aryldi-C_{1-4}alkylsilyl group (especially dimethylphenylsilyl), a diarylC_{1-4}alkylsilyl group (especially tert-butyldiphenylsilyl), a C_{1-4}alkyl group (especially methyl), a C_{2-4}alkenyl group (especially allyl), a C_{1-4}alkoxymethyl group (especially methoxymethyl) or a tetrahydropyranyl group (especially tetrahydroyran-2-yl). The deprotection conditions for the above protecting groups will necessary vary with the choice of protecting group. Thus, for an example, arylmethyl group such as a benzyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-charcoal. Alternatively a trialkylsilyl or an aryldialkylsilyl group such as tert-butydimethylsilyl or a dimethylphenylsilyl group may be removed, for example, by treatment with a suitable acid such as hydrochloric, sulphuric, phosphoric or trifluoroacetic acid, or with an alkali metal or ammonium fluoride such as sodium fluoride or, preferably tetrabutylammonium fluroide. Alternatively an alkyl group may be removed, for example, by treatment with an alkali metal C_{1-4}alkylsulphide such as sodium thioethoxide or, for example, by treatment with an alkali metal diarylphosphide such as lithium diphenylphosphide or, for example, by treatment with a boron or aluminium trihalide such as boron tribromide. Alternatively a C_{1-4}alkoxymethyl group or tetrahydropyranyl group may be removed, for example, by treatment with a suitable acid such as hydrochloric or trifluoroacetic acid.

Alternatively a suitable protecting group for a hydroxy group is, for example, an acyl group, for example a C_{2-4}alkanoyl group (especially acetyl) or an aroyl group (especially benzoyl). The deprotection conditions for the above protecting groups will necessarily vary with the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl or an aroyl group may be removed, for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide.

A suitable protecting group for an amino, imino or alkylamino group is, for example, an acyl group, for example a C_{2-4}alkanoyl group (especially acetyl), a C_{1-4}alkoxycarbonyl) group (especially methoxycarbonyl), ethoxycarbonyl or tert-butoxycarbonyl), an arylmethoxycarbonyl group (especially benzyloxycarbonyl) or an aroyl group (especially benzoyl). The deprotection conditions for the above protecting groups necessarily vary with

the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl, alkoxycarbonyl or aroyl group may be removed for example, by hydrolysis with a suitable base such as alkali metal hydroxide, for example lithium or sodium hydroxide. Alternatively an acyl group such as a tert-butoxycarbonyl group may be removed, for example, by treatment with a suitable acid such as hydrochloric, sulphuric or phosphoric acid or trifluoroacetic acid, and an arylmethoxycarbonyl group such as a benzyloxycarbonyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-charcoal.

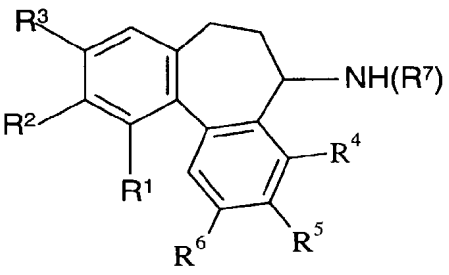
A suitable protecting group for a carboxy group is, for example, an esterifying group, for example a C_{1-4}alkyl group (especially methyl or ethyl) which may be removed, for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide; or for example, a tert-butyl group which may be removed, for example, by treatment with a suitable acid such as hydrochloric, sulphuric or phosphoric acid or trifluoroacetic acid.

The reader is referred to Advanced Organic Chemistry, 4th Edition, by J. March, published by John Wiley & Sons 1992, for general guidance on reaction conditions and reagents and to Protective Groups in Organic Synthesis, 2nd Edition, by T. Green et al., also published by John Wiley & Son, for general guidance on protecting groups.

In the following process description the symbols R^1 - R^7, A, B, D, R^a R^b , a and b are to be understood to represent those groups described above in relation to formulae (I) and (II) unless otherwise stated.

A compound of the formula (I), or a compound of the formula (I) wherein at least 1 functional group is protected, may be prepared using one of the following processes:

a) reacting a compound of the formula (X)



(X)

with a compound of the formula (XI):

L^1 - A - $[CH(R^a)]_a$-B-$[CH\ R^b)]_b$-D (XI)

wherein L^1 is a leaving group; or

b) converting one compound of the formula (I) into another compound of the formula (I); or

c) when a phosphoryloxy group is desired, reacting the corresponding hydroxy compound with a phosphoramidite;

wherein any functional groups are optionally protected.

and thereafter if necessary:

i) converting a compound of formula (I) into another compound of formula (I);

ii) removing any protecting groups;

iii) forming a pharmaceutically acceptable salt, solvate or pro-drug thereof.

The reaction between a compound of the formula (X) and a compound of the formula L^1 - A - $[CH(R^a)]_a$-B-$[CH\ R^b)]_b$-D is conveniently performed under standard acylation or sulphonylation conditions. L^1 is usually halogeno, for example chloro or bromo, hydroxy, mesyloxy, tosyloxy or an 'activated' hydroxy group. The precise conditions depending largely upon the nature of A.

For example, when -A- is -CO-, L^1 may be hydroxy and the reaction carried out in the presence of coupling agent such as dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide. Optionally, a base may be used, for example an organic base such as triethylamine. Suitable solvents are usually aprotic solvents, for example dimethylformamide, or chlorinated solvents, for example trichloromethane or dichloromethane. The temperature is usually in the range of about -30°C to about 60°C, conveniently at or near ambient temperature.

When -A- is -C(O)O-, L^1 is usually an "activated" hydroxy group. That is a group which acts as a leaving group in the same way as hydroxy, but is more labile. It can be formed in situ. An example of an activated hydroxy group is 4-nitrophenoxy, which can be formed by reacting a hydroxy group (HO-$[CH(R^a)]_a$-B-$[CH(R^b)]_b$-D) with 4-nitrophenylchloroformate. This reaction is usually carried out in an organic solvent such as dichloromethane, acetonitrile or tetrahydrofuran, in a temperature range of about -20°C to the reflux temperature of the solvent. In addition, organic base such as triethylamine or N-methylmorpholine is normally present. Alternatively, a compound of the formula (X) can be reacted with 4-nitrophenylchloroformate and the resulting intermediate reacted with

$HO-[CH(R^a)]_a-B-[CH(R^b)]_b-D$ under similar conditions to those described above for the reaction of a compound of the formula (X) with a compound of the formula $L^2-[CH(R^a)]_a-B-[CH(R^b)]_b-D$ wherein L^2 is 4-nitrophenoxy.

When -A- is $-CON(R^8)-$, L^1 is preferably halogeno, particularly chloro. Alternatively when -A- is -CONH-, a compound of the formula (X) can be reacted with an isocyanate of the formula $C\equiv N-[CH(R^a)]_a-B-[CH(R^b)]_b-D$. These reactions are conveniently carried out in the presence of a base, particularly an organic base, such as triethylamine, pyridine or N-methylmorpholine, in a solvent such as an ether solvent, for example tetrahydrofuran, or in a chlorinated solvent, for example dichloromethane, at a temperature in the range from about -20°C to the reflux temperature of the solvent. Alternatively, a compound of the formula (X) can be reacted with 4-nitrophenylchloroformate and the resulting intermediate reacted with $R^{17}-NH_2$ under similar conditions to those described above for the reaction of a compound of the formula (X) with a compound of the formula $L^2-[CH(R^a)]_a-B-[CH(R^b)]_b-D$ wherein L^2 is 4-nitrophenoxy.

When -A- is of the formula $-SO_2-$ or $-SO_2N(R^8)-$, L^1 is preferably halogeno, for example chloro. The reaction is conveniently carried out in the presence of a base such as dimethylaniline, in a chlorinated solvent such as trichloromethane and at a temperature in the range of -20°C to about 60 °C, more preferably in pyridine, at a temperature in the range from -20°C to about 60 °C.

A compound of formula (I) may be prepared from another compound of formula (I) by chemical modification. Examples of chemical modifications include standard alkylation, arylation, heteroarylation, acylation, sulphonylation, phosphorylation, aromatic halogenation and coupling reactions. These reactions may be used to add new substituents or to modify existing substituents. Alternatively , existing substituents in compounds of formula (I) may be modified by, for example, oxidation, reduction, elimination, hydrolysis or other cleavage reactions to yield other compounds of formula (I).

Thus for example a compound of formula (I) containing an amino group may be acylated on the amino group by treatment with, for example, an acyl halide or anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for example, a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In another general example of an interconversion process, an amino group in a compound of formula (I) may be sulphonylated by treatment with, for example, an alkyl or aryl sulphonyl chloride or an alkyl or aryl sulphonic anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for example a solvent such as a hydrocarbon solvent e.g. dichloromethane, at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In a further general example, a compound of formula (I) containing a hydroxy group can be converted into the corresponding dihydrogenphosphate ester by treatment with for example di-tert-butyl diisopropylphosphoramidite or di-tert-butyl diethylphosphoramidite in the presence of a suitable catalyst, for example tetrazole. A solvent such as an ether solvent, for example tetrahydrofuran can be used. The reaction is usually carried out at a temperature in the range -40°C to 40°C, conveniently at or near ambient temperature, followed by treatment with an oxidising agent for example 3-chloroperoxy benzoic acid at a temperature in the range -78°C to 40°C preferably -40°C to 10°C. The resulting intermediate phosphate triester is treated with an acid, for example trifluoroacetic acid, in a solvent such as a chlorinated solvent e.g. dichloromethane at a temperature in the range -30°C to 40°C, conveniently at or near 0°C, to give the compound of formula (I) containing a dihydrogenphosphate ester.

In a further general example a compound of formula (I) containing an amide can be hydrolysed by treatment with for example an acid such as hydrochloric acid in a solvent such as an alcohol, for example methanol at an elevated temperature conveniently at the reflux temperature.

In another general example an alkoxy group may be converted to the corresponding alcohol (OH) by reaction with boron tribromide in a solvent such as a chlorinated solvent e.g. dichloromethane at a low temperature e.g. around -78°C.

In a further general example a compound of formula (I) may be alkylated by reaction with a suitable alkylating agent such as an alkyl halide, an alkyl toluenesulphonate, an alkyl methanesulphonate or an alkyl triflate. The alkylation reaction can be carried out in the presence of a base, for example an inorganic base such as a carbonate e.g. caesium or potassium carbonate, a hydride such as sodium hydride or an alkoxide such as potassium t-butoxide, in a suitable solvent such as an aprotic solvent e.g. dimethylformamide or an ether solvent such as tetrahydrofuran, at a temperature of around -10°C to 80°C.

In a further example, an unsubstituted ring nitrogen in a saturated heterocyclic ring may be acylated using similar reaction conditions to those described above for the acylation of an amino group.

Synthesis of Intermediates

A compound of the formula (X) may be known or prepared according to processes described in International Patent Application No. PCT/GB98/01977.

A compound of the formula (XI) may be known or prepared by methods known in the art. For example, when A is of the formula -C(O)O- and L^1 is 4-nitrophenyloxy, the compound of the formula (XI) may be formed by reacting a compound of the formula:

$$HO\text{-}[CH(R^7)]_a\text{ - B -}[CH(R^7)]_b\text{-D}$$

with 4-nitrophenyl chloroformate, in the presence of a base, preferably an organic base such as triethylamine, in an inert organic solvent such as dichloromethane. The reaction is usually carried out in a temperature range of -30°C to 60 °C, most commonly at around ambient temperature.

Acid addition salts of the compounds of formula (I) are prepared in a conventional manner by treating a solution or suspension of the free base of a compound of formula (I) with about one equivalent of a pharmaceutically acceptable acid. Salts of compounds of formula (I) derived from inorganic or organic bases are prepared in a conventional manner by treating a solution or suspension of the free acid of a compound of formula (I) with about one equivalent of a pharmaceutically acceptable organic or inorganic base. Alternatively both acid addition salts and salts derived from bases may be prepared by treatment of the parent compound with the appropriate ion-exchange resin in a standard fashion. Conventional concentration and recrystallistion techniques are employed in isolating the salts.

Compounds according to the invention are able to destroy vasculature that has been newly formed such as tumour vasculature while leaving unaffected normal, mature vasculature. The identification of compounds which selectively, and preferably potently, damage newly-formed vasculature is desirable and is the subject of the present invention. The ability of the compounds to act in this way may be assessed, for example, using one or more of the procedures set out below:

(a) Activity against tumour vasculature measured by radioactive tracer

This assay demonstrates the ability of compounds to damage selectively tumour vasculature.

Subcutaneous CaNT tumours were initiated by injecting 0.05ml of a crude tumour cell suspension, approximately 10^6 cells, under the skin overlying the rear dorsum of 12-16 week-old mice. The animals were selected for treatment after approximately 3-4 weeks, when their tumours reached a geometric mean diameter of 5.5-6.5 mm. Compounds were dissolved in sterile saline and injected intraperitoneally in a volume of 0.1 ml per 10g body weight. Tumour perfusion was measured 6 hours after intraperitoneal administration in tumour, kidney, liver, skin, muscle, gut and brain by the ^{86}RbCl extraction technique (Sapirstein, Amer. Jnl. Physiol., 1958, 193, 161-168). Tissue radioactivity measured 1 minute after an intravenous injection of ^{86}RbCl was used to calculate relative blood flow as a proportion of cardiac output (Hill and Denekamp, Brit. Jnl. Radiol., 1982, 55, 905-913). Five animals were used in control and treated groups. Results were expressed as a percentage of the blood flow in the corresponding tissues in vehicle treated animals.

(b) Activity against tumour vasculature measured by fluorescent dye

This assay demonstrates the ability of compounds to damage tumour vasculature.

Tumour functional vascular volume in CaNT tumour-bearing mice was measured using the fluorescent dye Hoechst 33342 according to the method of Smith et al (Brit. Jnl. Cancer 1988, 57, 247-253). Five animals were used in control and treated groups. The fluorescent dye was dissolved in saline at 6.25mg/ml and injected intravenously at 10mg/kg 24 hours after intraperitoneal drug treatment. One minute later, animals were killed and tumours excised and frozen; 10μm sections were cut at 3 different levels and observed under UV illumination using an Olympus microscope equipped with epifluorescence. Blood vessels were identified by their fluorescent outlines and vascular volume was quantified using a point scoring system based on that described by Chalkley, (Jnl. Natl. Cancer Inst., 1943, 4, 47-53). All estimates were based on counting a minimum of 100 fields from sections cut at the 3 different levels.

The ability of the compounds to bind to preparations of mammalian tubulin can be evaluated by a number of methods available in the literature, for example by following temperature initiated tubulin polymerisation by turbidity in the absence and presence of the compound (for example O.Boye *et al* Med. Chem. Res., 1991, 1, 142-150).

The activity of *N*-[3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide, (V. Fernholz Justus Liebigs Ann., 1950, 568, 63-72), against tumour vasculature was measured by the fluorescent dye method described above. This compound decreased perfused vascular volume by 88% relative to control when dosed at 50mg/kg intraperitoneally. The IC_{50} of this compound in a tubulin polymerisation assay was 58 micromolar (O.Boye *et al* Med. Chem. Res., 1991, 1, 142-150).

(c) HUVEC detachment assay

This assay examined the effects of compounds on the adherence of HUVECs to tissue culture plasticware.

HUVECs were plated in 0.2% gelatin-coated 12 well tissue culture plates at a concentration of $3x10^4$ cells per well in 1ml TCS medium. After 24 hours, when the cells were at ~30% confluency, the cells were dosed with compound for 40 minutes at 37°C, 5% CO_2. After this incubation the medium containing drug was pipetted off, and the cells were then gently washed in 2mls of HBSS (Hanks' Balanced Salt Solution purchased from Life Technologies Ltd, Paisley UK; Catalogue # 24020-083) to remove any detached cells. The washing solution was then removed, and the adherent cells remaining were trypsinised using 300µl of 1x Trypsin-EDTA solution (Life Technologies Ltd, Paisley, UK; Catalogue # 43500-019) at ambient temperature for 2 minutes. The trypsinised cells were then made up to 1ml with TCS Biologicals medium, then centrifuged at 2000rpm for 2 minutes. The cell pellet was then resuspended in a volume of 50µl of TCS Biologicals medium. Total cell counts were obtained by counting the cells on a haemocytometer. The amount of cell detachment was calculated by comparing the number of cells remaining attached following treatment with the number in undosed control wells.

(d) Hras5 necrosis model

NIH 3T3 fibroblasts transfected with Harvey ras, clone 5, (Hras5 cells) were kept in continual passage in Dulbecco's modifed Eagles medium (DMEM) containing 10% foetal bovine serum (FBS) and 1% glutamine, at 37°C in a humidified incubator gassed with 7.5% carbon dioxide and 92.5% oxygen. Cells were implanted subcutaneously into the left flank of male nude mice (8-10weeks of age) at an inoculum of 2×10^5 cells/mouse. Tumours were measured using calipers and randomised into groups of 2-4 mice between days 9-14 after implant. Mice were dosed with compounds, either intravenously or intraperitoneally, once on

day of randomisation and culled 24 hours after dosing. Compounds were dissolved in 20% hydroxypropyl beta cyclodextrin in physiological saline at pH 7 and dosed in a volume of 0.1ml per 10g body weight. Tumours were excised, weighed and placed in buffered formalin. Area of necrosis in individual tumours was assessed from a haematoxylin/eosin stained-slide by a pathologist and scored from 0, meaning no significant change, to 10, meaning 91-100% necrosis. The activity of examples 5 and 7 (described hereinafter) against tumour vasculature was measured by the fluorescent dye method described hereinabove. Example 1 scored 6.0 at 100m/kg and example 4 scored 3.2 at 50m/kg.

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises a compound of the formula (I) as defined hereinbefore or a pharmaceutically acceptable salt, solvate or pro-drug thereof, in association with a pharmaceutically acceptable excipient or carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for nasal administration or administration by inhalation, for example as a powder or solution, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) for example as a sterile solution, suspension or emulsion, for topical administration for example as an ointment or cream or for rectal administration for example as a suppository. In general the above compositions may be prepared in a conventional manner using conventional excipients.

The compositions of the present invention are advantageously presented in unit dosage form. The compound will normally be administered to a warm-blooded animal at a unit dose within the range 5-5000mg per square metre body area of the animal, i.e. approximately 0.1-100mg/kg. A unit dose in the range, for example, 1-100mg/kg, preferably 1-50mg/kg is envisaged and this normally provides a therapeutically-effective dose. A unit dose form such as a tablet or capsule will usually contain, for example 1-250mg of active ingredient.

As stated above the size of the dose required for the therapeutic or prophylactic treatment of a particular disease state will necessarily be varied depending on the host treated, the route of administration and the severity of the illness being treated. Preferably a daily dose in the range of 1-50mg/kg is employed. However the daily dose will necessarily be varied depending upon the host treated, the particular route of administration, and the severity

of the illness being treated. Accordingly the optimum dosage may be determined by the practitioner who is treating any particular patient.

According to a further aspect of the present invention there is provided a compound of the formula (I) or a pharmaceutically acceptable salt, solvate or pro-drug thereof as defined hereinbefore for use in a method of treatment of the human or animal body by therapy.

A further feature of the present invention is a compound of formula (I), or a pharmaceutically acceptable salt, solvate or pro-drug thereof, for use as a medicament, conveniently a compound of formula (I), or a pharmaceutically acceptable salt, solvate or pro-drug thereof, for use as a medicament for producing a vascular damaging effect in a warm-blooded animal such as a human being.

Thus according to a further aspect of the invention there is provided the use of a compound of the formula (I), or a pharmaceutically acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm-blooded animal such as a human being.

According to a further feature of the invention there is provided a method for producing a vascular damaging effect in a warm-blooded animal, such as a human being, in need of such treatment which comprises administering to said animal an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt, solvate or pro-drug thereof as defined hereinbefore.

According to a further aspect of the present invention there is provided a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition, when dosed in divided doses (also known as split doses) produces a greater anti-tumour effect than when a single dose is given.

Anti-tumour effects of a method of treatment of the present invention include but are not limited to, inhibition of tumour growth, tumour growth delay, regression of tumour, shrinkage of tumour, increased time to re-growth of tumour on cessation of treatment, slowing of disease progression. It is expected that when a method of treatment of the present invention is administered to a warm-blooded animal such as a human, in need of treatment for cancer involving a solid tumour, said method of treatment will produce an effect, as measured by, for example, one or more of: the extent of the anti-tumour effect, the response rate, the time to disease progression and the survival rate.

According to a further aspect of the present invention there is provided a method for the production of a vascular damaging effect in a warm-blooded animal such as a human, which comprises administering to said animal in divided doses an effective amount of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition.

According to a further aspect of the present invention there is provided a method for the treatment of a cancer involving a solid tumour in a warm-blooded animal such as a human, which comprises administering to said animal in divided doses an effective amount of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition.

According to a further aspect of the present invention there is provided a medicament comprising two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition, which together add up to a total daily dose, for administration in divided doses for use in a method of treatment of a human or animal body by therapy.

According to a further aspect of the present invention there is provided a kit comprising two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition, which together add up to a total daily dose, for administration in divided doses.

According to a further aspect of the present invention there is provided a kit comprising:

a) two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, which together add up to a total daily dose, in unit dosage forms for administration in divided doses; and

b) container means for containing said dosage forms.

According to a further aspect of the present invention there is provided a kit comprising:

a) two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, which together add up to a total daily dose, together with a pharmaceutically acceptable excipient or carrier, in unit dosage forms; and

b) container means for containing said dosage forms.

According to a further aspect of the present invention there is provided the use of compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for administration in divided doses for use in the production of a vascular damaging effect in a warm-blooded animal such as a human.

According to a further aspect of the present invention there is provided the use of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for administration in divided doses for use in the production of an anti-cancer effect in a warm-blooded animal such as a human.

According to a further aspect of the present invention there is provided the use of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for administration in divided doses for use in the production of an anti-tumour effect in a warm-blooded animal such as a human.

Divided doses, also called split doses, means that the total dose to be administered to a warm-blooded animal, such as a human, in any one day period (for example one 24 hour period from midnight to midnight) is divided up into two or more fractions of the total dose and these fractions are administered with a time period between each fraction of about greater than 0 hours to about 10 hours, preferably about 1 hour to about 6 hours, more preferably about 2 hours to about 4 hours. The fractions of total dose may be about equal or unequal.

Preferably the total dose is divided into two parts which may be about equal or unequal.

The time intervals between doses may be for example selected from:
about 1 hour, about 1.5 hours, about 2 hours, about 2.5 hours, about 3 hours, about 3.5 hours, about 4 hours, about 4.5 hours, about 5 hours, about 5.5 hours and about 6 hours.

The time intervals between doses may be any number (including non-integers) of minutes between greater than 0 minutes and 600 minutes, preferably between 45 and 375 minutes inclusive. If more than two doses are administered the time intervals between each dose may be about equal or unequal.

Preferably two doses are given with a time interval in between them of greater than or equal to 1 hour and less than 6 hours.

More preferably two doses are given with a time interval in between them of greater than or equal to two hours and less than 5 hours.

Yet more preferably two doses are given with a time interval in between them of greater than or equal to two hours and less than or equal to 4 hours.

Particularly the total dose is divided into two parts which may be about equal or unequal with a time interval between doses of greater than or equal to about two hours and less than or equal to about 4 hours.

More particularly the total dose is divided into two parts which may be about equal with a time interval between doses of greater than or equal to about two hours and less than or equal to about 4 hours.

For the avoidance of doubt the term 'about' in the description of time periods means the time given plus or minus 15 minutes, thus for example about 1 hour means 45 to 75 minutes, about 1.5 hours means 75 to 105 minutes. Elsewhere the term 'about' has its usual dictionary meaning.

The antiangiogenic treatment defined hereinbefore may be applied as a sole therapy or may involve, in addition to a compound of the invention, one or more other substances and/or treatments. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate administration of the individual components of the treatment. In the field of medical oncology it is normal practice to use a combination of different forms of treatment to treat each patient with cancer. In medical oncology the other component(s) of such conjoint treatment in addition to the antiangiogenic treatment defined hereinbefore may be: surgery, radiotherapy or chemotherapy. Such chemotherapy may include the following categories of therapeutic agent:

(i) other antiangiogenic agents that work by different mechanisms from those defined hereinbefore (for example linomide, inhibitors of integrin $\alpha v\beta 3$ function, angiostatin, endostatin, razoxin, thalidomide) and including vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitors (RTKIs) (for example those described in International Patent Applications Publication Nos. WO 97/22596, WO 97/30035, WO 97/32856 and WO 98/13354 the entire disclosure of which documents is incorporated herein by reference);

(ii) cytostatic agents such as antioestrogens (for example tamoxifen,toremifene, raloxifene, droloxifene, iodoxyfene), progestogens (for example megestrol acetate), aromatase inhibitors (for example anastrozole, letrazole, vorazole, exemestane), antiprogestogens, antiandrogens (for example flutamide, nilutamide, bicalutamide, cyproterone acetate), LHRH agonists and antagonists (for example goserelin acetate, luprolide), inhibitors of testosterone 5α-dihydroreductase (for example finasteride), anti-invasion agents (for example metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator

receptor function) and inhibitors of growth factor function, (such growth factors include for example epidermal growth factor (EGF), platelet derived growth factor and hepatocyte growth factor such inhibitors include growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors and serine/threonine kinase inhibitors);

(iii) biological response modifiers (for example interferon);

(iv) antibodies (for example edrecolomab); and

(v) antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as antimetabolites (for example antifolates like methotrexate, fluoropyrimidines like 5-fluorouracil, purine and adenosine analogues, cytosine arabinoside); antitumour antibiotics (for example anthracyclines like doxorubicin, daunomycin, epirubicin and idarubicin, mitomycin-C, dactinomycin, mithramycin); platinum derivatives (for example cisplatin, carboplatin); alkylating agents (for example nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide, nitrosoureas, thiotepa); antimitotic agents (for example vinca alkaloids like vincristine and taxoids like taxol, taxotere); enzymes (for example asparaginase); thymidylate synthase inhibitors (for example raltitrexed); topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan, irinotecan).

As stated above the compounds defined in the present invention are of interest for their vascular damaging effects. Such compounds of the invention are expected to be useful in the prophylaxis and treatment of a wide range of disease states where inappropriate angiogenesis occurs including cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation, endometriosis, dysfunctional uterine bleeding and ocular diseases with retinal vessel proliferation. In particular such compounds of the invention are expected to slow advantageously the growth of primary and recurrent solid tumours of, for example, the colon, breast, prostate, lungs and skin.

In addition to their use in therapeutic medicine, the compounds of formula (I) and their pharmaceutically acceptable salts, solvates or pro-drugs are also useful as pharmacological tools in the development and standardisation of in vitro and in vivo test systems for the evaluation of the effects of vascular damaging agents in laboratory animals such as cats, dogs, rabbits, monkeys, rats and mice, as part of the search for new therapeutic agents.

It is to be understood that where the term "ether" is used anywhere in this specification it refers to diethyl ether.

The invention will now be illustrated in the following non-limiting Examples in which, unless otherwise stated:

(i) evaporations were carried out by rotary evaporation in vacuo and work-up procedures were carried out after removal of residual solids such as drying agents by filtration;

(ii) operations were carried out at ambient temperature, that is in the range 18-25°C and under an atmosphere of an inert gas such as argon or nitrogen;

(iii) yields are given for illustration only and are not necessarily the maximum attainable;

(iv) the structures of the end-products of the formula (I) were confirmed by nuclear (generally proton) magnetic resonance (NMR) and mass spectral techniques; proton magnetic resonance chemical shift values were measured on the delta scale and peak multiplicities are shown as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; q, quartet, quin, quintet;

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

Abbreviations

4-Dimethylaminopyridine	DMAP
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride	EDCI
Dimethyl sulphoxide	DMSO
Trifluoroacetic acid	TFA

Example 1

N-[(5S) -3,9,10,11-Tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-2-[2-aminoacetylamino]acetamide



A solution of N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-2-[2-(butoxycarbonylamino)acetylamino]acetamide (0.9 g ; 0.64 mmol) in dichloromethane (6 ml) was treated with TFA (6 ml) at ambient temperature for 0.5 hour. After evaporation to dryness, the residue was neutralised to pH 6.5 with solid sodium hydrogen carbonate and purified on reverse phase silica eluting with a gradient of 30-40 % methanol / ammonium carbonate buffer (2 g / l, pH 7). The appropriate fractions were evaporated to dryness and triturated in ether to give the title compound.

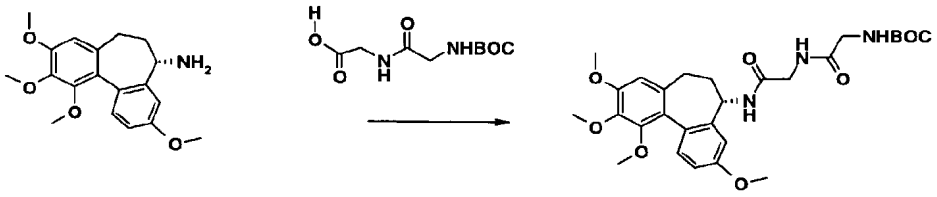
Yield : 65 %

^{1}H NMR (DMSO-d_6) : 1.88-2.21 (m, 3H) ; 2.58 partially obscured by DMSO peak (m, 1H) ; 3.10 (s, 2H) ; 3.46 (s, 3H) ; 3.79 (s, 3H) ; 3.82 (s, 3H) ; 3.83 (s, 3H) ; 3.84 (s, 3H) ; 4.47-4.58 (m, 1H) ; 6.77 (s, 1H) ; 6.87 (dd, 1H) ; 6.91 (d, 1H) ; 7.25 (d, 1H) ; 8.06 (m, 1H) ; 8.41 (d, 1H).

MS-ESI : 444 $[MH]^+$

Elemental analysis	Found	C 59.14	H 6.44	N 9.08
$C_{23}H_{29}N_3O_6$, 1.2 H_2O	Requires	C 59.39	H 6.80	N 9.03

The starting material was prepared as follows:



A solution of (5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine [Collect. Czech. Chem. Commun. 1999, 64(2), 217-228] (0.329 g ; 1.36 mmol),

EDCI (0.230 g ; 1.2 mmol); DMAP (0.025 g, 0.2 mmol) and 2[2-(tert-butoxycarbonylamino) acetylamino] acetic acid (0.189 g ; 1.2 mmol) in dichloromethane was stirred under argon atmosphere overnight. The resulting precipitate was filtered and washed with ether to give N-[(5S) -3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo [a,c] cyclohepten-5-yl]-2-[2-(butoxycarbonylamino)acetylamino]acetamide as a white solid.

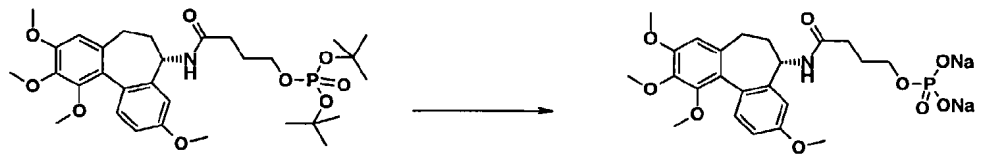
Yield : 65 %.

^{1}H NMR (DMSO-d_6) : 1.33 (s, 9H) ; 1.94-2.24 (m, 3H) ; 2.97-3.08 (m, 1H) ; 3.35 (s, 3H) ; 3.56 (t, 3H) ; 3.71-3.77 (m, 1H) ; 3.75 (s, 3H) ; 3.78 (s, 3H) ; 3.80 (s, 3H) ; 4.48-4.59 (m, 1H) ; 6.79 (s, 1H) ; 6.87 (dd, 1H) ; 6.93 (d, 1H) ; 7.14 (t, 1H) ; 7.25 (d, 1H) ; 8.17 (t, 1H) ; 8.21 (d, 1H).

MS-ESI : 544 $[MH]^+$

Example 2

4-Oxo-4-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]amino]butyl disodium phosphate

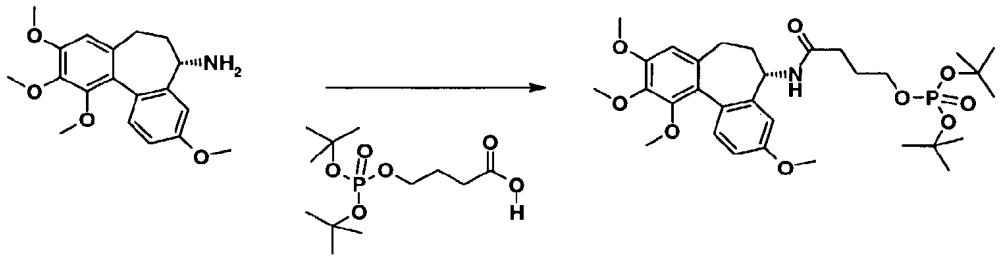


A solution of N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-4-[di(tert-butoxy)phosphoryloxy]butanamide (0.529g; 0.892 mmol) in a mixture of (12N) HCl (5 ml) and dioxan (25 ml) was stirred under argon atmosphere for 4 hours. After evaporation of the dioxan, the pH was adjusted at 7.2 with sodium hydroxide solution (2N) and the residue purified on HP20SS resin eluting with a 0-40 % gradient of methanol/water to give the title compound after freeze drying.

Yield : 75 %

^{1}H NMR (DMSO-d_6) : 1.71-2.36 (m, 7H) ; 2.58 partially obscured by DMSO peak (m, 1H) ; 3.49 (s, 3H) ; 3.78 -3.85 (m, 11H) ; 5.20 (dd, 1H) ; 5.00 (s, 1H) ; 6.77 (s, 1H) ; 6.88 (dd, 1H) ; 6.91 (d, 1H) ; 6.26 (d, 1H).

The starting material was prepared as follows:



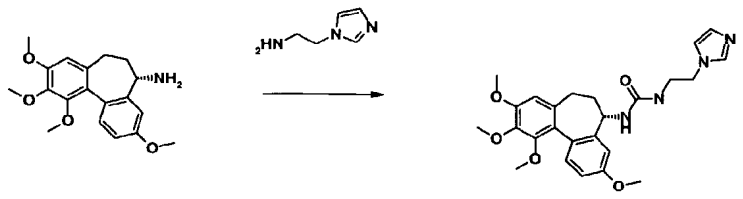
N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-4-[di-(tert-butoxy)phosphoryloxy]butanamide was prepared using a similar method to that of Example 1 by reacting (5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine with 4-[di(tert-butoxy)phosphoryloxy]butanoic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

Yield : 89 %

^{1}H NMR (DMSO-d_6): 1.40 (s, 18H) ; 1.80 (t, 2H) ; 1.82-1.94 (m, 1H) ; 2.00-2.20 (m, 2H ; 2.23-2.33 (m, 2H) ; 2.52-2.58 (m, 1H) ; 3.48 (s, 3H) ; 3.78 (s, 3H) ; 3.80-3.85 (m, 8H) ; 4.50-4.59 (m, 1H) ; 6.78 (s, 1H) ; 6.89 (dd, 1H) ; 6.90 (d, 1H) ; 7.26 (d, 1H) ; 8.42 (d, 1H).

Example 3

N-{N-[2-(Imidazol-1-yl)ethyl]carbamoyl}-5(S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine



A solution of (5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine (0.263 g ; 0.8 mmol), 4-nitrophenol chloroformate (0.177 g ; 0.88 mmol) and triethylamine (0.123 ml ; 0.88 mmol) in dichloromethane was stirred under argon atmosphere for 1 hour. 2-(Imidazol-1-yl)ethylamine (0.145 ml ; 1.2 mmol) was added. After stirring for 2 hours, the mixture was evaporated to dryness and the residue purified on reverse phase silica

eluting with a 40-60 % gradient of methanol / ammonium carbonate buffer (2 g/l, pH 7) to give the title compound after evaporation and trituration in ether.

Yield : 52 %.

^{1}H NMR (DMSO-d_6) : 1.66-1.77 (m, 1H) ; 1.97-2.10 (m, 1H) ; 2.13-2.25 (m, 1H) ; 2.53 partially obscured by DMSO peak (m, 1H) ; 3.12-3.32 (m, 2H) ; 3.47 (s, 3H) ; 3.77 (s, 3H) ; 3.79 (s, 3H) ; 3.83 (s, 1H) ; 3.94 (t, 3H) ; 4.32-4.42 (m, 1H) ; 5.97 (t, 1H) ; 6.63 (d, 1H) ; 6.77 (s, 1H) ; 6.83-6.92 (m, 3H) ; 7.11 (s, 1H) ; 7.24 (d, 1H) ; 7.54 (s, 1H).

MS-ESI : 481 $[MH]^+$

Elemental analysis	Found	C 64.68	H 6.89	N 11.55
$C_{26}H_{32}N_4O_5$	Requires	C 64.98	H 6.71	N 11.66

Example 4

2-{N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamoyloxy}ethyl disodium phosphate



A solution of 2-[di-(benzyloxy)phosphoryloxy]ethyl N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamate (0.576 g ; 0.85 mmol) in solution in methanol (10 ml) and ethyl acetate (5 ml) was hydrogenated in the presence of 10 % C/Pd (0.165 mg) for 4 hours. After filtration on celite and evaporation, the residue was purified on HP2O SS resin eluting with a 0-80 % gradient of methanol/distilled water. The pH of the corresponding fractions was adjusted at 8 with aqueous sodium hydroxide solution (2N), after evaporation of the methanol. After freeze drying the title compound was obtained as a white solid.

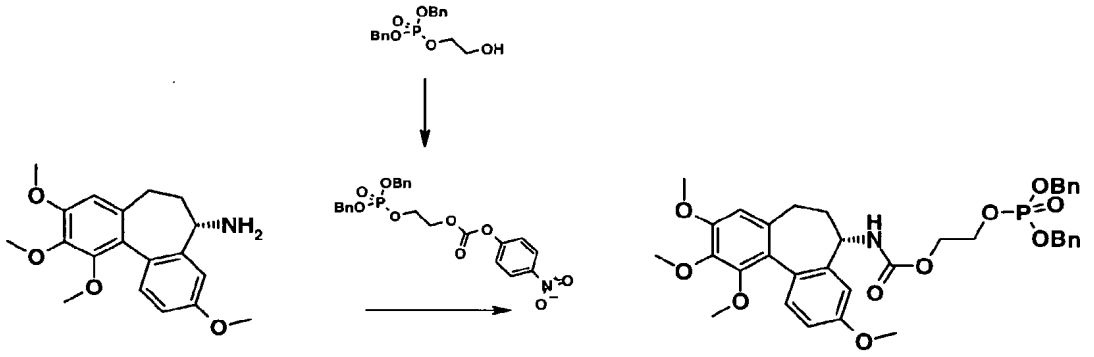
Yield : 83 %

^{1}H NMR (DMSO-d_6 + TFA-d) : 1.85-1.97 (m, 1H) ; 1.98-2.09 (m, 1H) ; 2.13-2.27 (m, 1H) ; 2.42-2.52 (m, 1H) ; 3.48 (s, 3H) ; 3.79 (s, 3H) ; 3.80 (s, 3H) ; 3.84 (s, 3H) ; 3.98 (m, 2H) ;

4.03-4.18 (m, 2H) ; 4.04-4.17 (m, 2H) ; 4.24-4.35 (m, 1H) ; 6.77 (s, 1H) ; 6.89 (dd, 1H) ; 6.96 (d, 1H) ; 7.27 (d, 1H).

MS-ESI : 498 $[MH]^+$

The starting material was prepared as follows:



4-Nitrophenyl chloroformate (1.01 g ; 5.04 mmol) was added at 0°C under argon atmosphere to a solution of 2-[di (benzyloxy)phosphoryloxy]ethanol (1.62 g ; 5.09 mmol) and triethylamine (0.7 ml ; 5 mmol) in dichloromethane (20 ml). The mixture was stirred at ambient temperature for 30 minutes, evaporated and purified by flash chromatography, eluting with petroleum ether / ethyl acetate (40/60) to give 2-[di(benzyloxy)phosphoryloxy]ethyl 4-nitrophenyl carbonate.

Yield : 45 %

^{1}HNMR ($CDCl_3$) : 4.21-4.30 (m, 2H) ; 4.41 (m, 2H) ; 5.01-5.15 (m, 4H) ; 7.29-7.42 (m, 12H) ; 8.25 (d, 2H).

A solution of (5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine (0.329 ; 1 mmol) and 2-[di-(benzyloxy)phosphoryloxy]ethyl 4-nitrophenyl carbonate (0.633 g ; 1.3 mmol) in acetonitrile (8 ml) was heated at 65°C, under argon atmosphere for 8 hours. After evaporation to dryness, the residue was purified by flash chromatography eluting with a 50-80 % gradient of ethyl acetate / petroleum ether to give 2-[di(benzyloxy) phosphoryloxy]ethyl N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamate.

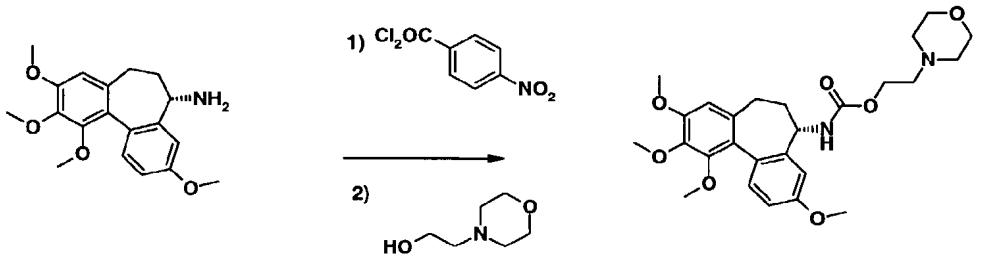
Yield : 85 %

^{1}H NMR (DMSO-d_6) : 1.81-1.93 (m, 1H) ; 1.94-2.06 (m, 1H) ; 2.06-2.20 (m, 1H) ; 2.40-2.52 (m, 1H) ; 3.43 (s, 3H) ; 3.73 (s, 3H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 4.11 (m, 4H) ; 4.20-4.33 (m,

4H); 5.02 (d, 4H); 6.76 (s, 1H); 6.86 -dd, 1H) ; 6.93 (d, 1H) ; 7.25 (d, 1H) . 7.35 (s, 10H) ; 7.99 (d, 1H).

Example 5

2-Morpholinoethyl *N*-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5*H*-dibenzo [a,c]cyclohepten-5-yl]carbamate



A solution of (5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine [Collect. Czech. Chem. Commun. 1999, 64(2), 217-228] (0.263 g ; 0.8 mmol), 4-nitrophenylchloroformate (0.177 g ; 0.88 mmol) and triethylamine (0.123 ml ; 0.88 mmol) in acetonitrile (5 ml) was stirred under argon atmosphere for 2 hours at ambient temperature ; 4-(2-hydroxyethyl)morpholine (0.145 ml ; 1.2 mmol) in solution in acetonitrile (2 ml) was then added to the above solution. The mixture was heated at 60°C overnight. After evaporation to dryness, the residue was purified by flash chromatography, eluted with ethanol/dichloromethane (4/96) to give the title compound.

Yield : 64 %

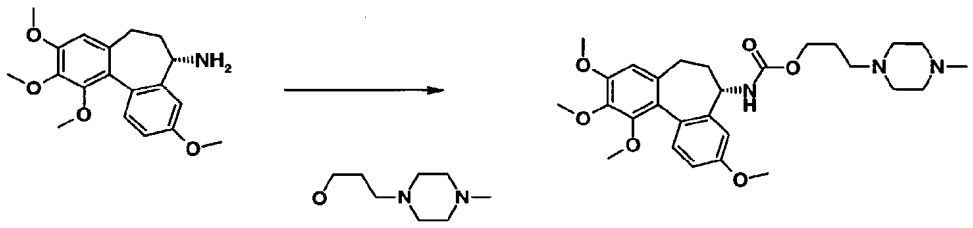
^{1}H NMR Spectrum (DMSO-d_6 + AcO-d_4) : 1.82-2.31 (m, 3H) ; 2.44 (m, 4H) ; 2.49 (m, 2H) ; 2.57 partially obscured by DMSO peak (m, 1H) ; 3.47 (s, 3H) ; 3.56 (m, 4H) ; 3.78 (s, 3H) ; 3.79 (s, 3H) ; 3.83 (s, 3H) ; 4.03 (m, 2H) ; 4.17-4.33 (m, 1H) ; 6.76 (s, 1H) ; 6.88 (dd, 1H) ; 6.93 (d, 1H) ; 7.26 (d, 1H) ; 7.86 (d, 1H).

MS-ESI : 487 $[MH]^+$

Elemental analysis :	Found	C 63.38	H 7.04	N 5.74
$C_{26}H_{34}N_2O_7$,0.3 H_2O	Requires	C 63.48	H 7.09	N 5.69

Example 6

3-(1-Methylpiperazin-4-yl)propyl N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo [a,c]cyclohepten-5-yl] carbamate



Using a similar procedure to that described for Example 5, (5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine was reacted with 4-(3-hydroxypropyl)-1-methylpiperazine to give the title compound.

Yield : 40 %.

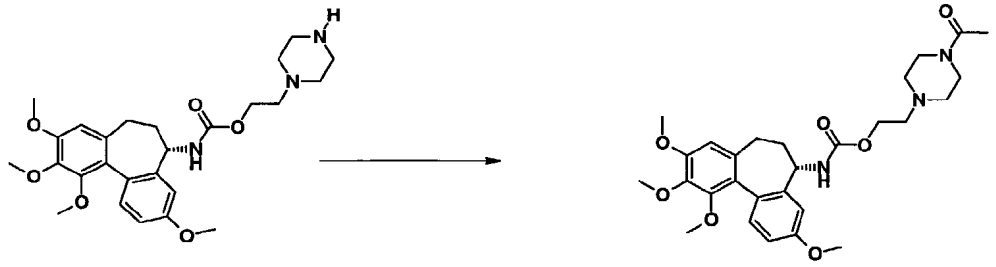
^{1}H NMR (DMSO-d_6) : 1.62-2.44 (m, 20H) ; 2.54 partially obscured by DMSO peak (m, 1H) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.78 (s, 3H) ; 3.82 (s, 3H) ; 6.78 (s, 1H) ; 6.89 (dd, 1H) ; 6.93 (d, 1H) ; 7.27 (d, 1H) ; 7.80 (d, 1H).

MS-ESI : 514 $[MH]^+$

Elemental analysis :	Found	C 65.42	H 7.54	N 8.18
$C_{28}H_{39}N_3O_6$	Requires	C 65.48	H 7.65	N 8.18

Example 7

2-(1-Acetylpiperazin-4-yl)ethyl-N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl] carbamate



A solution of 2-(piperazin-4-yl)ethyl-N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamate (0.255 g ; 0.525 mmol), acetyl chloride (0.038 ml ; 0.53 mmol) and triethylamine (0.073 ml ; 0.525 mmol) in dichloromethane (10 ml) was stirred at ambient temperature under argon atmosphere for 2 hours. After evaporation to

dryness, the residue was purified by flash chromatography, eluting with dichhloromethane/ethanol (93/7) to give the title compound.

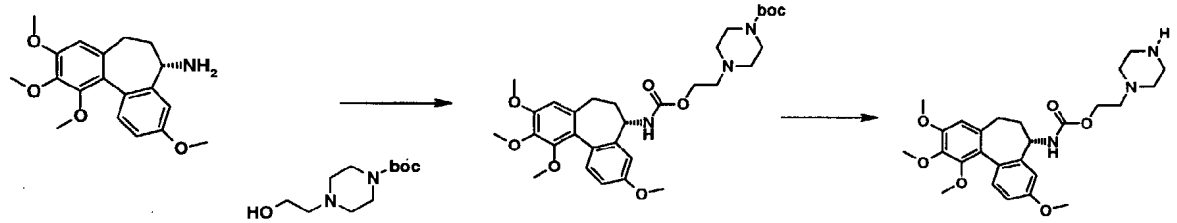
Yield : 90 %

^{1}H NMR (DMSO-d_6) : 1.81-2.32 (m, 3H) ; 1.97 (s, 3H) ; 2.33 (m, 2H) ; 2.40 (m, 2H) ; 2.47 partially obscured by DMSO peak (m, 2H) ; 2.58 partially obscured by DMSO peak (m, 1H) ; 3.38 (m, 4H) ; 3.46 (s, 3H) ; 3.77 (m, 3H) ; 3.80 (s, 3H) ; 3.82 (s, 3H) ; 3.92-4.10 (m, 2H) ; 4.20-4.30 (m, 1H) ; 6.77 (s, 1H) ; 6.88 (dd, 1H) ; 6.92 (d, 1H) ; 7.25 (d, 1H) ; 7.87 (d, 1H).

MS-ESI : 528 $[MH]^+$

Elemental analysis :	Found	C 63.21	H 7.30	N 7.86
$C_{28}H_{37}N_3O_7$	Requires	C 63.52	H 7.08	N 7.94

The starting material was prepared as follows :



2-(1-tert-Butoxycarbonylpiperazin-4-yl)ethyl-N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamate was prepared using a similar method to that described for Example 1, but using 2-[1-(tert-butoxycarbonyl)piperazin-4-yl]ethanol.

Yield : 75 %

^{1}H NMR (DMSO-d_6) : 1.78-1.94 (m, 1H) ; 1.95-2.07 (m, 1H) ; 2.10-2.23 (m, 1H) ; 2.30 (m, 4H) ; 2.38-2.53 (m, 3H) ; 2.65 (t, 2H) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.78 (s, 3H) ; 3.82 (s, 3H) ; 3.99 (t, 2H) ; 4.20-4.32 (m, 1H) ; 6.77 (s, 1H) ; 6.88 (dd, 1H) ; 6.92 (d, 1H) ; 7.25 (d, 1H) ; 7.86 (d, 1H).

2-(-1- tert-Butoxycarbonylpiperazin-4-yl)ethyl-N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo [a,c]cyclohepten-5-yl] carbamate (0.617 g ; 1.05 mmol) in dichloromethane (10 ml) was treated with TFA (5 ml) at ambient temperature for 1 hour. After evaporation to dryness, the residue was neutralised to pH 8 with sodium hydroxide solution and purified on reverse phase silica eluting with a gradient of 30 - 40 % methanol / ammonium carbonate

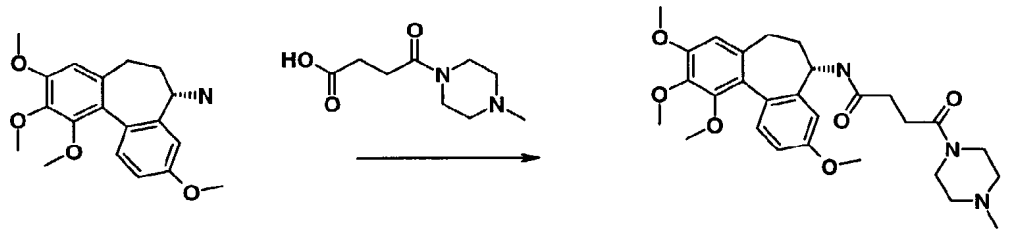
buffer (2 g/l, pH 7) to give 2-(piperazin-4-yl) ethyl- N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl] carbamate.

Yield : 60 %

^{1}H NMR (DMSO-d_6) : 1.39 (s, 9H) ; 1.81-1.94 (m, 1H) ; 1.95-2.07 (m, 1H) ; 2.09-2.27 (m, 1H) ; 2.34 (m, 4H) ; 2.52-2.64 (m, 1H) ; 3.28 (m, 2H) ; 3.36 (s, 3H) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.82 (s, 3H) ; 3.94-4.09 (m, 2H) ; 4.20-4.30 (m, 1H) ; 6.77 (s, 1H) ; 6.87 (dd, 1H) ; 6.92 (d, 1H) ; 7.25 (d, 1H) ; 7.86 (d, 1H).

Example 8

N-[(5S)-3,9,10,11-Tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl] -4-(1-methylpiperazin-4-yl)-4-oxobutan-l-amide



A solution of 4-(1-methylpiperazin-4-yl)-4-oxo butanoic acid (0.356 g ; 1.78 mmol), EDCI (0.367 g ; 1.78 mmol) and DMAP (0.05 g ; 0.41 mmol) in dichloromethane (30 ml) was stirred for 35 minutes under argon atmosphere. (5S)-3,9,10,11-Tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine 0.45 g ; 1.37 mmol) was then added and the mixture stirred at ambient temperature overnight. After evaporation of the solvent, the residue was purified by flash chromatography, eluting with dichloromethane/ethanol (95/5) to give after evaporation and trituration in pentane, the title compound as a white solid.

Yield : 60 %

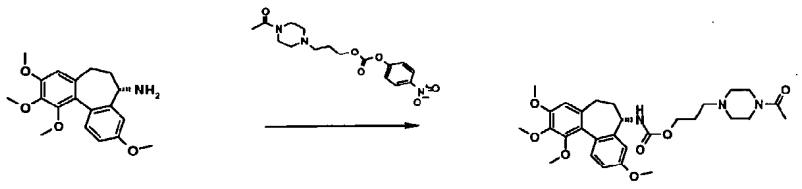
^{1}H NMR (DMSO-d_6) : 1.85-1.96 (m, 1H) ; 2.01-2.15 (m, 1H) ; 2.16 (s, 3H) ; 2.22 (t, 2H) ; 2.26 (t, 2H) ; 2.33-2.42 (m, 1H) ; 2.47-2.53 (m, 1H) ; 3.35-3.46 (m, 4H) ; 3.46 (s, 3H) ; 3.79 (s, 3H) ; 3.82 (s, 3H) ; 3.84 (s, 3H) ; 4.44-4.56 (m, 1H) . 6.79 (s, 1H) ; 6.86 (dd, 1H) ; 6.98 (d, 1H) ; 7.25 (d, 1H) ; 8.40 (d, 1H).

MS-ESI : 512 $[MH]^+$

Elemental analysis	Found	C 65.51	H 7.40	N 8.19
$C_{28}H_{37}N_3O_6$	Requires	C 65.73	H 7.29	N 8.21

Example 9

3-(1-Acetylpiperazin-4-yl) propyl-N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamate



A solution of (5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine (0.329 g ; 1 mmol) and 3-(-4-acetylpiperazino)propyl 4-nitrophenylcarbonate (0.456 g; 1.3 mmol) in acetonitrile (8 ml) was heated under argon atmosphere at 70°C for 6 hours. After evaporation to dryness, the residue was purified by flash chromatography eluting with dichloromethane/ethanol (93/7) to give the title compound.

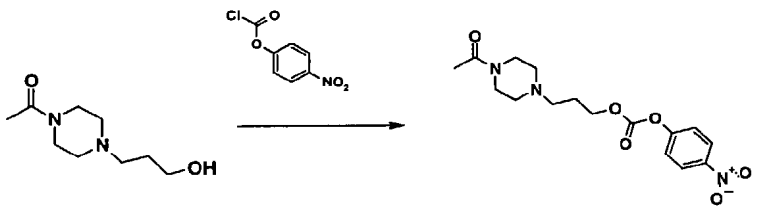
Yield : 80 %

^{1}H NMR (DMSO-d_6) : 1.62-2.51 (m, 12H) ; 1.97 (s, 3H) ; 3.32-3.44 (m, 4H) ; 3.46 (s, 3H) ; 3.77 (s, 3H) ; 3.78 (s, 3H) ; 3.82 (s, 3H) ; 3.87-4.01 (m, 2H) ; 4.19-4.31 (m, 1H) ; 6.77 (s, 1H) ; 6.88 (dd, 1H) ; 6.92 (d, 1H) ; 7.25 (d, 1H) ; 7.80 (d, 1H).

MS-ESI : 542 $[MH]^+$

Elemental analysis	Found	C 63.48	H 7.25	N 7.72
$C_{29}H_{39}N_3O_7$, 0.4 H_2O	Requires	C 63.46	H 7.31	N 7.66

The starting material was prepared as follows :

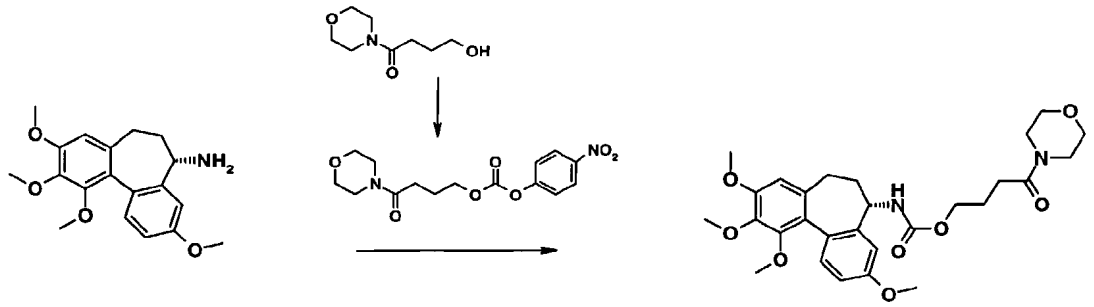


4-Nitrophenyl chloroformate (0.733 g ; 3.63 mmol) was added to a solution of 3-(4-acetylpiperazin-1-yl) propanol [Synthesis (1997), 6, 643-648] (0.645 g ; 3.46 mmol) and triethylamine (0.51 ml ; 3.36 mmol) in dichloromethane (7 ml) under argon atmosphere at 0°C. The mixture was stirred at ambient temperature for 1 hour, evaporated to dryness and purified by flash chromatography, eluting with dichloromethane/ethanol (95/5) to give 3(-4-acetylpiperazino)propyl 4-nitrophenyl carbonate.

^{1}H NMR ($CDCl_3$) : 1.96 (m, 2H) ; 2.09 (s, 3H) ; 2.39-2.48 (m, 4H) ; 2.51 (t, 2H) ; 3.47 (t, 3H); 3.63 (t, 2H) ; 3.68-3.78 (m, 2H) ; 4.38 (t, 2H) ; 7.39 (d, 2H) ; 8.29 (d, 2H).

Example 10

4-Morpholino-4-oxobutyl-N-[(5S)-3,9,10, 11-tetramethoxy-6,7-dihydro-5H-dibenzo [a-c]cyclohepten-5-yl]carbamate



The compound was prepared using a similar method to that of Example 9, but replacing 3-(4-acetylpiperazino)propyl 4-nitrophenyl carbonate by 4-morpholino-4-oxobutyl 4-nitrophenylcarbonate.

Yield : 55 %.

^{1}H NMR (DMSO-d_6) : 1.71-1.81 (m, 2H) ; 1.82-1.94 (m, 1H) ; 1.95-2.07 (s, 1H) ; 2.11-2.24 (s, 1H) ; 2.34 (t, 2H) ; 2.46 (m, 1H) ; 3.31-3.44 (m, 4H) ; 3.45 (s, 3H) ; 3.52 (m, 4H) ; 3.77 (s, 3H) ; 3.78 (s, 3H) ; 3.82 (s, 3H) ; 3.86-3.98 (m, 2H) ; 4.20-4.32 (m, 1H) ; 6.77 (s, 1H) ; 6.88 (dd, 1H) ; 6.92 (d, 1H) ; 7.25 (d, 1H) ; 7.80 (d, 1H).

MS-ESI : 529 $[MH]^+$

Elemental analysis	Found	C 62.81	H 6.95	N 5.27
$C_{28}H_{36}N_2O_8$, 0.3 H_2O	Requires	C 62.98	H 6.91	N 5.25

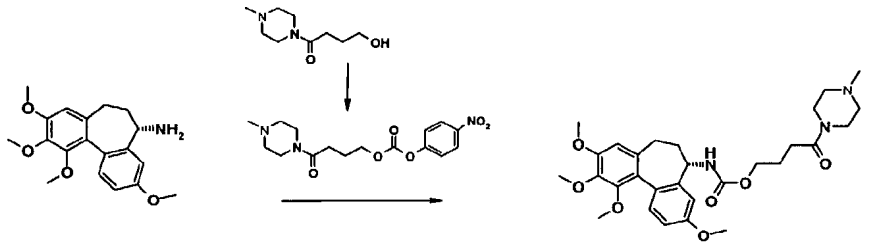
The starting material was prepared using a similar method to that of example 9, starting from 4-morpholino-4-oxobutyl 4-nitrophenyl carbonate.

Yield : 92 %

^{1}H NMR ($CDCl_3$) : 2.15 (m, 2H) ; 2.50 (t, 2H) ; 3.46-3.53 (m, 2H) ; 3.51-3.75 (m, 6H) ; 3.38 (t, 2H) ; 7.33 (d, 2H) ; 8.29 (d, 2H).

Example 11

4-(1-Methylpiperazin-4-yl)-4-oxobutyl-N-[(5S)-3,9,10, 11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cylcohepten-5-yl]carbamate



The title compound was prepared using a similar method to that of Example 9, but replacing 3-(4-acetylpiperazino)propyl 4-nitrophenyl carbonate by 4-methylpiperazin-l-yl)-4-oxobutyl 4-nitrophenyl carbonate.

Yield : 65%

^{1}H NMR (DMSO-d_6) : 1.75 (m, 2H) ; 1.81-2.07 (m, 2H) ; 2.08-2.40 (m, 7H) ; 2.15 (s, 3H) ; 2.50-2.60 (m, 1H) ; 3.22-3.56 (m, 4H) ; 3.45 (s, 3H) ; 3.77 (s, 3H) ; 3.78 (s, 3H) ; 3.82 (s, 3H) ; 3.82-3.99 (m, 2H) ; 4.12-4.32 (m, 1H) ; 6.76 (s, 1H) ; 6.87 (dd, 1H) ; 6.92 (d, 1H) ; 7.25 (d, 1H) ; 7.80 (d, 1H).

MS-ESI : 542 $[MH]^+$

Elemental analysis	Found	C 63.38	H 7.58	N 7.64
$C_{29}H_{39}N_3O_7$, 0.4 H_2O	Requires	C 63.46	H 7.31	N 7.66

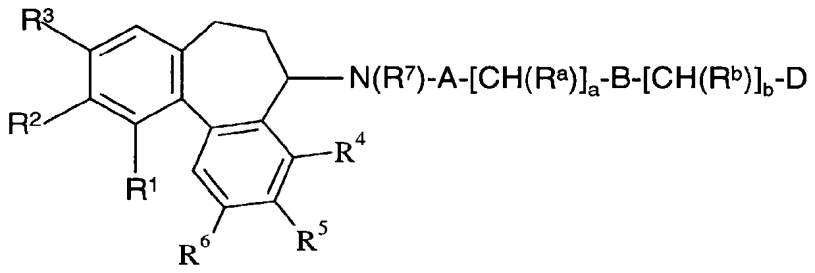
The starting material was prepared using a similar method to that of Example 9, from 4-(4-methylpiperazin-l-yl)-4-oxobutanol.

Yield : 65 %.

^{1}H NMR ($CDCl_3$) : 2.08-2.19 (m, 2H) ; 2.32 (s, 3H) ; 2.35-2.46 (m, 4H) ; 2.49 (t, 2H) ; 3.51 (t, 2H) ; 2.66 (t, 2H) ; 4.38 (t, 2H) ; 7.39 (d, 2H) ; 8.29 (d, 2H).

Claims

1. A compound of the formula (I):



(I)

wherein:

$\mathbf{R^1}$, $\mathbf{R^2}$ and $\mathbf{R^3}$ are each independently hydroxy, phosphoryloxy ($-OPO_3H_2$), C_{1-4}alkoxy or an in vivo hydrolysable ester of hydroxy, with the proviso that at least 2 of R^1, R^2 and R^3 are C_{1-4}alkoxy;

A is - CO-, -C(O)O-, $-CON(R^8)-$, $-SO_2-$ or $-SO_2N(R^8)-$ (wherein $\mathbf{R^8}$ is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl or hydroxyC_{1-3}alkyl);

a is an integer from 1 to 4 inclusive;

$\mathbf{R^a}$ and $\mathbf{R^b}$ are independently selected from hydrogen, hydroxy and amino;

B is -O-, -CO-, $-N(R^9)CO-$, $-CON(R^9)-$, -C(O)O-, $-N(R^9)-$, $-N(R^9)C(O)O-$, $-N(R^9)CON(R^{10})-$, $-N(R^9)SO_2-$, $-SO_2N(R^9)-$ or a direct single bond (wherein $\mathbf{R^9}$ and $\mathbf{R^{10}}$ are independently selected from hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl and hydroxyC_{1-3}alkyl);

b is 0 or an integer from 1 to 4 inclusive, (provided that when b is 0, B is a single direct bond);

D is carboxy, sulpho, tetrazolyl, imidazolyl, phosphoryloxy, hydroxy, amino, N-(C_{1-4}alkyl)amino, N,N-di(C_{1-3}alkyl)amino or of the formula $-Y^1-(CH_2)_cR^{11}$ or $-NHCH(R^{12})COOH$; [wherein $\mathbf{Y^1}$ is a direct single bond, -O-, -C(O)-, $-N(R^{13})-$, $-N(R^{13})C(O)-$ or $-C(O)N(R^{13})-$ (wherein $\mathbf{R^{13}}$ is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{2-3}alkyl, aminoC_{2-3}alkyl or hydroxyC_{2-3}alkyl); **c** is 0 or an integer from 1 to 4 inclusive; $\mathbf{R^{11}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from

O, S and N, or a 5-6-membered unsaturated or partially unsaturated heteroaryl group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, which heterocyclic group or heteroaryl group may bear 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-(C_{1-4}alkyl)carbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, aminoC_{1-4}alkyl, N-C_{1-4}alkylaminoC_{1-4}alkyl, di-N,N-(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein **R^{14}** is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl);

R^{12} is an amino acid side chain;

R^5 is C_{1-4}alkoxy;

R^4 and **R^6** are each independently selected from: hydrogen, fluoro, nitro, amino, N-C_{1-4}alkylamino, N,N-di-(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy and C_{1-4}alkyl;

R^7 is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl or hydroxyC_{1-3}alkyl;

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

2. A compound according to claim 1 where R^1, R^2 and R^3 are all methoxy.
or a pharmaceutically acceptable salt, solvate or pro-drug thereof.

3 A compound according to claim 1 wherein:

R^1 , R^2, and R^3 are all C_{1-4}alkoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, C_{1-3} alkoxy, and C_{1-3}alkyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 1, 2 or 3;

B is -CO-, -NHCO-, -CONH, -C(O)O-, -NH-, -NHC(O)O-, NHCONH- or a single direct bond;

b is 0, 1 or 2;

D is carboxy, sulpho, phosphoryloxy, hydroxy, amino, N-C_{1-4} alkylamino, N,N-di(C_{1-4} alkyl)amino or of the formula $-Y^1(CH_2)_cR^{11}$ (wherein $\mathbf{Y^1}$ is -NHC(O)- or -C(O)NH-; **c** is 1 or 2; $\mathbf{R^{11}}$ is a 5-6-membered saturated heterocyclic group (linked via nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O and N, which heterocyclic group may bear 1 or 2 substituents selected from:

C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

R^7 is hydrogen;

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

4. A compound according to claim 1 wherein:

R^1 , R^2, and R^3 are all methoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, methoxy and methyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

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

b is 0 or 1;

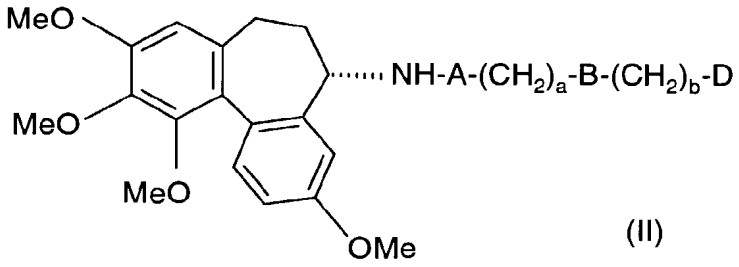
D is carboxy, phosphoryloxy, hydroxy, amino, N-C_{1-4} alkylamino, N,N-di(C_{1-4} alkyl)amino or of the formula $-Y^1(CH_2)_cR^{11}$ (wherein $\mathbf{Y^1}$ is -NHC(O)- or -C(O)NH-; **c** is 1 or 2; $\mathbf{R^{11}}$ is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring nitrogen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

C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

R^7 is hydrogen;

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

5. A compound of formula (II):



(II)

wherein a, b, A, B and D are as defined in claim 1;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

6. A compound according to claim 5 wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

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

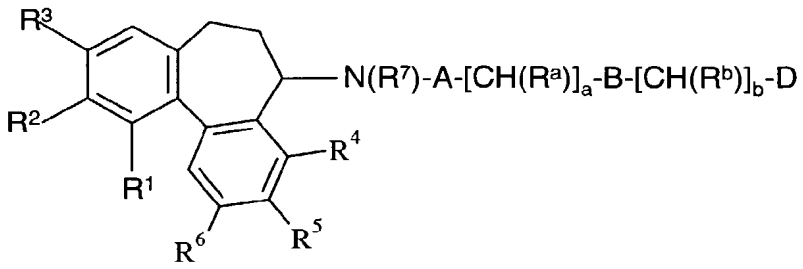
b is 0 or 1;

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

C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

7. A compound of formula (III):



(III)

wherein:

R^1, R^2 and **R^3** are each independently hydroxy, phosphoryloxy (-OPO_3H_2), C_{1-4}alkoxy or an in vivo hydrolysable ester of hydroxy, with the proviso that at least 2 of R^1, R^2 and R^3 are C_{1-4}alkoxy;

A is - CO-, -C(O)O-, -$CON(R^8)$-, -SO_2- or -$SO_2N(R^8)$- (wherein **R^8** is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{2-3}alkyl, aminoC_{2-3}alkyl or hydroxyC_{2-3}alkyl);

a is an integer from 1 to 4 inclusive;

R^a and **R^b** are independently selected from hydrogen, hydroxy and amino;

B is -O-, -CO-, -$N(R^9)$CO-, -$CON(R^9)$ -, -C(O)O-, -$N(R^9)$ -, - $N(R^9)$C(O)O-, -$N(R^9)CON(R^{10})$-, -$N(R^9)SO_2$-, -$SO_2N(R^9)$- or a direct single bond (wherein **R^9** and **R^{10}** are independently selected from hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{2-3}alkyl, aminoC_{2-3}alkyl and hydroxyC_{2-3}alkyl);

b is 0 or an integer from 1 to 4 inclusive;

D is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O and N, which heterocyclic group may bear 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-(C_{1-4}alkyl)carbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, aminoC_{1-4}alkyl, N-C_{1-4}alkylaminoC_{1-4}alkyl, di-N,N-(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein **R^{14}** is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl);

R^5 is C_{1-4}alkoxy;

R^4 and **R^6** are each independently selected from:

hydrogen, halogeno, nitro, amino, N-C_{1-4}alkylamino, N,N-di-(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy and C_{1-4}alkyl;

R^7 is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aminoC_{1-3}alkyl or hydroxyC_{1-3}alkyl;

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

8. A compound according to claim 7 wherein:

R^1 , R^2, and R^3 are all C_{1-4}alkoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, C_{1-3} alkoxy, and C_{1-3}alkyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 1, 2 or 3;

B is -CO-, -NHCO-, -CONH, -C(O)O-, -NH-, -NHC(O)O-, NHCONH- or a single direct bond;

b is 0, 1 or 2;

D is piperazinyl or morpholinyl or piperidinyl, each ring being optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl;

R^7 is hydrogen;

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

9. A compound according to claim 7 wherein:

R^1 , R^2, and R^3 are all methoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, methoxy and methyl;

R^5 is methoxy;

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

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

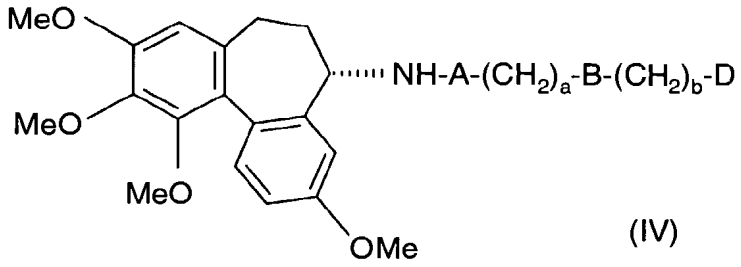
b is 0 or 1;

D is piperazino or morpholino, each ring being optionally substituted by 1 or 2 substituents selected from methyl, ethyl, acetyl, propionyl, carbamoyl and 2-hydroxyethyl;

R^7 is hydrogen;

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

10. A compound according to claim 7 wherein:



(IV)

wherein a, b, A, B and D are as hereinabove defined in claim 7;

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

11. A compound according to claim 10 wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

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

b is 0 or 1;

D is piperazino or morpholino, each ring being optionally substituted by 1 or 2 substituents selected from methyl, ethyl, acetyl, propionyl, carbamoyl and 2-hydroxyethyl;

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

12. A compound according to claim 10 wherein:

A is -CO-, -C(O)O- or -CONH-;

a is 2 or 3;

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

b is 0 or 1;

D is morpholino, 4-methylpiperazin-1-yl or 4-acetylpiperazin-1-yl;

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

13. A compound selected from:

N-[(5S) -3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-2-[2-aminoacetylamino]acetamide;

4-oxo-4-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]amino]butyl disodium phosphate;

N-{N-[2-(imidazol-1-yl)ethyl]carbamoyl}-5(S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine; and

2-{N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamoyloxy}ethyl disodium phosphate;

2-morpholinoethyl *N*-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5*H*-dibenzo [a,c]cyclohepten-5-yl]carbamate;

3-(1-methylpiperazin-4-yl)propyl N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo [a,c]cyclohepten-5-yl] carbamate;

N-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-2-[2-aminoacetylamino]acetamide;

2-(1-acetylpiperazin-4-yl)ethyl-N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl] carbamate;

N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]-4-(1-methylpiperazin-4-yl)-4-oxobutan-l-amide;

4-oxo-4-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]amino]butyl disodium phosphate;

N-{N-[2-(imidazol-1-yl)ethyl]carbamoyl}-5(S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-ylamine;

3-(1-acetylpiperazin-4-yl) propyl-N-[(5S)-3,9,10,11-tetramethoxy-6-7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamate;

N-l-[(5S)-3,9,10,11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-5-yl]carbamoyloxy]ethyl disodiumphosphate;

4-morpholino-4-oxobutyl-N-[(5S)-3,9,10, 11-tetramethoxy-6,7-dihydro-5H-dibenzo [a-c]cyclohepten-5-yl]carbamate; and

4-(1-methylpiperazin-4-yl)-4-oxobutyl-N-[(5S)-3,9,10, 11-tetramethoxy-6,7-dihydro-5H-dibenzo[a,c]cylcohepten-5-yl]carbamate;

and pharmaceutically-acceptable salts, solvates or pro-drugs thereof.

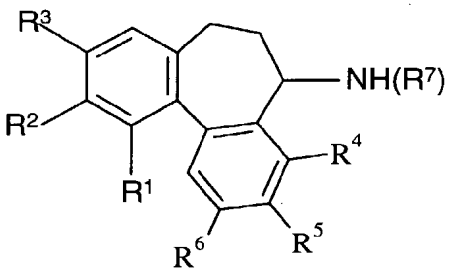
14. A pharmaceutical composition comprising a compound according to any one of claims 1 to 13 or a pharmaceutically acceptable salt, solvate or pro-drug thereof, in association with a pharmaceutically acceptable carrier.

15. The use of a compound according any one claims 1 to 13, or a pharmaceutically-acceptable salt, solvate or pro-drug thereof, in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm-blooded animal.

16. The use of a compound according to any one of claim 1 to 13 or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for administration in divided doses for use in the production of a vascular damaging effect in a warm-blooded animal.

17. A process for preparing a compound of the formula (I), or a compound of the formula (I) wherein at least 1 functional group is protected, wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, A, B, D, a, b and c are as defined in claim 1, comprising:

a) reacting a compound of the formula (X)



(X)

with a compound of the formula (XI):

$$L^1 - A - [CH(R^a)]_a\text{-}B\text{-}[CH\,R^b)]_b\text{-}D$$

(XI)

wherein L^1 is a leaving group; or

b) converting one compound of the formula (I) into another compound of the formula (I);

c) when a phosphoryloxy group is desired, reacting the corresponding hydroxy compound with a phosphoramidite;

wherein any functional groups are optionally protected.

and thereafter if necessary:

i) converting a compound of formula (I) into another compound of formula (I);

ii) removing any protecting groups;

iii) forming a pharmaceutically acceptable salt, solvate or pro-drug thereof.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 01/02964

A. CLASSIFICATION OF SUBJECT MATTER
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

(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 1: wherein the substituents are as defined in the description or a pharmaceutically-acceptable salt, solvate or pro-drug thereof. The invention also relates to processes for preparing compounds of formula (1), pharmaceutical compositions of compounds of formula (1) and the use of compounds of formula (1), in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm blooded animal.

COLCHINOL DERIVATIVES AS VASCULAR DAMAGING AGENTS

The present invention relates to vascular damaging agents, to the use of compounds of the invention in the manufacture of medicaments for use in the production of antiangiogenic effects in warm-blooded animals such as humans, to processes for the preparation of such compounds, to pharmaceutical compositions containing such compounds as active ingredient, to methods for the treatment of disease states associated with angiogenesis and to the use of such compounds as medicaments.

Normal angiogenesis plays an important role in a variety of processes including embryonic development, wound healing and several components of female reproductive function. Undesirable or pathological angiogenesis has been associated with disease states including diabetic retinopathy, psoriasis, cancer, rheumatoid arthritis, atheroma, Kaposi's sarcoma and haemangioma (Fan et al, 1995, Trends Pharmacol. Sci. 16: 57-66; Folkman, 1995, Nature Medicine 1: 27-31). Formation of new vasculature by angiogenesis is a key pathological feature of several diseases (J. Folkman, New England Journal of Medicine 333, 1757-1763 (1995)). For example, for a solid tumour to grow it must develop its own blood supply upon which it depends critically for the provision of oxygen and nutrients; if this blood supply is mechanically shut off the tumour undergoes necrotic death. Neovascularisation is also a clinical feature of skin lesions in psoriasis, of the invasive pannus in the joints of rheumatoid arthritis patients and of atherosclerotic plaques. Retinal neovascularisation is pathological in macular degeneration and in diabetic retinopathy.

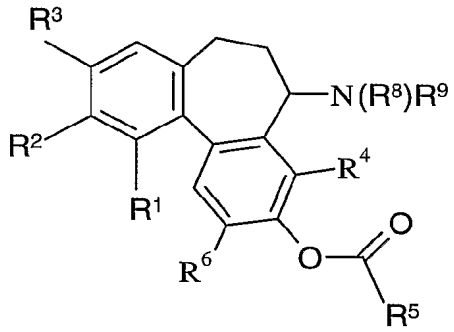
Reversal of neovascularisation by damaging the newly-formed vascular endothelium is expected to have a beneficial therapeutic effect. The present invention is based on the discovery of tricyclic compounds that surprisingly specifically damage newly formed vasculature without affecting the normal, established vascular endothelium of the host species, a property of value in the treatment of disease states associated with angiogenesis such as cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation, endometriosis, dysfunctional uterine bleeding and ocular diseases with retinal vessel proliferation.

Compounds of the present invention are colchinol derivatives. Colchinol derivatives for example *N*-acetyl-colchinol are known. Anti-tumour effects have been noted on animal

models (see for example - Jnl. Natl. Cancer Inst. 1952, 13, 379-392). However, the effect studied was that of gross damage (haemorrhage, softening and necrosis) and there is no suggestion of treatment of inappropriate angiogenesis by destruction of neovasculature.

It is believed, though this is not limiting on the invention, that the use of compounds of the invention damages newly-formed vasculature, for example the vasculature of tumours, thus effectively reversing the process of angiogenesis as compared to known anti-angiogenic agents which tend to be less effective once the vasculature has formed.

According to one aspect of the present invention there is provided a compound of the formula I:



(I)

wherein:

R^1, R^2 and **R^3** are each independently hydroxy, phosphoryloxy ($-OPO_3H_2$), C_{1-4}alkoxy or an in vivo hydrolysable ester of hydroxy; with the proviso that at least two of R^1, R^2 and R^3 are C_{1-4}alkoxy;

R^4 and **R^6** are each independently selected from:

hydrogen, nitro, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, fluoro, C_{1-4}alkoxy and C_{1-4}alkyl;

R^5 is selected from one of the following groups:

1) of the formula -A -X^1-Y^1 -B, wherein:

A is C_{1-4}alkylene or $-(CH_2)_p$-Q- (wherein **p** is 0, 1 or 2 and **Q** is phenylene or thienylene);

X^1 is -O-, -CO-, -C(O)O-, $-CON(R^{10})-$, $-N(R^{10})-$, $-N(R^{10})CO-$, $N(R^{10})C(O)O-$, $-N(R^{10})CON(R^{11})-$, $-N(R^{10})SO_2-$, $-SO_2N(R^{10})-$ or $OC(O)N(R^{10})-$

(wherein **R^{10}** is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

Y^1 is C_{1-3}alkylene;

B is carboxy, sulpho, phosphoryloxy, hydroxy, amino, N-(C_{1-4}alkyl)amino, N,N-di(C_{1-3}alkyl)amino (wherein the C_{1-}alkyl group in the alkylated amino groups is optionally substituted by hydroxy or amino), -R^{12} or -NHC(R^{13})COOH;

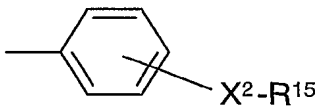
(wherein $\mathbf{R^{12}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, aminoC_{1-4}alkyl, N,N-di(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein $\mathbf{R^{14}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkoxyC_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl);

R^{13} is an amino acid side chain;

2) of the formula:



wherein: the phenyl ring is substituted by -$\mathbf{X^2}$-$\mathbf{R^{15}}$ in the 3- or 4-position;

$\mathbf{X^2}$ is -CO- or of the formula -$(CH_2)_r$- (wherein **r** is 0, 1, 2 or 3) and $\mathbf{R^{15}}$ is a 5-6 membered saturated heterocyclic group (linked via a ring carbon or nitrogen atom) containing 1 or 2 ring heteroatoms selected from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from

oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, C_{1-4}aminoalkyl, N,N-di(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein $\mathbf{R^{14}}$ is as hereinabove defined);

provided that the heterocyclic group (R^{15}) is substituted by at least one substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N,N-di(C_{1-4}alkyl)carbamoyl;

3) -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15} (wherein **a** is 0, 1, 2, 3 or 4; **b** is 0, 1, 2, 3 or 4; $\mathbf{Y^2}$ is a direct bond, -O-, -C(O)-, -N(R^{16})-, -N(R^{16})C(O)- or -C(O)N(R^{16})- (wherein $\mathbf{R^{16}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and wherein 1 or 2 of the $(CH_2)_a$ or $(CH_2)_b$ groups are optionally substituted by 1 or 2 substituents selected from hydroxy and amino and $\mathbf{R^{15}}$ is as hereinabove defined; provided that when a is 0, then Y^2 is a single direct bond;

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl- (wherein the alkyl groups are independently optionally substituted by 1 or 2 substituents selected from:

amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, C_{1-4}alkoxy, C_{1-4}alkanoyl, carboxy, sulpho and phosphoryloxy);

provided that:

a) when A is C_{1-4}alkylene and X^1 is of the formula -CO-, -N(R^{10})-, -N(R^{10})CO- or -CON(R^{10})- then when B is R^{12}, R^{12} is as defined hereinabove for R^{15} ;

b) when A is C_{1-4}alkylene and X^1 is of the formula -N(R^{10})CO-, -CON(R^{10})- , or -C(O)O-, then B is not carboxy;

c) when A is C_{1-4}alkylene and X^1 is -CONH- or -NHCO-, then B is not carboxy, hydroxy, phosphoryloxy, amino, N-C_{1-4}alkylamino or N,N-di-C_{1-4}alkylamino;

$\mathbf{R^8}$ is a group -Y^3R^{17} (wherein $\mathbf{Y^3}$ is a direct bond, -C(O)-, -C(O)O-, -N(R^{18})-, -C(O)N(R^{18})-, -SO_2- or -SO_2NR^{18}- (wherein $\mathbf{R^{18}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and $\mathbf{R^{17}}$ is selected from one of the following 4 groups:

1) hydrogen, C_{1-4}alkyl, phenyl, C_{1-4}alkylY^4C_{1-4}alkyl (wherein $\mathbf{Y^4}$ is -C(O)-, -NR^{19}C(O)- or -C(O)NR^{19}- (wherein $\mathbf{R^{19}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl));

[which alkyl, alkylY^4alkyl or phenyl group is optionally substituted by 1 or 2 substituents selected from:

halogeno, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, carboxy, -CON(R^{23})R^{24} (wherein $\mathbf{R^{23}}$ **and** $\mathbf{R^{24}}$ are independently selected from hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl and C_{1-3}alkoxyC_{2-3}alkyl), C_{1-4}alkoxy, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, sulpho, phosphoryloxy, R^{12} (wherein $\mathbf{R^{12}}$ is as hereinabove defined), and a group -$\mathbf{Y^5R^{20}}$ [wherein $\mathbf{Y^5}$ is -NR^{21}C(O)- or -OC(O)- (wherein

$\mathbf{R^{21}}$ represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and $\mathbf{R^{20}}$ is C_{1-4}alkyl or a group R^{22} (wherein $\mathbf{R^{22}}$ is a 5 or 6 membered aromatic heterocyclic group containing 1 to 4, inclusive, ring heteroatoms selected independently from O, N and S, which aromatic heterocyclic group is optionally substituted by 1 or 2 substituents selected from hydroxy, amino, C_{1-4}alkyl, aminoC_{1-4}alkyl, N-C_{1-4}alkylaminoC_{1-4}alkyl, N, N-di(C_{1-4}alkyl)aminoC_{1-4}alkyl, carboxy, -$CONR^{25}R^{26}$ and -$NR^{25}COR^{27}$ (wherein $\mathbf{R^{25}}$ **and** $\mathbf{R^{26}}$, which may be the same or different, are hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl and $\mathbf{R^{27}}$ is C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))];

2) R^{22} (wherein R^{22} is as hereinabove defined);

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

4) $R^{12}Y^7C_{1-4}$alkyl- (wherein R^{12} is as hereinabove defined and Y^7 is -C(O)-, -NR^{23}C(O)-, -NR^{23}C(O)C_{1-4}alkyl-, -C(O)NR^{23}- or -C(O)$NR^{23}C_{1-4}$alkyl- (wherein R^{23} is as hereinabove defined))];

and $\mathbf{R^9}$ is hydrogen or C_{1-3}alkyl;

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

In another aspect, the invention relates to a compound of the formula (I) as hereinabove defined or to a pharmaceutically-acceptable salt thereof.

In this specification the generic term "alkyl" includes both straight-chain and branched-chain alkyl groups. However references to individual alkyl groups such as "propyl" are specific for the straight-chain version only and references to individual branched-chain alkyl groups such as "isopropyl" are specific for the branched-chain version only. An analogous convention applies to other generic terms.

R^{13} is an amino acid side chain. This includes amino acid side chains from natural and non-natural amino acids and includes the possibility of R^{13} joining to the NH group so as to form a ring as in the amino acid proline. It includes α-amino acids β-amino acids and γ-amino acids. In addition, the amino acids may be L-isomers or D-isomers, but preferably L-isomers. Preferred amino acids include glycine, alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tryptophan, serine, threonine, cysteine, tyrosine, asparaginine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, β-alanine and ornithine. More preferred amino acids include glutamic acid, serine, threonine, arginine, glycine, alanine, β-alanine and lysine. Especially preferred amino acids include glutamic acid, serine, threonine, arginine, alanine and β-alanine. Specific values for R^{12} include hydrogen,

C_{1-4}alkyl, C_{1-4}alkylthioC_{1-4}alkyl, hydroxyC_{1-4}alkyl, thioC_{1-4}alkyl, phenylC_{1-4}alkyl (optionally substituted by hydroxy), guanidinoC_{1-4}alkyl, carboxyC_{1-4}alkyl, carbamoylC_{1-4}alkyl, aminoC_{1-4}alkyl and imidazolyl C_{1-4}alkyl and R^{12} forming a pyrrolidinyl ring with the NH group. Preferred values for R^{13} include hydrogen, C_{1-4}alkyl, C_{1-4}alkylthioC_{1-4}alkyl, hydroxyC_{1-4}alkyl, thioC_{1-4}alkyl, guanidinoC_{1-4}alkyl, carboxyC_{1-4}alkyl, carbamoylC_{1-4}alkyl and aminoC_{1-4}alkyl.

In this specification, the term heteroaryl is used to describe fully saturated heterocyclic rings. Examples of 5- or 6-membered heteroaryl rings include pyrrolyl, imidazolyl, pyrazolyl, isothiazolyl, isoxazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazolyl, tetrazolyl, thiazolyl, thiadiazolyl, thienyl, furyl and oxazolyl.

It is to be understood that, insofar as certain of the compounds of Formula I defined above may exist in optically active or racemic forms by virtue of one or more asymmetric carbon atoms, the invention includes in its definition any such optically active or racemic form which possesses vascular damaging activity. The synthesis of optically active forms may be carried out by standard techniques of organic chemistry well known in the art, for example by synthesis from optically active starting materials or by resolution of a racemic form. Similarly, the above-mentioned activity may be evaluated using the standard laboratory techniques referred to hereinafter.

Suitable values for the generic radicals referred to above include those set out below. Within the present invention it is to be understood that a compound of the formula I or a salt thereof may exhibit the phenomenon of tautomerism and that the formulae drawings within this specification can represent only one of the possible tautomeric forms. It is to be understood that the invention encompasses any tautomeric form which has vascular damaging activity and is not to be limited merely to any one tautomeric form utilised within the formulae drawings.

It is also to be understood that certain compounds of the formula I and salts thereof can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms which have vascular damaging activity.

The present invention relates to the compounds of formula I as hereinbefore defined as well as to the salts thereof. Salts for use in pharmaceutical compositions will be pharmaceutically acceptable salts, but other salts may be useful in the production of the

compounds of formula I and their pharmaceutically acceptable salts. Pharmaceutically acceptable salts of the invention may, for example, include acid addition salts of the compounds of formula I as hereinbefore defined which are sufficiently basic to form such salts. Such acid addition salts include for example salts with inorganic or organic acids affording pharmaceutically acceptable anions such as with hydrogen halides (especially hydrochloric or hydrobromic acid of which hydrochloric acid is particularly preferred) or with sulphuric or phosphoric acid, or with trifluoroacetic, citric or maleic acid. Suitable salts include hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates and tartrates. In addition where the compounds of formula I are sufficiently acidic, pharmaceutically acceptable salts may be formed with an inorganic or organic base which affords a pharmaceutically acceptable cation. Such salts with inorganic or organic bases include for example an alkali metal salt, such as a sodium or potassium salt, an alkaline earth metal salt such as a calcium or magnesium salt, an ammonium salt or for example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

Various forms of prodrugs are known in the art. For examples of such prodrug derivatives, see:

a) Design of Prodrugs, edited by H. Bundgaard, (Elsevier, 1985) and Methods in Enzymology, Vol. 42, p. 309-396, edited by K. Widder, *et al.* (Academic Press, 1985);

b) A Textbook of Drug Design and Development, edited by Krogsgaard-Larsen and H. Bundgaard, Chapter 5 "Design and Application of Prodrugs", by H. Bundgaard p. 113-191 (1991);

c) H. Bundgaard, Advanced Drug Delivery Reviews, 8, 1-38 (1992);

d) H. Bundgaard, *et al.*, Journal of Pharmaceutical Sciences, 77, 285 (1988); and

e) N. Kakeya, *et al.*, Chem. Pharm. Bull., 32, 692 (1984).

Examples of such pro-drugs may be used to form in-vivo-cleavable esters of a compound of the Formula I. An in-vivo-cleavable ester of a compound of the Formula I containing a carboxy group is, for example, a pharmaceutically-acceptable ester which is cleaved in the human or animal body to produce the parent acid. Suitable pharmaceutically-acceptable esters for carboxy include C_{1-6}alkoxymethyl esters, for example

methoxymethyl; C_{1-6}alkanoyloxymethyl esters, for example pivaloyloxymethyl; phthalidyl esters; C_{3-8}cycloalkoxycarbonyloxy C_{1-6}alkyl esters, for example 1-cyclohexylcarbonyloxyethyl; 1,3-dioxolan-2-ylmethyl esters, for example 5-methyl-1,3-dioxolan-2-ylmethyl; and C_{1-6}alkoxycarbonyloxyethyl esters, for example 1-methoxycarbonyloxyethyl; and may be formed at any carboxy group in the compounds of this invention.

Suitable values for R^1, R^2, R^3 R^4, R^5, R^6, R^7, R^8, R^9, R^{10} or R^{16} or for various substituents on D, R^{12}, R^{14} or R^{15} include:

for halogeno	fluoro, chloro, bromo and iodo;
for C_{1-3}alkyl:	methyl, ethyl, propyl, and isopropyl;
for C_{1-4}alkyl:	methyl, ethyl, propyl, isopropyl and tert-butyl;
for N-C_{1-4}alkylamino:	methylamino, ethylamino, propylamino, isopropylamino and butylamino;
for N,N-di-(C_{1-4}alkyl)amino:	dimethylamino, diethylamino, N-ethyl-N-methylamino and diisopropylamino;
for C_{2-4}alkanoyl:	acetyl and propionyl;
for C_{2-4}alkanoylamino:	acetamido and propionamido;
for C_{1-4}alkoxy:	methoxy and ethoxy;
for C_{1-3}alkoxyC_{2-3}alkyl:	methoxyethyl and ethoxypropyl;
for cyanoC_{1-4}alkyl:	cyanomethyl and 2-cyanoethyl;
for N-C_{1-4}alkylcarbamoyl:	N-methylcarbamoyl, N-ethylcarbamoyl and N-propylcarbamoyl;
for N,N-di-(C_{1-4}alkyl)carbamoyl:	N,N-dimethylcarbamoyl, N-ethyl-N-methylcarbamoyl and N,N-diethylcarbamoyl;
for C_{1-4}alkylsulphonylalkyl:	methylsulphonylmethyl and ethylsulphonylmethyl;
for hydroxyC_{1-4}alkyl:	hydroxymethyl, 2-hydroxyethyl, 1-hydroxyethyl and 3-hydroxypropyl as appropriate;
for hydroxyC_{2-3}alkyl:	2-hydroxyethyl, 1-hydroxyethyl and 3-hydroxypropyl as appropriate;
for C_{1-4}alkoxyC_{1-4}alkyl:	methoxymethyl, ethoxymethyl, 1-methoxyethyl, 2-methoxyethyl, 2-ethoxyethyl and 3-methoxypropyl as appropriate;

for aminoC_{1-4}alkyl or	aminomethyl, 2-aminoethyl, 1-aminoethyl and 3-aminopropyl as appropriate;
for aminoC_{2-3}alkyl or	2-aminoethyl, 1-aminoethyl and 3-aminopropyl as appropriate;
for N-C_{1-4}alkylaminoC_{1-4}alkyl:	methylaminomethyl, ethylaminomethyl, 1-methylaminoethyl, 2-methylaminoethyl, 2-ethylaminoethyl and 3-methylaminopropyl as appropriate;
for N,N-di-(C_{1-4}alkyl)aminoC_{1-4}alkyl:	dimethylaminomethyl, diethylaminomethyl, 1-dimethylaminoethyl, 2-dimethylaminoethyl and 3-dimethylaminopropyl as appropriate:
for carboxyC_{1-4}alkyl:	carboxymethyl, 1-carboxyethyl, 2-carboxyethyl, 3-carboxypropyl and 4-carboxybutyl;
for C_{1-4}alkoxycarbonylC_{1-4}alkyl:	methoxycarbonylmethyl, ethoxycarbonylmethyl, tert-butoxycarbonylmethyl, 1-methoxycarbonylethyl, 1-ethoxycarbonylethyl, 2-methoxycarbonylethyl, 2-ethoxycarbonylethyl, 3-methoxycarbonylpropyl and 3-ethoxycarbonylpropyl;
for C_{1-4}alkoxycarbonylamino:	methoxycarbonylamino and ethoxycarbonylamino;
for carbamoylC_{1-4}alkyl:	carbamoylmethyl, 1-carbamoylethyl, 2-carbamoylethyl and 3-carbamoylpropyl;

Examples of 5- or 6-membered saturated heterocyclic ring ring systems include pyrrolidinyl, imidazolidinyl, pyrazolidinyl, piperidyl, piperazinyl and morpholinyl.

Preferably at least 2 of R^1, R^2, and R^3 are methoxy.

Preferably R^1 , R^2, and R^3 are all C_{1-4}alkoxy.

Most preferably R^1, R^2, and R^3 are all methoxy.

Preferably A is ethylene, propylene, benzylene or phenylene. More preferably A is ethylene or phenylene.

More preferably A is phenylene.

Most preferably A is 1,4-phenylene.

Preferably X^1 is -CO-, -CON(R^{10})-, -N(R^{10}) -, -N(R^{10})CO- or -OC(O)N(R^{10})-.

Most preferably X^1 is -CO- or -N(R^{10})CO-.

Preferably R^{10} is hydrogen or methyl. Most preferably R^{10} is hydrogen.

Preferably Y^1 is propylene or ethylene.

More preferably Y^1 is ethylene.

Preferably B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} wherein R^{12} is as hereinabove defined.

Yet more preferably B is phosphoryloxy or -R^{12}. Most preferably, B is -R^{12}.

Preferably R^{12} is a 5 or 6 membered saturated heterocyclic ring containing 1 or 2 ring heteratoms sclected 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:

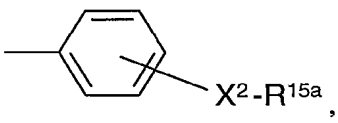
A is ethylene or phenylene;

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

R^8 is a group $-Y^3R^{17}$ (wherein Y^3 is -C(O)-, -C(O)O- or -C(O)NH-;

and **R^{17}** is selected from one of the following 4 groups:

1) hydrogen, C_{1-4}alkyl, phenyl or C_{1-4}alkylY^4C_{1-4}alkyl (wherein Y^4 is -NHCO- or -CONH-); [which alkyl, alkylY^4alkyl or phenyl group is optionally substituted by 1 or 2 substituents selected from:

halogeno, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, C_{1-4}alkoxy, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, phosphoryloxy, R^{12a} (wherein R^{12a} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl), $-Y^5-R^{20}$ [wherein Y^5 is -NHCO-; and R^{20} is C_{1-4}alkyl or R^{22a} (wherein R^{22a} is imidazolyl, pyridyl, pyrimidyl, thiazolyl or pyrazinyl, each of which is optionally substituted by C_{1-4}alkyl)];

2) R^{22a} (wherein R^{22a} is as hereinabove defined);

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

4) $R^{12a}Y^7C_{1-4}$alkyl- (wherein R^{12a} is as hereinabove defined and Y^7 is Y^7 is -NHC(O)- or -CONH-)];

and $\mathbf{R^9}$ is hydrogen;

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

Another preferred class of compound is of the formula (I) wherein:

R^1 , R^2, and R^3 are all methoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, methoxy and methyl;

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

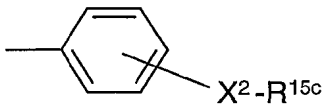
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, -CON(R^{10})-, -N(R^{10}) -, -N(R^{10})CO- or -OC(O)N(R^{10})-;

B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the -X^2-R^{15c} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 0, 1 and 2; and

R^{15c} is morpholinyl, piperazinyl or piperidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15d}, wherein:

a is 2 or 3;

b is 0 or 1; and

Y^2 is a single direct bond, -C(O)- or -NHC(O)-; and

R^{15d} is morpholinyl, piperazinyl or piperidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino, N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy; and

R^8 is a group -Y^3R^{17} (wherein Y^3 is -C(O)- or -C(O)O-;

and **R^{17}** is selected from one of the following 4 groups:

1) C_{1-4}alkyl [which alkyl, group is optionally substituted by 1 or 2 substituents selected from: fluoro, chloro and bromo;

2) R^{22b} (wherein R^{22b} is imidazolyl, pyridyl, pyrimidyl, thiazolyl or pyrazinyl, each of which is optionally substituted by C_{1-4}alkyl);

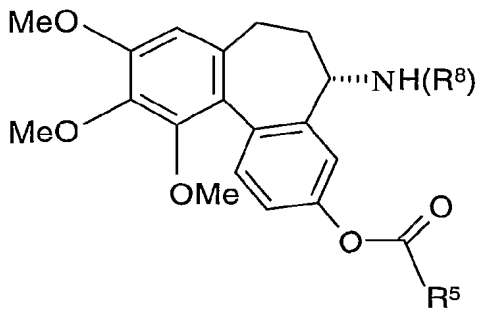
3) R^{22b} -C_{1-4}alkyl- (wherein R^{22b} is as hereinabove defined); or

4) $R^{12b}Y^7C_{1-4}$alkyl- (wherein R^{12b} is morpholinyl, piperidinyl or piperazinyl each of which is optionally substituted by methyl, ethyl, acetyl, propionyl, carbamoyl or 2-hydroxyethyl; and Y^7 is -NHC(O)- or -CONH-)];

and **R^9** is hydrogen;

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

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

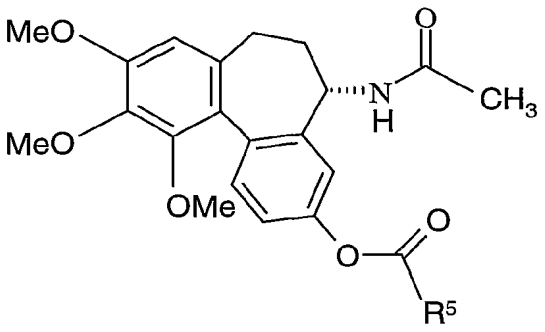


(II)

wherein R^5 and R^8 are as hereinabove defined;

or a pharmacetically-acceptable salt, solvate or pro-drug thereof.

Another preferred class of compounds is that of the formula (III) wherein:



(III)

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

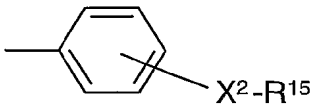
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, -CONH-, -NH-, -NHCO- or -OC(O)NH-;

B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the -X^2-R^{15} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 0, 1 and 2; and

R^{15} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted as immediately hereinabove defined for R^{12}, and substituted by at least 1 substituent selected from C_{2-4}alkanyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15}, wherein:

a is 2 or 3;

b is 0, 1, or 2; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino, N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Another preferred class of compounds is that of the formula (III) wherein:

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

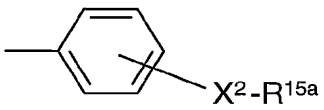
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, -NHCO-;

B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the -X^2-R^{15a} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 1 and 2; and

R^{15a} is as hereinabove defined;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15b}, wherein:

a is 2 or 3;

b is 0; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; and

R^{15b} is as hereinabove defined; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, hydroxy and phosphoryloxy;

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

Another preferred class of compounds is that of the formula (III) wherein:

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

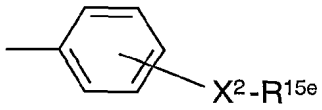
A is phenylene;

X^1 is -CO-, -NHCO-;

Y^1 is methylene or ethylene;

B is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group;

2) of the formula



wherein:

the -X^2-R^{15e} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 1; and

R^{15e} is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15f}, wherein:

a is 2 or 3;

b is 0; and

Y^2 is -C(O)-;

R^{15f} is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group; or

4) 2-[N,N-di(C_{1-4}alkyl)carbamoyl]ethyl- or 3-[N,N-di(C_{1-4}alkyl)carbamoyl]propyl-, wherein the C_{1-4}alkyl group is optionally substituted by 1 hydroxy group;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Yet another preferred class of compounds is that of the formula (III) wherein:

R^5 is 2-[N,N-di(C_{1-4}alkyl)carbamoyl]ethyl- or 3-[N,N-di(C_{1-4}alkyl)carbamoyl]propyl-, wherein the C_{1-4}alkyl group is optionally substituted by 1 hydroxy group;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Particular compounds of the present invention include:

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 5-(4-acetylpiperazin-l-yl)-5-oxopentanoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c}cyclohepten-3-yl 4-(4-acetylpiperazin-1-yl)-4-oxobutanoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-acetylpiperazin-1-ylmethyl)benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[3-(4-methylpiperazin-l-yl)propionylamino]benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-carbamoylpiperazin-1-ylmethyl)benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl N-acetylpiperidin-1-ylcarboxylate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-[N,N-di-(2-hydroxyethyl)carbamoyl]propanoate; and

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[N,N-di(2-hydroxyethyl)carbamoyl]butanoate;

and pharmaceutically-acceptable salts, solvates or pro-drugs thereof.

Synthesis of Compounds of the Formula I

Compounds of Formula I may be prepared by a number of processes as generally described herein below and more specifically in the Examples hereinafter. Processes for the preparation of novel compounds of formula I, are provided as a further feature of the invention and are as described hereinafter. Necessary starting materials may be obtained by standard procedures of organic chemistry. The preparation of such starting materials is described within the accompanying non-limiting Examples. Alternatively necessary starting materials are obtainable by analogous procedures to those illustrated which are within the ordinary skill of an organic chemist.

Thus according to another aspect of the invention, a compound of the formula (I) may be formed by deprotecting a compound of the formula (I) wherein at least 1 functional group is protected. For example, amino, hydroxy, carboxy or phosphoryloxy groups may be protected during the reaction sequence used to prepare a compound of the formula (I).

Protecting groups may in general be chosen from any of the groups described in the literature or known to the skilled chemist as appropriate for the protection of the group in question, and may be introduced by conventional methods.

Protecting groups may be removed by any convenient method as described in the literature or known to the skilled chemist as appropriate for the removal of the protecting group in question, such methods being chosen so as to effect removal of the protecting group with minimum disturbance of groups elsewhere in the molecule.

A suitable protecting group for a hydroxy group is, for example, an arylmethyl group (especially benzyl), a tri-(1-4C)alkylsilyl group (especially trimethylsilyl or tert-butyldimethylsilyl), an aryldi-(1-4C)alkylsilyl group (especially dimethylphenylsilyl), a diaryl-(1-4C)alkylsilyl group (especially tert-butyldiphenylsilyl), a (1-4C)alkyl group (especially methyl), a (2-4C)alkenyl group (especially allyl), a (1-4C)alkoxymethyl group (especially methoxymethyl) or a tetrahydropyranyl group (especially tetrahydroyran-2-yl). The deprotection conditions for the above protecting groups will necessarily vary with the choice of protecting group. Thus, for example, an arylmethyl group such as a benzyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-charcoal. Alternatively a trialkylsilyl or an aryldialkylsilyl group such as a tert-butyldimethylsilyl or a dimethylphenylsilyl group may be removed, for example, by treatment with a suitable acid such as hydrochloric, sulphuric, phosphoric or trifluoroacetic acid, or with an alkali metal or ammonium fluoride such as sodium fluoride or, preferably, tetrabutylammonium fluoride. Alternatively an alkyl group may be removed, for example, by treatment with an alkali metal (1-4C)alkylsulphide such as sodium thioethoxide or, for example, by treatment with an alkali metal diarylphosphide such as lithium diphenylphosphide or, for example, by treatment with a boron or aluminium trihalide such as boron tribromide. Alternatively a (1-4C)alkoxymethyl group or tetrahydropyranyl group may be removed, for example, by treatment with a suitable acid such as hydrochloric or trifluoroacetic acid.

Alternatively a suitable protecting group for a hydroxy group is, for example, an acyl group, for example a (2-4C)alkanoyl group (especially acetyl) or an aroyl group (especially benzoyl). The deprotection conditions for the above protecting groups will necessarily vary with the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl or an aroyl group may be removed, for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide.

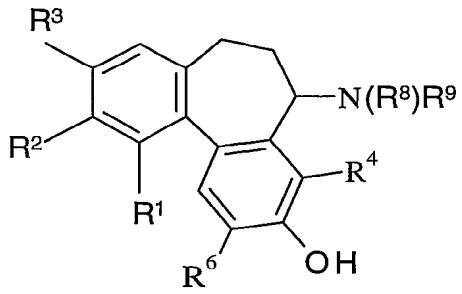
A suitable protecting group for an amino, imino or alkylamino group is, for example, an acyl group, for example a (2-4C)alkanoyl group (especially acetyl), a (1-4C)alkoxycarbonyl group (especially methoxycarbonyl, ethoxycarbonyl or tert-butoxycarbonyl), an arylmethoxycarbonyl group (especially benzyloxycarbonyl) or an aroyl group (especially benzoyl). The deprotection conditions for the above protecting groups necessarily vary with the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl, alkoxycarbonyl or aroyl group may be removed for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide. Alternatively an acyl group such as a tert-butoxycarbonyl group may be removed, for example, by treatment with a suitable acid such as hydrochloric, sulphuric or phosphoric acid or trifluoroacetic acid, and an arylmethoxycarbonyl group such as a benzyloxycarbonyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-charcoal.

A suitable protecting group for a carboxy group is, for example, an esterifying group, for example a (1-4C)alkyl group (especially methyl or ethyl) which may be removed, for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide; or, for example, a tert-butyl group which may be removed, for example, by treatment with a suitable acid such as hydrochloric, sulphuric or phosphoric acid or trifluoroacetic acid.

In the following process description (the symbols R^1-R^7, A, B, D, R^a, R^b, a and b are to be understood to represent those groups described above in relation to formulae (I), (II) and (III) unless otherwise stated.

A compound of the formula (I), or a compound of the formula (I) wherein at least 1 functional group is protected, may be prepared using one of the following processes:

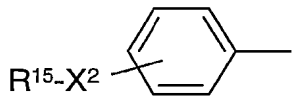
(a) reacting a compound of the formula (X):



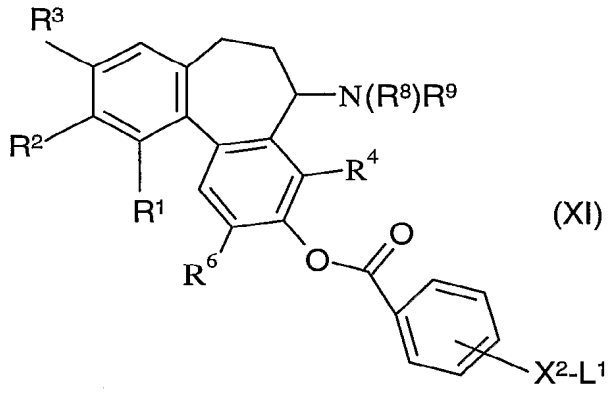
(X)

with a compound of the formula R^5-COOH or an activated derivative thereof;

(b) when R^5 is of the formula:



reacting a compound of the formula (XI):



(XI)

with R^{15} (wherein L^1 is a leaving group) ;

(c) introducing substituents onto a ring nitrogen atom in R^{12} or R^{15};

(d) converting one compound of the formula (I) into another compound of the formula (I);

(e) when a phosphoryloxy group is desired, reacting the corresponding hydroxy compound with a phosphoramidite;

wherein any functional groups are optionally protected.

and thereafter if necessary:

i) converting a compound of formula (I) into another compound of formula (I);

ii) removing any protecting groups;

iii) forming a pharmaceutically acceptable salt, solvate or pro-drug thereof.

The reaction between a compound of the formula (X) and a compound of the formula R^5-COOH or an activated derivative thereof is performed under standard coupling conditions. For example, in the presence of a coupling agent such as dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, and optionally a base, for example an organic base such as triethylamine or DMAP. The reaction is conveniently carried out in a solvent such as an aprotic solvent, for example dimethylformamide, or in a chlorinated solvent, for example trichloromethane or dichloromethane, and at a temperature in the range of about -30°C to about 60°C. Conveniently at or near ambient temperature.

When X^2 is -(CH_2)r, the reaction between a compound of the formula (XI) and R^{15} is conveniently carried out in an inert organic solvent such as acetonitrile, in a temperature range of 0°C to 60°C, normally at ambient temperature. Suitable leaving groups (L^1) include halogeno, mesyloxy and tosyloxy. Preferably halogeno, and particularly chloro or iodo.

When X^2 is -CO-, L^1 is usually chloro and the reaction is normally carried out in a chlorinated solvent such as dichloromethane. The reaction is carried out in the presence of a base such as triethylamine and in a temperature range of 0 to 60°C, normally about ambient temperature.

Substituents, such as C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl and alkylated carbamoyl groups, can be in introduced onto a ring nitrogen atom in R^{12} or R^{15}, using standard conditions known in the art for alkylation and acylation of an amino group. Alkylations are normally carried out by reacting a ring-nitrogen containing R^{12} or R^{15} with the appropriate alkylating agent, such as an alkyl halide, an alkyl toluenesulphonate, an alkyl methanesulphonate or an alkyl triflate. The alkylation reaction can be carried out in the presence of a base for example an inorganic base such as a carbonate e.g. caesium or potassium carbonate, a hydride such as sodium hydride or an alkoxide such as potassium tert-butoxide in a suitable solvent such as an aprotic solvent e.g. dimethylformamide or an ether solvent such as tetrahydrofuran at a temperature of around -10°C to 80°C.

Acylation of a ring nitrogen in R^{12} or R^{15} is carried out by reacting the saturated heterocyclic ring with an acylating agent, for example, an acyl halide or anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for example, a solvent such as a hydrocarbon solvent e.g. dichloromethane, at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

A carbamoyl group can be introduced by reacting the saturated heterocyclic ring with a tri(alkyl)silyl isocyanate in an inert organic solvent such as dichloromethane.

A compound of formula I may also be prepared from another compound of formula I by chemical modification. Examples of such chemical modifications include standard alkylation, arylation, heteroarylation, acylation, sulphonylation, phosphorylation, aromatic halogenation and coupling reactions. These reactions may be used to add new substituents or to modify existing substituents. Alternatively, existing substituents in compounds of formula I may be modified by, for example, oxidation, reduction, elimination, hydrolysis or other cleavage reaction to yield other compounds of formula I. For example a substituent can be introduced onto a ring nitrogen atom in R^{12} using similar processes to those described above, for the alkylation or acylation of a ring nitrogen.

In another general example an alkoxy group may be cleaved to the corresponding hydroxy group by reaction with boron tribromide, in a solvent such as a chlorinated solvent e.g. dichloromethane, at a low temperature e.g. around -78°C.

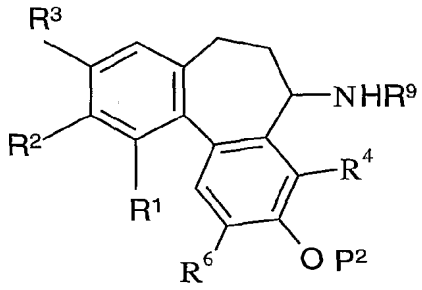
A amino group can be alkylated or acylated using similar reaction conditions to those described above for alkylation or acylation of a ring nitrogen atom in R^{12} or R^{15}.

A compound containing a hydroxy group can be converted into the corresponding phosphoryloxy compound by treatment with for example di-tert-butyl diisopropylphoramidite or di-tert-butyl diethylphosphoramidite, in the presence of a suitable catalyst for example tetrazole. A solvent, such as an ether solvent, for example tetrahydrofuran can be used at a temperature in the range of -40°C to 40°C, conveniently at or near ambient temperature, followed by treatment with an oxidising agent, such as 3-chloroperoxy benzoic acid. The reaction is carried out at a temperature in the range -78°C to 40°C, preferably -40°C to 10°C. The resulting intermediate phosphate triester is treated with an acid for example trifluoroacetic acid in a solvent such as a chlorinated solvent e.g. dichloromethane at a temperature in the range -30°C to 40°C conveniently at or near 0°C to give the phosphoryloxy compound.

Synthesis of Intermediates

A compound of the formula (X) may be known in the art or may be prepared from by

(f) reacting a compound of the formula (XII):



(XII)

wherein P^2 is an hydroxy-protecting group, with a compound of the formula L^2-R^8 , wherein L^2 is a leaving group;

The reaction between a compound of the formula (XII) and a compound of the formula L^2-R^8 is conveniently performed under standard acylation or sulphonylation conditions. L^1 is usually halogeno, for example chloro or bromo, hydroxy, mesyloxy or tosyloxy or an 'activated' hydroxy group. The precise conditions depending largely upon the nature of R^8.

For example, when Y^3 is -CO-, L^2 may be hydroxy and the reaction is normally carried out in the presence of coupling agent such as dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide. Optionally, a base may be used, for example an organic base such as triethylamine. Suitable solvents are usually aprotic solvents, for example dimethylformamide, or chlorinated solvents, for example trichloromethane or dichloromethane. The temperature is usually in the range of about -30°C to about 60°C, conveniently at or near ambient temperature.

When Y^3 is -C(O)O-, L^2 is usually an 'activated' hydroxy group. That is a group which acts as a leaving group in the same way as hydroxy, but is more labile. It can be formed in situ. An example, of an activated hydroxy group is 4-nitrophenoxy, in which case the compound R^8-L^2 can be formed by reacting a hydroxy group (R^{17}-OH) with 4-nitrophenylchloroformate. The reaction is usually carried out in an organic solvent such as dichloromethane, acetonitrile or tetrahydrofuran, in a temperature range of about -20°C to the reflux temperature of the solvent. In addition an organic base such as triethylamine or N-methylmorpholine is normally present. Alternatively, a compound of the formula (XII) can be reacted with 4-nitrophenylchloroformate and the resulting intermediate reacted with R^{17}-OH under similar conditions to those described above for the reaction of a compound of the formula (XII) with a compound of the formula R^8-L^2 wherein L^2 is 4-nitrophenoxy.

When Y^3 is -CON(R^{18})-, L^2 is preferably halogeno, particularly chloro. Alternatively when -A- is -CONH-, a compound of the formula (XII) can be reacted with an isocyanate of

the formula $C\equiv N\text{-}R^{17}$. These reactions are conveniently carried out in the presence of a base, particularly an organic base, such as triethylamine, pyridine or N-methylmorpholine, triethylamine, pyridine or N-methylmorpholine in a solvent such as an ether solvent for example tetrahydrofuran or in a chlorinated solvent for example dichloromethane at a temperature in the range from about -20°C to the reflux temperature of the solvent. Alternatively, a compound of the formula (XII) can be reacted with 4-nitrophenylchloroformate and the resulting intermediate reacted with $R^{17}\text{-}NH_2$ under similar conditions to those described above for the reaction of a compound of the formula (XII) with a compound of the formula $R^8\text{-}L^2$ wherein L^2 is 4-nitrophenoxy.

When $-X^1-$ is of the formula $-SO_2N(R^8)-$, L^2 is preferably halogeno, for example chloro. The reaction is conveniently carried out in the presence of a base such as dimethylaniline, in a chlorinated solvent such as trichloromethane and at a temperature in the range from about -20°C to about 60°C. More preferably in pyridine, at a temperature in the range from about -20°C to about 60°C. A compound of the formula (XI) may be prepared by reacting a compound of the formula (X) with a compound of $L^1\text{-}X^2\text{-phenyl-COOH}$ in which L^1 is protected or a precursor of L^1 is used, using similar conditions to those described for the formation of a compound of the formula (I) from a compound of the formula (X).

L^1 can be deprotected or the precursor converted to L^1 following the coupling with the compound of the formula (X).

A compound of the formula (XII) can be formed from a compound of the formula (X) or (XI) wherein R^8 is hydrogen, using similar conditions to those described above for the formation of a compound of the formula (I).

Compounds of the formula $R^5\text{-COOH}$ may be known in the art or prepared by methods known in the art or analogous to those illustrated in the specific examples. The following description gives some general processes for preparing some compounds of the formula $R^5\text{-COOH}$.

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

formula $-N(R^{10})SO^2-$ or $SO^2N(R^{10})-$ a compound of the formula R^5-COOH can be formed by reacting the appropriate amine and sulphonyl chloride under conditions known for the formation of a sulphonamide. Likewise a compound of the formula R^5-COOH wherein X^1 is -C(O)O- can be formed by reacting together the appropriate carboxylic acid and alcohol and when X^1 is $-N(R^{10})C(O)O-$, by reacting together the appropriate amine and ROC(O)OR compound.

When R^5 is of the formula R^{15}-X^2-phenyl, a compound of the formula R^5-COOH can be formed by reacting together the appropriate L^1-$(CH_2)_r$- or L^1-CO-substituted benzoic acid, wherein the carboxylic group in the benzoic acid is protected during the course of the reaction, under similar conditions to that described for the reaction between a compound of the formula (XI) with R^{15}.

When R^5 is of the formula $-(CH_2)a-Y^2-(CH_2)b$ and $-R^{15}$ a is 2 or 3 and b is 0, a compound of the formula R^5-COOH can be formed by reacting R^{15} with succinic anhydride or glutaric anhydride, as appropriate. The reaction is normally carried out in an inert organic solvent such as dichloromethane, in a temperature range of 0° to 60°C, usually around ambient temperature.

When R^5 is N,N-di-(C_{1-4}alkyl)carbamoylC_{1-4}alkyl and C_{1-4}alkyl is ethyl or propyl, a compound of the formula R^5-COOH can be formed by reacting the $HN(C_{1-4}alkyl)_2$ compound with succinic anhydride or glutaric anhydride as appropriate. The reaction is normally carried out in an inert organic solvent such as dichloromethane, in a temperature range of 0° to 60°C, usually around ambient temperature.

Acid addition salts of the compounds of formula I are prepared in a conventional manner by treating a solution or suspension of the free base I with about one equivalent of a pharmaceutically acceptable acid. Salts of compounds of formula I derived from inorganic or organic bases are prepared in a conventional manner by treating a solution or suspension of the free acid I with about one equivalent of a pharmaceutically acceptable organic or inorganic base. Alternatively both acid addition salts and salts derived from bases may be prepared by treatment of the parent compound with the appropriate ion-exchange resin in a standard fashion. Conventional concentration and recrystallistion techniques are employed in isolating the salts.

Compounds according to the invention are able to destroy vasculature that has been newly formed such as tumour vasculature while leaving unaffected normal, mature

vasculature. The identification of compounds which selectively, and preferably potently, damage newly-formed vasculature is desirable and is the subject of the present invention. The ability of the compounds to act in this way may be assessed, for example, using one or more of the procedures set out below:

(a) Activity against tumour vasculature measured by radioactive tracer

This assay demonstrates the ability of compounds to damage selectively tumour vasculature.

Subcutaneous CaNT tumours were initiated by injecting 0.05ml of a crude tumour cell suspension, approximately 10^6 cells, under the skin overlying the rear dorsum of 12-16 week-old mice. The animals were selected for treatment after approximately 3-4 weeks, when their tumours reached a geometric mean diameter of 5.5-6.5 mm. Compounds were dissolved in sterile saline and injected intraperitoneally in a volume of 0.1 ml per 10g body weight. Tumour perfusion was measured 6 hours after intraperitoneal administration in tumour, kidney, liver, skin, muscle, gut and brain by the ^{86}RbCl extraction technique (Sapirstein, Amer. Jnl. Physiol., 1958, 193, 161-168). Tissue radioactivity measured 1 minute after an intravenous injection of ^{86}RbCl was used to calculate relative blood flow as a proportion of cardiac output (Hill and Denekamp, Brit. Jnl. Radiol., 1982, 55, 905-913). Five animals were used in control and treated groups. Results were expressed as a percentage of the blood flow in the corresponding tissues in vehicle treated animals.

(b) Activity against tumour vasculature measured by fluorescent dye

This assay demonstrates the ability of compounds to damage tumour vasculature.

Tumour functional vascular volume in CaNT tumour-bearing mice was measured using the fluorescent dye Hoechst 33342 according to the method of Smith et al (Brit. Jnl. Cancer 1988, 57, 247-253). Five animals were used in control and treated groups. The fluorescent dye was dissolved in saline at 6.25mg/ml and injected intravenously at 10mg/kg 24 hours after intraperitoneal drug treatment. One minute later, animals were killed and tumours excised and frozen; 10μm sections were cut at 3 different levels and observed under UV illumination using an Olympus microscope equipped with epifluorescence. Blood vessels were identified by their fluorescent outlines and vascular volume was quantified using a point scoring system based on that described by Chalkley, (Jnl. Natl. Cancer Inst., 1943, 4, 47-53). All estimates were based on counting a minimum of 100 fields from sections cut at the 3 different levels.

The ability of the compounds to bind to preparations of mammalian tubulin can be evaluated by a number of methods available in the literature, for example by following temperature initiated tubulin polymerisation by turbidity in the absence and presence of the compound (for example O.Boye *et al* Med. Chem. Res., 1991, 1, 142-150).

The activity of *N*-[3-amino-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl]acetamide, (V. Fernholz Justus Liebigs Ann., 1950, 568, 63-72), against tumour vasculature was measured by the fluorescent dye method described above. This compound decreased perfused vascular volume by 88% relative to control when dosed at 50mg/kg intraperitoneally. The IC_{50} of this compound in a tubulin polymerisation assay was 58 micromolar (O.Boye *et al* Med. Chem. Res., 1991, 1, 142-150).

(c) HUVEC detachment assay

This assay examined the effects of compounds on the adherence of HUVECs to tissue culture plasticware.

HUVECs were plated in 0.2% gelatin-coated 12 well tissue culture plates at a concentration of $3x10^4$ cells per well in 1ml TCS medium. After 24 hours, when the cells were at ~30% confluency, the cells were dosed with compound for 40 minutes at 37°C, 5% CO_2. After this incubation the medium containing drug was pipetted off, and the cells were then gently washed in 2mls of HBSS (Hanks' Balanced Salt Solution purchased from Life Technologies Ltd, Paisley UK; Catalogue # 24020-083) to remove any detached cells. The washing solution was then removed, and the adherent cells remaining were trypsinised using 300µl of 1x Trypsin-EDTA solution (Life Technologies Ltd, Paisley, UK; Catalogue # 43500-019) at ambient temperature for 2 minutes. The trypsinised cells were then made up to 1ml with TCS Biologicals medium, then centrifuged at 2000rpm for 2 minutes. The cell pellet was then resuspended in a volume of 50µl of TCS Biologicals medium. Total cell counts were obtained by counting the cells on a haemocytometer. The amount of cell detachment was calculated by comparing the number of cells remaining attached following treatment with the number in undosed control wells.

(d) Hras5 necrosis model

NIH 3T3 fibroblasts transfected with Harvey ras, clone 5, (Hras5 cells) were kept in continual passage in Dulbecco's modifed Eagles medium (DMEM) containing 10% foetal bovine serum (FBS) and 1% glutamine, at 37°C in a humidified incubator gassed with 7.5%

carbon dioxide and 92.5% oxygen. Cells were implanted subcutaneously into the left flank of male nude mice (8-10weeks of age) at an inoculum of 2 x 10^5 cells/mouse. Tumours were measured using calipers and randomised into groups of 2-4 mice between days 9-14 after implant. Mice were dosed with compounds, either intravenously or intraperitoneally, once on day of randomisation and culled 24 hours after dosing. Compounds were dissolved in 20% hydroxypropyl beta cyclodextrin in physiological saline at pH 7 and dosed in a volume of 0.1ml per 10g body weight. Tumours were excised, weighed and placed in buffered formalin. Area of necrosis in individual tumours was assessed from a haematoxylin/eosin stained-slide by a pathologist and scored from 0, meaning no significant change, to 10, meaning 91-100% necrosis. The activity of examples 5 and 7 (described hereinafter) against tumour vasculature was measured by the fluorescent dye method described hereinabove. Example 1 scored 6.6 at 25mg/kg.

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises a compound of the formula I as defined hereinbefore or a pharmaceutically acceptable salt, solvate or pro-drug thereof, in association with a pharmaceutically acceptable excipient or carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for nasal administration or administration by inhalation, for example as a powder or solution, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) for example as a sterile solution, suspension or emulsion, for topical administration for example as an ointment or cream or for rectal administration for example as a suppository. In general the above compositions may be prepared in a conventional manner using conventional excipients.

The compositions of the present invention are advantageously presented in unit dosage form. The compound will normally be administered to a warm-blooded animal at a unit dose within the range 5-5000mg per square metre body area of the animal, i.e. approximately 0.1-100mg/kg. A unit dose in the range, for example, 1-100mg/kg, preferably 1-50mg/kg is envisaged and this normally provides a therapeutically-effective dose. A unit dose form such as a tablet or capsule will usually contain, for example 1-250mg of active ingredient.

As stated above the size of the dose required for the therapeutic or prophylactic treatment of a particular disease state will necessarily be varied depending on the host treated,

the route of administration and the severity of the illness being treated. Preferably a daily dose in the range of 1-50mg/kg is employed. However the daily dose will necessarily be varied depending upon the host treated, the particular route of administration, and the severity of the illness being treated. Accordingly the optimum dosage may be determined by the practitioner who is treating any particular patient.

According to a further aspect of the present invention there is provided a compound of the formula I or a pharmaceutically acceptable salt, solvate or pro-drug thereof as defined hereinbefore for use in a method of treatment of the human or animal body by therapy.

A further feature of the present invention is a compound of formula I, or a pharmaceutically acceptable salt, solvate or pro-drug thereof, for use as a medicament, conveniently a compound of formula I, or a pharmaceutically acceptable salt, solvate or pro-drug thereof, for use as a medicament for producing a vascular damaging effect in a warm-blooded animal such as a human being.

Thus according to a further aspect of the invention there is provided the use of a compound of the formula I, or a pharmaceutically acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm-blooded animal such as a human being.

According to a further feature of the invention there is provided a method for producing a vascular damaging effect in a warm-blooded animal, such as a human being, in need of such treatment which comprises administering to said animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt, solvate or pro-drug thereof as defined hereinbefore.

According to a further aspect of the present invention there is provided a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition, when dosed in divided doses (also known as split doses) produces a greater anti-tumour effect than when a single dose is given.

Anti-tumour effects of a method of treatment of the present invention include but are not limited to, inhibition of tumour growth, tumour growth delay, regression of tumour, shrinkage of tumour, increased time to re-growth of tumour on cessation of treatment, slowing of disease progression. It is expected that when a method of treatment of the present invention is administered to a warm-blooded animal such as a human, in need of treatment for cancer involving a solid tumour, said method of treatment will produce an effect, as measured

by, for example, one or more of: the extent of the anti-tumour effect, the response rate, the time to disease progression and the survival rate.

According to a further aspect of the present invention there is provided a method for the production of a vascular damaging effect in a warm-blooded animal such as a human, which comprises administering to said animal in divided doses an effective amount of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition.

According to a further aspect of the present invention there is provided a method for the treatment of a cancer involving a solid tumour in a warm-blooded animal such as a human, which comprises administering to said animal in divided doses an effective amount of a compound of formula (I) or pharmaceutically-acceptable salt or solvate thereof, preferably in the form of a pharmaceutical composition.

According to a further aspect of the present invention there is provided a medicament comprising two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition, which together add up to a total daily dose, for administration in divided doses for use in a method of treatment of a human or animal body by therapy.

According to a further aspect of the present invention there is provided a kit comprising two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, preferably in the form of a pharmaceutical composition, which together add up to a total daily dose, for administration in divided doses.

According to a further aspect of the present invention there is provided a kit comprising:

a) two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, which together add up to a total daily dose, in unit dosage forms for administration in divided doses; and

b) container means for containing said dosage forms.

According to a further aspect of the present invention there is provided a kit comprising:

a) two or more fractions of doses of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof, which together add up to a total daily dose, together with a pharmaceutically acceptable excipient or carrier, in unit dosage forms; and

b) container means for containing said dosage forms.

According to a further aspect of the present invention there is provided the use of compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for administration in divided doses for use in the production of a vascular damaging effect in a warm-blooded animal such as a human.

According to a further aspect of the present invention there is provided the use of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for administration in divided doses for use in the production of an anti-cancer effect in a warm-blooded animal such as a human.

According to a further aspect of the present invention there is provided the use of a compound of formula (I) or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for administration in divided doses for use in the production of an anti-tumour effect in a warm-blooded animal such as a human.

Divided doses, also called split doses, means that the total dose to be administered to a warm-blooded animal, such as a human, in any one day period (for example one 24 hour period from midnight to midnight) is divided up into two or more fractions of the total dose and these fractions are administered with a time period between each fraction of about greater than 0 hours to about 10 hours, preferably about 1 hour to about 6 hours, more preferably about 2 hours to about 4 hours. The fractions of total dose may be about equal or unequal.

Preferably the total dose is divided into two parts which may be about equal or unequal.

The time intervals between doses may be for example selected from:

about 1 hour, about 1.5 hours, about 2 hours, about 2.5 hours, about 3 hours, about 3.5 hours, about 4 hours, about 4.5 hours, about 5 hours, about 5.5 hours and about 6 hours.

The time intervals between doses may be any number (including non-integers) of minutes between greater than 0 minutes and 600 minutes, preferably between 45 and 375 minutes inclusive. If more than two doses are administered the time intervals between each dose may be about equal or unequal.

Preferably two doses are given with a time interval in between them of greater than or equal to 1 hour and less than 6 hours.

More preferably two doses are given with a time interval in between them of greater than or equal to two hours and less than 5 hours.

Yet more preferably two doses are given with a time interval in between them of greater than or equal to two hours and less than or equal to 4 hours.

Particularly the total dose is divided into two parts which may be about equal or unequal with a time interval between doses of greater than or equal to about two hours and less than or equal to about 4 hours.

More particularly the total dose is divided into two parts which may be about equal with a time interval between doses of greater than or equal to about two hours and less than or equal to about 4 hours.

For the avoidance of doubt the term 'about' in the description of time periods means the time given plus or minus 15 minutes, thus for example about 1 hour means 45 to 75 minutes, about 1.5 hours means 75 to 105 minutes. Elsewhere the term 'about' has its usual dictionary meaning.

The antiangiogenic treatment defined hereinbefore may be applied as a sole therapy or may involve, in addition to a compound of the invention, one or more other substances and/or treatments. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate administration of the individual components of the treatment. In the field of medical oncology it is normal practice to use a combination of different forms of treatment to treat each patient with cancer. In medical oncology the other component(s) of such conjoint treatment in addition to the antiangiogenic treatment defined hereinbefore may be: surgery, radiotherapy or chemotherapy. Such chemotherapy may include the following categories of therapeutic agent:

(i) other antiangiogenic agents that work by different mechanisms from those defined hereinbefore (for example linomide, inhibitors of integrin $\alpha v\beta 3$ function, angiostatin, endostatin, razoxin, thalidomide) and including vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitors (RTKIs) (for example those described in International Patent Applications Publication Nos. WO 97/22596, WO 97/30035, WO 97/32856 and WO 98/13354 the entire disclosure of which documents is incorporated herein by reference);

(ii) cytostatic agents such as antioestrogens (for example tamoxifen,toremifene, raloxifene, droloxifene, iodoxyfene), progestogens (for example megestrol acetate), aromatase inhibitors (for example anastrozole, letrazole, vorazole, exemestane), antiprogestogens, antiandrogens (for example flutamide, nilutamide, bicalutamide, cyproterone acetate), LHRH agonists and antagonists (for example goserelin acetate, luprolide), inhibitors of testosterone 5α-

dihydroreductase (for example finasteride), anti-invasion agents (for example metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator receptor function) and inhibitors of growth factor function, (such growth factors include for example epidermal growth factor (EGF), platelet derived growth factor and hepatocyte growth factor such inhibitors include growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors and serine/threonine kinase inhibitors);

(iii) biological response modifiers (for example interferon);

(iv) antibodies (for example edrecolomab); and

(v) antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as antimetabolites (for example antifolates like methotrexate, fluoropyrimidines like 5-fluorouracil, purine and adenosine analogues, cytosine arabinoside); antitumour antibiotics (for example anthracyclines like doxorubicin, daunomycin, epirubicin and idarubicin, mitomycin-C, dactinomycin, mithramycin); platinum derivatives (for example cisplatin, carboplatin); alkylating agents (for example nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide, nitrosoureas, thiotepa); antimitotic agents (for example vinca alkaloids like vincristine and taxoids like taxol, taxotere); enzymes (for example asparaginase); thymidylate synthase inhibitors (for example raltitrexed); topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan, irinotecan).

As stated above the compounds defined in the present invention are of interest for their vascular damaging effects. Such compounds of the invention are expected to be useful in the prophylaxis and treatment of a wide range of disease states where inappropriate angiogenesis occurs including cancer, diabetes, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, arterial restenosis, autoimmune diseases, acute inflammation, endometriosis, dysfunctional uterine bleeding and ocular diseases with retinal vessel proliferation. In particular such compounds of the invention are expected to slow advantageously the growth of primary and recurrent solid tumours of, for example, the colon, breast, prostate, lungs and skin.

In addition to their use in therapeutic medicine, the compounds of formula I and their pharmaceutically acceptable salts, solvates and pro-drugs are also useful as pharmacological tools in the development and standardisation of in vitro and in vivo test systems for the evaluation of the effects of vascular damaging agents in laboratory animals

such as cats, dogs, rabbits, monkeys, rats and mice, as part of the search for new therapeutic agents.

It is to be understood that where the term "ether" is used anywhere in this specification it refers to diethyl ether.

The invention will now be illustrated in the following non-limiting Examples in which, unless otherwise stated:

(i) evaporations were carried out by rotary evaporation in vacuo and work-up procedures were carried out after removal of residual solids such as drying agents by filtration;

(ii) operations were carried out at ambient temperature, that is in the range 18-25°C and under an atmosphere of an inert gas such as argon or nitrogen;

(iii) yields are given for illustration only and are not necessarily the maximum attainable;

(iv) the structures of the end-products of the formula I were confirmed by nuclear (generally proton) magnetic resonance (NMR) and mass spectral techniques; proton magnetic resonance chemical shift values were measured on the delta scale and peak multiplicities are shown as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; q, quartet, quin, quintet;

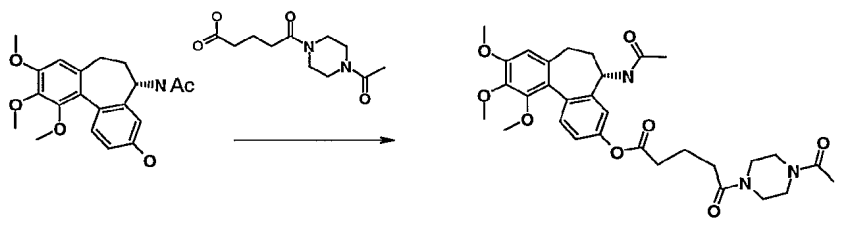
(v) intermediates were not generally fully characterised and purity was assessed by thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), infra-red (IR) or NMR analysis;

Abbreviations

4-Dimethylaminopyridine	DMAP
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride	EDCI
Dimethyl sulphoxide	DMSO
Trifluoroacetic acid	TFA

Example 1

(5S)-5-Acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 5-(4-acetylpiperazin-1-yl)-5-oxopentanoate



A solution of 5-(4-acetylpiperazin-1-yl)-5-oxopentanoic acid (0.308 g ; 1.27 mmol), EDCI (0.244 g ; 1.27 mmol), DMAP (0.036 g ; 0.29 mmol) in dichloromethane (30 ml) was stirred under argon atmosphere for 30 minutes. N-acetyl colchinol [International Patent Application No. PCT/GB98/01977] (0.350 g ; 0.98 mmol) was then added and the mixture was stirred overnight. After evaporation to dryness, the residue was purified by flash chromatography, eluting with dichloromethane/methanol (95/5) to give the title compound after evaporation of the appropriate fractions and trituration in ether / pentane.

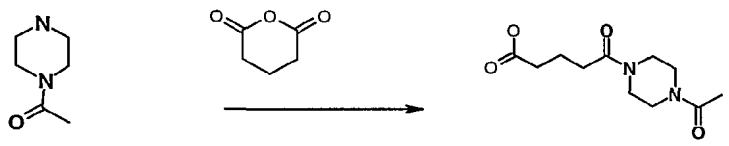
Yield : 82 %

^{1}H NMR (DMSO-d_6) : 1.45-1.50 (m, 1H) ; 1.51-1.75 (m, 1H) ; 1.87 (s, 3H) ; 1.79-1.94 (m, 1H) ; 1.94-2.11 (m, 2H) ; 2.02 (s, 3H) ; 2.10-2.24 (m, 1H) ; 2.52-2.62 (m, 1H, signal partially obscured by DMSO peak) ; 2.74-2.85 (m, 1H) ; 2.88-2.98 (m, 1H) ; 3.14-3.24 (m, 1H) ; 3.28-3.33 (m, 1H) ; 3.51 (s, 3H) ; 3.78 (s, 3H) ; 3.78-3.89 (m, 1H) ; 3.85 (s, 3H) ; 4.24-4.33 (m, 1H) ; 4.49-4.59 (m, 1H) ; 6.80 (s, 1H) ; 7.07 (s, 1H) ; 7.09 (dd, 1H) ; 7.35 (d, 1H) ; 3.39 (d, 1H).

MS-ESI : 582 $[MH]^+$

Elemental analysis	Found	C 62.54	H 6.92	N 6.93
$C_{31}H_{39}N_3O_8$, 0.8 H_2O	Requires	C 62.47	H 6.87	N 7.05

The starting material as follows :



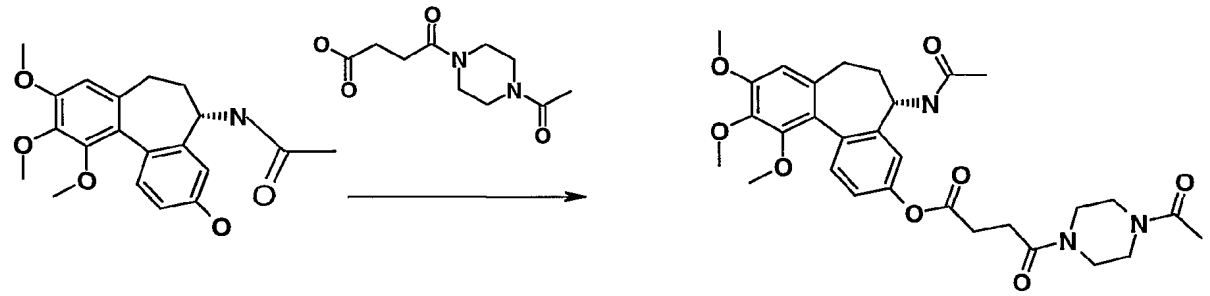
A solution of glutaric anhydride (1.6 g ; 14 mmol) and N-acetylpiperazine (1.5 g ; 12 mmol) in dichloromethane (20 ml) was stirred overnight. The resulting precipitate was filtered, washed with ether and dried to give 5-(4-acetylpiperazin-l-yl)-5-oxopentanoic acid as a white solid.

Yield : 83 %

^{1}H NMR ($CDCl_3$) : 1.98 (m, 2H) ; 2.13 (s, 3H) ; 2.46 (m, 4H) ; 3.47 (m, 4H) ; 3.64 (m, 4H).

Example 2

(5S)-5-Acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c}cyclohepten-3-yl 4-(4-acetylpiperazin-1-yl)-4-oxobutanoate



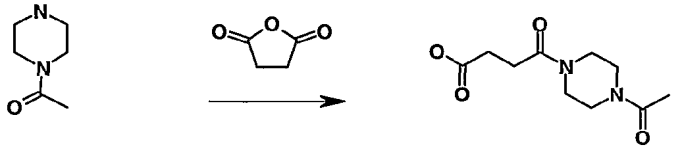
The compound was prepared using a similar method to that described in Example 1, but using 4-(4-acetylpiperazin-1-yl)-4-oxobutanoic acid in place of 5-(4-acetylpiperazin-l-yl)-5-oxopentanoic acid.

Yield : 67 %

^{1}H NMR (DMSO-d_6) : 1.81-1.93 (m, 1H) ; 1.86 (s, 3H) ; 1.98-2.10 (m, 1H) ; 2.02 (d, 3H) ; 2.11-2.23 (m, 1H) ; 2.52-2.59 (m, 1H, signal partially obscured by DMSO peak) ; 2.71-2.85 (m, 4H) ; 3.27-3.54 (m, 8H) ; 3.51 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.47-4.58 (m, 1H) ; 6.80 (s, 1H) ; 7.04 (dd, 1H) ; 7.06 (d, 1H) ; 7.34 (d, 1H) ; 8.41 (d, 1H).

MS-ESI : 568 $[MH]^+$

The starting material was prepared as follows:



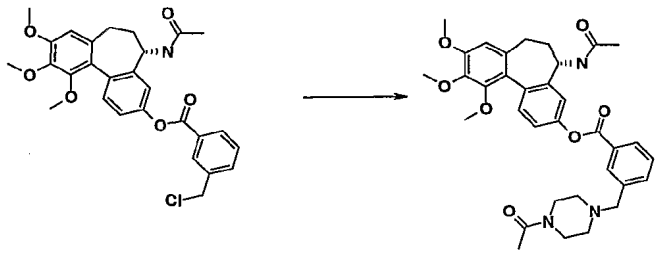
A solution of succinic anhydride (1.72 g ; 17 mmol) and N-acetyl piperazine (2 g ; 15.6 mmol) in dichloromethane (40 ml) was stirred overnight. After evaporation to dryness, the residue was triturated in ether / pentane to give 4-(4-acetylpiperazin-1-yl)-4-oxobutanoic acid as a solid.

Yield : 95 %

^{1}H NMR ($CDCl_3$) : 2.13 (s, 3H) ; 2.70 (m, 4H) ; 3.48 (m, 4H) ; 3.66 (m, 4H).

Example 3

(5S)-5-Acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-acetylpiperazin-1-ylmethyl)benzoate



A solution of (5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-chloromethylbenzoate (0.408 g ; 0.8 mmol), N-acetylpiperazine (0.144 g ; 1.12 mmol) and sodium iodide (0.06 g ; 0.4 mmol) in acetonitrile (6 ml) was stirred under argon atmosphere at room temperature overnight. After evaporation to dryness, the mixture was purified by flash chromatography eluting with dichloromethane/ethanol (92/8) to give the title compound as a white solid.

Yield : 69 %

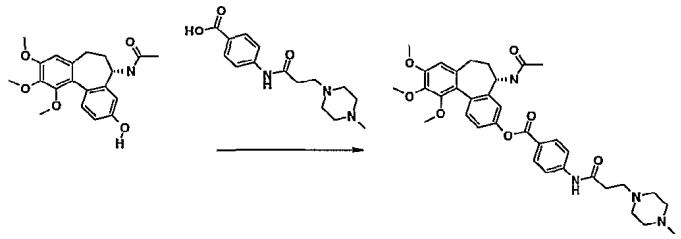
^{1}H NMR (DMSO-d_6) : 1.81-1.97 (m, 1H) ; 1.87 (s, 3H) ; 1.99 (s, 3H) ; 2.04-2.28 (m, 2H) ; 2.28-2.38 (m, 2H) ; 2.39-2.48 (m, 2H) ; 2.48-2.55 (m, 1H, signal partially obscured by DMSO peak) ; 3.42-3.51 (m, 4H) ; 3.56 (s, 3H) ; 3.64 (s, 2H) ; 3.81 (s, 3H) ; 3.87 (s, 3H) ; 4.54-4.67 (m, 1H) ; 6.84 (s, 1H) ; 7.25 (s, 1H) ; 7.26 (dd, 1H) ; 7.43 (d, 1H) ; 7.52 (dd, 1H) ; 7.72 (d, 1H) ; 8.10 (d, 1H) ; 8.12 (s, 1H) ; 8.40 (d, 1H).

MS-ESI : 602 $[MH]^+$

Elemental analysis	Found	C 67.00	H 6.76	N 6.81
$C_{34}H_{39}N_3O_7$, 0.3 H_2O	Requires	C 67.27	H 6.57	N 6.92

Example 4

(5S)-5-Acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[3-(4-methylpiperazin-1-yl)propionylamino]benzoate



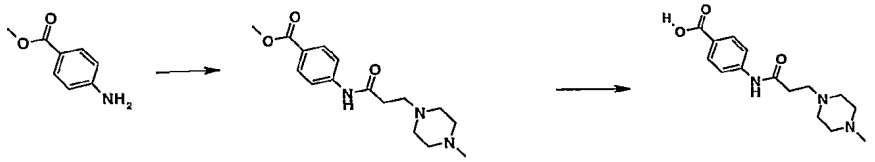
The title compound was prepared using a similar method to that in Example 1 but replacing 5-(4-acetylpiperazin-1-yl)-5-oxopentanoic acid by 4-{3-(4-methylpiperazin-1-yl)propionylamino]benzoic acid.

Yield : 55 %

^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 $[MH]^+$

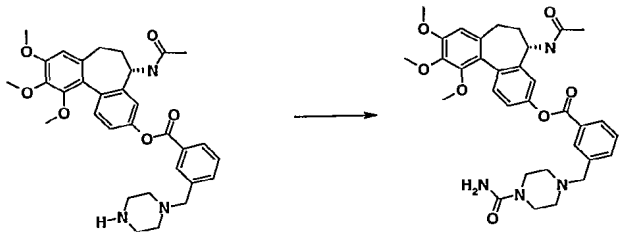
A solution of methyl 4-[3-(4-methylpiperazin-1-yl) propionylamino]benzoate (0.69 g ; 2.26 mmol) in methanol (10 ml) was treated with 2N sodium hydroxide solution (1.25 ml ; 2.48 mmol) at 60°C for 6 hours. After evaporation to dryness, the residue was triturated in acetone. The insoluble material was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

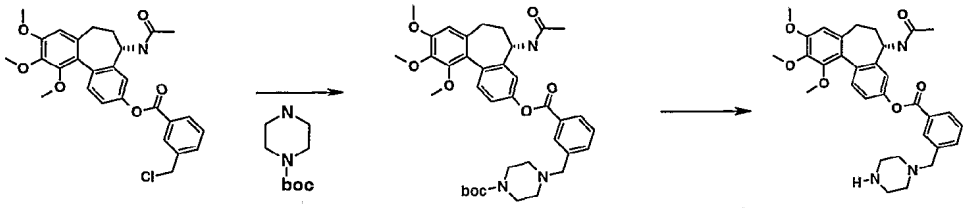
Yield : 87 %

^{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-1-ylmethyl)benzoate.

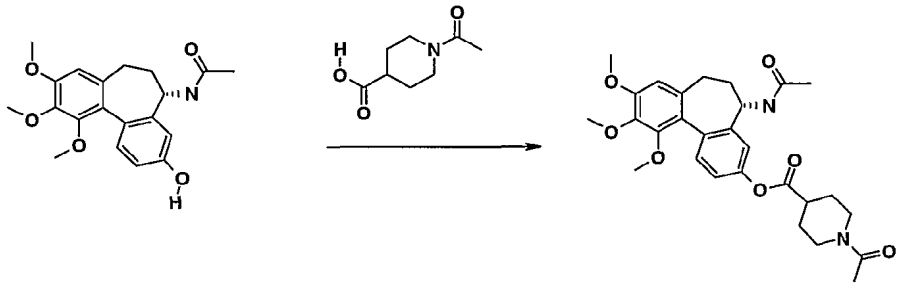
Yield : 55 %

^{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-1-yl)-5-oxopentanoic acid :

Yield : 79 %

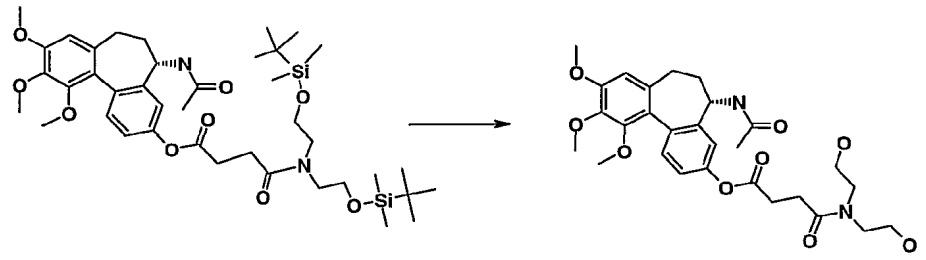
^{1}H NMR (DMSO-d_6) : 1.45-1.60 (m, 1H) ; 1.61-1.75 (m, 1H) ; 1.88-1.93 (m, 2H) ; 1.87 (s, 3H) ; 1.94-2.09 (m, 2H) ; 2.02 (s, 3H) ; 2.10-2.24 (m, 1H) ; 2.53-2.60 (m , 1H, signal partially obscured by DMSO peak) ; 2.75-2.85 (m, 1H) ; 2.88-2.98 (m, 1H) ; 3.14-3.25 (m, 1H) ; 3.26 (bs, 1H) ; 3.50 (s, 3H) ; 3.78 (s, 3H) ; 3.81 (bs, 1H) ; 3.84 (s, 3H) ; 4.23-4.33 (m, 1H) ; 4.49-4.59 (m, 1H) ; 6.80 (s, 1H) ; 7.06 (s, 1H) ; 7.08 (dd, 1H) ; 7.35 (d, 1H) ; 8.39 (d, 1H).

MS-ESI : 511 $[MH]^+$

Elemental analysis	Found	C 64.65	H 6.85	N 5.43
$C_{28}H_{33}N_2O_7$, 0.5 H_2O	Requires	C 64.85	H 6.61	N 5.40

Example 7

(5S)-5-Acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-[N,N-di-(2-hydroxyethyl)carbamoyl]propanoate



A 2.4 N solution of sulphuric acid in methanol (4 ml) was added at 3°C under argon atmosphere, to a solution of (5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-{N-N-di-[2-(tert-butyldimethylsilyloxy)ethyl]carbamoyl} propanoate (0.7 g ; 0.906 mmol) in methanol (15 ml). After stirring at 3° C for 45 mins, a mixture of ethyl acetate (100 ml) and saturated aqueous sodium hydrogen carbonate solution (75 ml) was added. The organic phase was washed with water, dried and was purified by flash chromatography eluting dichloromethane/methanol (92/8) to give the title compound.

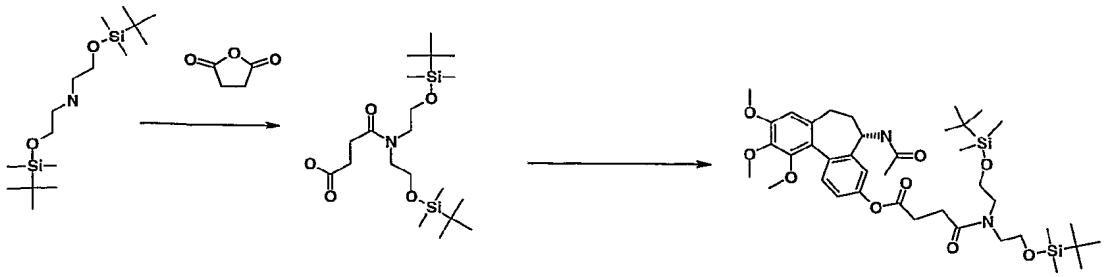
Yield : 66 %

^{1}H NMR (DMSO-d_6) : 1.81-1.96 (m, 1H) ; 1.89 (s, 3H) ; 1.98-2.56 (m, 2H) ; 2.55-2.65 (m, 1H, signal partially obscured by DMSO peak) ; 2.80 (bs, 4H) ; 4.37-4.63 (m, 8H) ; 3.53 (s, 3H) ; 3.80 (s, 3H) ; 3.86 (s, 3H) ; 4.49-4.60 (m, 1H) ; 4.69 (t, 1H) ; 4.88 (t, 1H) ; 6.80 (s, 1H) ; 7.02-7.07 (m, 2H) ; 7.34 (d, 1H). 8.40 (d, 1H).

MS-ESI : 545 $[MH]^+$

Elemental analysis	Found	C 59.27	H 6.62	N 4.89
$C_{28}H_{36}N_2O_9$, 1.2 H_2O	Requires	C 59.40	H 6.84	N 4.95

The starting material was prepared as follows :



Succinic anhydride (2.64 g ; 0.026 mol) was added under argon atmosphere to a solution of N-N-di-[2-(tert-butyldimethylsilyloxy)ethyl]amino [Synthesis (19997), 6, 643-648] (8 g ; 0.024 mol) in dichloromethane. The mixture was stirred at ambient temperature overnight. After filtration of the insoluble, the filtrate was evaporated and dried to give 3-{N,N-di[2-(tert-butyldimethylsilyloxy)ethyl] carbamoyl}propanoic acid.

Yield : 96 %

^{1}H NMR ($CDCl_3$) : 0.04 (s, 6H) ; 0.05 (s, 6H) ; 0.87 (s, 9H) ; 0.88 (s, 9H) ; 2.62-2.69 (m, 2H) ; 2.83-2.90 (m, 2H) ; 3.52 (t, 2H) ; 3.58 (t, 2H) ; 3.75 (t, 2H) ; 3.78 (t, 2H).

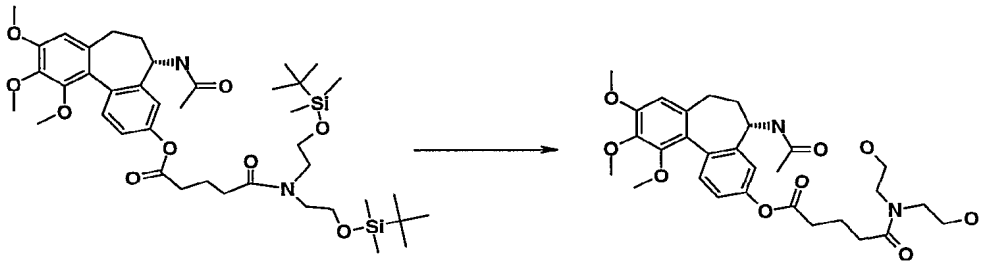
A solution of 3-{N,N-di[2-(tert-butyldimethylsilyloxy)ethyl]carbamoyl}propanoic acid (0.552 g ; 1.27 mmol), EDCI (0.244 g ; 1.27 mmol), DMAP (0.036 g ; 0.294 mmol) in dichloromethane (30 ml) was stirred under argon atmosphere for 30 mins. N-acetyl colchinol (0.35 g ; 0.98 mmol) was added and the mixture was stirred at ambient temperature overnight. After evaporation to dryness, the residue was purified by flash chromatography, eluting with ethyl acetate/ petroleum ether (70/30) to give 5(S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3{N-N-di[2-(tert-butyl-dimethylsilyloxy]ethyl}carbamoyl}propanoate.

Yield : 74 %

^{1}H NMR (DMSO-d_6) : 0.03 (s, 6H) ; 0.04 (s, 6H) ; 0.86 (s, 9H) ; 0.87 (s, 9H) ; 1.83-1.94 (m, 1H) ; 1.80 (s, 3H) ; 2.00-2.11 (m, 1H) ; 2.12-2.24 (m, 1H) ; 2.53-2.60 (m 1H, signal partially obscured by DMSO peak) ; 2.72-2.78 (m, 2H) ; 2.78-2.86 (m, 2H) ; 3.42 (t, 2H) ; 3.52 (s, 3H) ; 3.53 (t, 2H) ; 3.57 (t, 2H) ; 3.75 (t, 2H) ; 3.80 (s, 3H) ; 3.85 (s, 3H) ; 4.50-4.60 (m, 1H) ; 6.81 (s, 1H) ; 7.03 (dd, 1H) ; 7.07 (d, 1H) ; 7.34 (d, 1H) ; 8.41 (d, 1H).

Example 8

(5S)-5-Acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[N,N-di(2-hydroxyethyl)carbamoyl]butanoate



The title compound was prepared using a similar method to that described in Example 7, but using (5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c}cyclohepten-3-yl 4-N,N-di[2-(tert-butyldimethylsilyloxy)ethyl]carbamoyl}butanoate in place of (5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3{N,N-di[2-(tert-butyl-dimethylsilyloxy)ethyl]carbamoyl}propanoate.

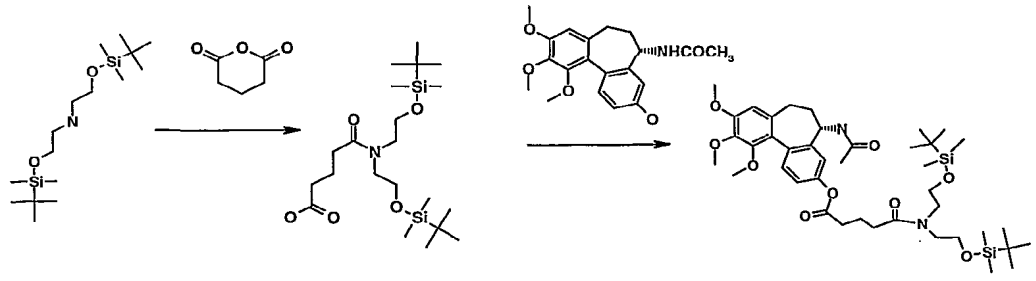
Yield : 37 %

^{1}H NMR (DMSO-d_6) : 1.83-1.92 (m, 2H) ; 1.87 (s, 3H) ; 1.98-2.10 (m, 1H) ; 2.11-2.22 (m, 1H) ; 2.48 (t , 2H, signal partially obscured by DMSO peak) ; 2.52-2.56 (m, 1H, signal partially obscured by DMSO peak) ; 2.63 (t, 2H) ; 3.35 (t, 2H) ; 3.41 (t, 2H) ; 3.44-3.50 (m, 2H) ; 3.50-3.57 (m, 2H) ; 3.51 (s, 3H) ; 3.78 (s, 3H) ; 3.84 (s, 3H) ; 4.50-4.60 (m, 1H) ; 4.67 (t, 1H) ; 4.85 (t, 1H) ; 6.80 (s, 1H) ; 7.07 (s, 1H) ; 7.09 (dd, 1H) ; 7.34 (d, 1H). 8.38 (d, 1H).

MS - ESI : 581 $[MNa]^+$

Elemental analysis	Found	C 61.22	H 7.21	N 4.89
$C_{29}H_{38}N_2O_9$, 0.5 H_2O	Required	C 61.36	H 6.93	N 4.94

The starting material was prepared as follows :



A solution of glutaric anhydride (0.753 g ; 6.6 mmol) and N,N-di{2-(tert-butyldimethylsilyloxy)ethyl]amino (2 g ; 6.6 mmol) in dichloromethane (40 ml) was stirred overnight. After evaporation to dryness the residue was triturated in pentane to give a solid which was filtered. The filtrate was evaporated to give 4-{N,N-di[2-(tert-butyldimethylsilyloxy)ethyl]carbamoyl}butanoic acid as an oil.

Yield : 95 %

^{1}H NMR (DMSO-d_6) : 0.03 (s, 6H) ; 0.04 (s, 6H) ; 0.86 (s, 9H) ; 0.87 (s, 9H) ; 1.70 (m, 2H) ; 2.23 (t, 2H) ; 2.38 (t, 2H) ; 3.38 (t, 2H) ; 3.46 (t, 2H) ; 3.65 (t, 2H) ; 3.70 (t, 2H).

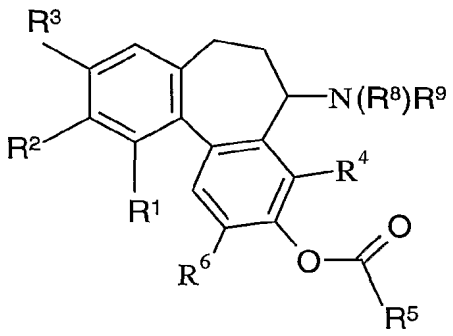
4-{N,N-di[2-(tert-Butyldimethylsilyloxy)ethyl]carbamoyl}butanoic acid was reacted with N-acetyl colchinol using the similar conditions to that in Example 7, but using 4-{N,N-di[2-(tert-butyldimethylsilyloxy)ethyl]carbamoyl}butanoic acid in place of 3-{N,N-di[2-(tert-butyldimethylsilyloxy)ethyl]carbamoyl}propanoic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

Yield : 92 %

^{1}H NMR (DMSO-d_6) : 0.04 (s, 12H) ; 0.86 (s, 9H) ; 0.87 (s, 9H) ; 1.83-1.94 (m, 2H) ; 1.88 (s, 3H) ; 2.00-2.11 (m, 1H) ; 2.12-2.24 (m, 1H) ; 2.48 (t , 2H, signal partially obscured by DMSO peak) ; 2.51-2.54 (m, 1H, signal partially obscured by DMSO peak) ; 2.65 (t, 2H) ; 3.41 (t, 2H) ; 3.50 (t, 2H) ; 3.53 (s, 3H) ; 3.64-3.76 (m, 2H) ; 3.80 (s, 3H) ; 3.86 (s, 3H) ; 4.51-4.61 (m, 1H) ; 6.81 (s, 1H) ; 7.08 (dd, 1H) ; 7.09 (d, 1H) ; 7.36 (d, 1H) ; 7.99 (s, 1H) ; 8.40 (d, 1H).

Claims

1. A compound of the formula I:



(I)

wherein:

R^1, R^2 and **R^3** are each independently hydroxy, phosphoryloxy ($-OPO_3H_2$), C_{1-4}alkoxy or an in vivo hydrolysable ester of hydroxy; with the proviso that at least two of R^1, R^2 and R^3 are C_{1-4}alkoxy;

R^4 and **R^6** are each independently selected from: hydrogen, nitro, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, fluoro, C_{1-4}alkoxy and C_{1-4}alkyl;

R^5 is selected from one of the following groups:

1) of the formula -A -X^1-Y^1 -B, wherein:

A is C_{1-4}alkylene or $-(CH_2)_p$-Q- (wherein **p** is 0, 1 or 2 and **Q** is phenylene or thienylene);

X^1 is -O-, -CO-, -C(O)O-, $-CON(R^{10})$-, $-N(R^{10})$-, $-N(R^{10})CO$-, $N(R^{10})C(O)O$-, $-N(R^{10})CON(R^{11})$-, $-N(R^{10})SO_2$-, $-SO_2N(R^{10})$- or $OC(O)N(R^{10})$- (wherein **R^{10}** is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

Y^1 is C_{1-3}alkylene;

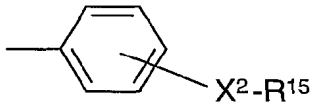
B is carboxy, sulpho, phosphoryloxy, hydroxy, amino, N-(C_{1-4}alkyl)amino, N,N-di (C_{1-3}alkyl)amino, $-R^{12}$ or $-NHC(R^{13})COOH$; (wherein **R^{12}** is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O,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

which heterocyclic group is optionally substituted by 1 or 2 substituents selected from: oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, aminoC_{1-4}alkyl, N,N-di(C_{1-4}alkyl)amino-C_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein $\mathbf{R^{14}}$ is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkoxy-C_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl);

$\mathbf{R^{13}}$ is an amino acid side chain;

2) of the formula:



wherein:

the phenyl ring is substituted by $-\mathbf{X^2}-\mathbf{R^{15}}$ in the 3- or 4-position;

$\mathbf{X^2}$ is -CO- or of the formula $-(CH_2)_r-$ (wherein **r** is 0, 1, 2 or 3) and

$\mathbf{R^{15}}$ is a 5-6 membered saturated heterocyclic group (linked via a ring carbon or nitrogen atom) containing 1 or 2 ring heteroatoms selected from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, C_{1-4}aminoalkyl, N,N-di(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein $\mathbf{R^{14}}$ is as hereinabove defined); provided that the heterocyclic group (R^{15}) is substituted by at least one substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N,N-di(C_{1-4}alkyl)carbamoyl;

3) $-(CH_2)_a-Y^2-(CH_2)_b-R^{15}$

(wherein **a** is 0, 1, 2, 3 or 4; **b** is 0, 1, 2, 3 or 4; $\mathbf{Y^2}$ is a direct bond, -O-, -C(O)-, $-N(R^{16})-$, $-N(R^{16})C(O)-$ or $-C(O)N(R^{16})-$ (wherein $\mathbf{R^{16}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and wherein 1 or 2 of the $(CH_2)_a$ or $(CH_2)_b$ groups are optionally substituted by 1 or 2 substituents selected from hydroxy and amino and $\mathbf{R^{15}}$ is as hereinabove defined; provided that when a is 0, then Y^2 is a single direct bond;

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-

(wherein the alkyl groups are independently optionally substituted by 1 or 2 substituents selected from: amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkanoyl, carboxy, sulpho and phosphoryloxy);

provided that:

a) when A is C_{1-4}alkylene and X^1 is of the formula -CO-, $-N(R^{10})-$, $-N(R^{10})CO-$ or $-CON(R^{10})-$ then when B is R^{12}, $R^{12}$is defined hereinabove for R^{15} ;

b) when A is C_{1-4}alkylene and X^1 is of the formula $-N(R^{10})CO-$, $-CON(R^{10})-$, or -C(O)O-, then B is not carboxy;

c) when A is C_{1-4}alkylene and X^1 is -CONH- or -NHCO-, then B is not carboxy, hydroxy, phosphoryloxy, amino, N-C_{1-4}alkylamino or N,N-di-C_{1-4}alkylamino;

$\mathbf{R^8}$ is a group $-Y^3R^{17}$

(wherein $\mathbf{Y^3}$ is a direct bond, -C(O)-, -C(O)O-, $-N(R^{18})-$, $-C(O)N(R^{18})-$, $-SO_2-$ or $-SO_2NR^{18}-$ (wherein $\mathbf{R^{18}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and $\mathbf{R^{17}}$ is selected from one of the following 4 groups:

1) hydrogen, C_{1-4}alkyl, phenyl, C_{1-4}alkylY^4C_{1-4}alkyl (wherein $\mathbf{Y^4}$ is -C(O)-, $-NR^{19}C(O)-$ or $-C(O)NR^{19}-$ (wherein $\mathbf{R^{19}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl));

[which alkyl, alkylY^4alkyl or phenyl group is optionally substituted by 1 or 2 substituents selected from: halogeno, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, carboxy, $-CON(R^{23})R^{24}$ (wherein $\mathbf{R^{23}}$ **and** $\mathbf{R^{24}}$ are independently selected from hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl and C_{1-3}alkoxyC_{2-3}alkyl), C_{1-4}alkoxy, C_{1-4}alkoxycarbonylamino,

C_{1-4}alkanoyl, sulpho, phosphoryloxy, R^{12} (wherein $\mathbf{R^{12}}$ is as hereinabove defined), and a group $\mathbf{-Y^5R^{20}}$ [wherein $\mathbf{Y^5}$ is $-NR^{21}C(O)-$ or $-OC(O)-$ (wherein $\mathbf{R^{21}}$ represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and $\mathbf{R^{20}}$ is C_{1-4}alkyl or a group R^{22} (wherein $\mathbf{R^{22}}$ is a 5 or 6 membered aromatic heterocyclic group containing 1 to 4, inclusive, ring heteroatoms selected independently from O, N and S, which aromatic heterocyclic group is optionally substituted by 1 or 2 substituents selected from hydroxy, amino, C_{1-4}alkyl, aminoC_{1-4}alkyl, N-C_{1-4}alkylaminoC_{1-4}alkyl, N, N-di(C_{1-4}alkyl)aminoC_{1-4}alkyl, carboxy, $-CONR^{25}R^{26}$ and $-NR^{25}COR^{27}$ (wherein $\mathbf{R^{25}}$ **and** $\mathbf{R^{26}}$, which may be the same or different, are hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl and $\mathbf{R^{27}}$ is C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))];

2) R^{22} (wherein R^{22} is as hereinabove defined);

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

4) $R^{12}Y^7C_{1-4}$alkyl- (wherein R^{12} is as hereinabove defined and Y^7 is -C(O)-, $-NR^{23}C(O)-$, $-NR^{23}C(O)C_{1-4}$alkyl-, $-C(O)NR^{23}-$ or $-C(O)NR^{23}C_{1-4}$alkyl- (wherein R^{23} is as hereinabove defined))];

and $\mathbf{R^9}$ is hydrogen or C_{1-3}alkyl;

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

2. A compound according to claim 1 where R^1, R^2 and R^3 are all methoxy or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

3. A compound according to claim 1 wherein:

R^1 , R^2, and R^3 are all C_{1-4}alkoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, C_{1-3} alkoxy, and C_{1-3}alkyl;

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

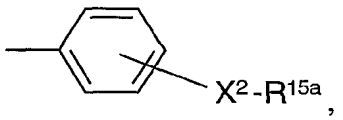
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is $-CO-$, $-CON(R^{10})-$, $-N(R^{10})-$, $-N(R^{10})CO-$ or $-OC(O)N(R^{10})-$;

B is carboxy sulpho, phosphoryloxy or of the formula $-R^{12}$ (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the $-X^2-R^{15a}$ substituent is in the 3, or 4-position of the phenyl ring;

X^2 is $-(CH_2)_r-$;

r is 0, 1 and 2; and

R^{15a} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula $-(CH_2)_a-Y^2-(CH_2)_b-R^{15b}$, wherein:

a is 2 or 3;

b is 0, 1, or 2; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; and

R^{15b} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino,

N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy; and

$\mathbf{R^8}$ is a group $-Y^3R^{17}$ (wherein Y^3 is -C(O)-, -C(O)O- or -C(O)NH-; and

$\mathbf{R^{17}}$ is selected from one of the following 4 groups:

1) hydrogen, C_{1-4}alkyl, phenyl or C_{1-4}alkylY^4C_{1-4}alkyl (wherein Y^4 is -NHCO- or -CONH-); [which alkyl, alkylY^4alkyl or phenyl group is optionally substituted by 1 or 2 substituents selected from: halogeno, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, C_{1-4}alkoxy, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, phosphoryloxy, R^{12a} (wherein R^{12a} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl), $-Y^5-R^{20}$ [wherein Y^5 is -NHCO-; and R^{20} is C_{1-4}alkyl or R^{22a} (wherein R^{22a} is imidazolyl, pyridyl, pyrimidyl, thiazolyl or pyrazinyl, each of which is optionally substituted by C_{1-4}alkyl)];

2) R^{22a} (wherein R^{22a} is as hereinabove defined);

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

4) $R^{12a}Y^7C_{1-4}$alkyl- (wherein R^{12a} is as hereinabove defined and Y^7 is Y^7 is -NHC(O)- or -CONH-)];

and $\mathbf{R^9}$ is hydrogen;

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

4. A compound according to claim 1 wherein:

R^1 , R^2, and R^3 are all methoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, methoxy and methyl;

R^5 is selected from one of the following groups:

1) of the formula $-A-X^1-Y^1-B$, wherein:

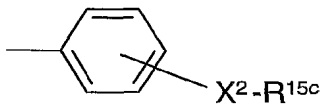
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, $-CON(R^{10})-$, $-N(R^{10})$ -, $-N(R^{10})CO-$ or $-OC(O)N(R^{10})-$, wherein R^{10} is as defined in claim 1;

B is carboxy sulpho, phosphoryloxy or of the formula $-R^{12}$ (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the $-X^2-R^{15c}$ substituent is in the 3, or 4-position of the phenyl ring;

X^2 is $-(CH_2)_r-$;

r is 0, 1 and 2; and

R^{15c} is morpholinyl, piperazinyl or piperidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula $-(CH_2)_a-Y^2-(CH_2)_b-R^{15d}$, wherein:

a is 2 or 3;

b is 0 or 1; and

Y^2 is a single direct bond, -C(O)- or -NHC(O)-; and

R^{15d} is morpholinyl, piperazinyl or piperidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino, N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy; and

$\mathbf{R^8}$ is a group $-Y^3R^{17}$ (wherein Y^3 is -C(O)- or -C(O)O-; and

$\mathbf{R^{17}}$ is selected from one of the following 4 groups:

1) C_{1-4}alkyl [which alkyl, group is optionally substituted by 1 or 2 substituents selected from: fluoro, chloro and bromo;

2) R^{22b} (wherein R^{22b} is imidazolyl, pyridyl, pyrimidyl, thiazolyl or pyrazinyl, each of which is optionally substituted by C_{1-4}alkyl);

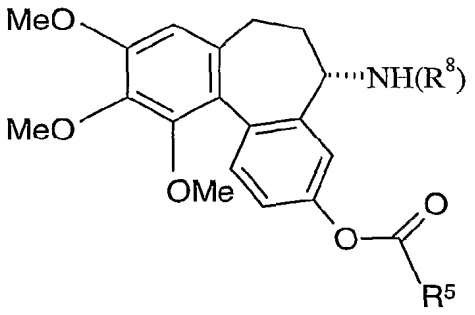
3) R^{22b} -C_{1-4}alkyl- (wherein R^{22b} is as hereinabove defined); or

4) $R^{12b}Y^7C_{1-4}$alkyl- (wherein R^{12a} is morpholinyl, piperidinyl or piperazinyl each of which is optionally substituted by methyl, ethyl, acetyl, propionyl, carbamoyl or 2-hydroxyethyl; and Y^7 is -NHC(O)- or -CONH-)];

and $\mathbf{R^9}$ is hydrogen;

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

5. A compound of formula (II):

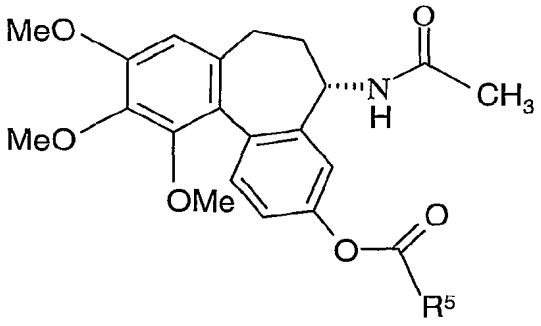


(II)

wherein R^5 and R^8 are as defined in claim 1;

or a pharmacetically-acceptable salt, solvate or pro-drug thereof.

6. A compound of the formula (III) wherein:



(III)

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

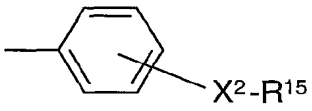
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, -CONH-, -NH-, -NHCO- or -OC(O)NH-;

B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the -X^2-R^{15} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 0, 1 and 2; and

R^{15} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted as immediately hereinabove defined for R^{12}, and substituted by at least 1 substituent selected from C_{2-4}alkanyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15}, wherein:

a is 2 or 3;

b is 0, 1, or 2; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino, N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

7. A compound according to Claim 6 wherein:

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

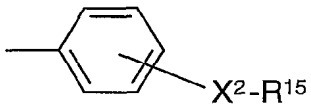
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, -NHCO-;

B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the -X^2-R^{15} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 1 and 2; and

R^{15a} is as hereinabove defined;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15b}, wherein:

a is 2 or 3;

b is 0; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; and

R^{15b} is as hereinabove defined; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, hydroxy and phosphoryloxy;

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

8. A compound according to Claim 6 wherein:

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

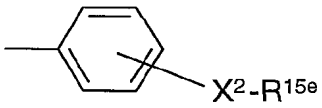
A is phenylene;

X^1 is -CO-, -NHCO-;

Y^1 is methylene or ethylene;

B is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group;

2) of the formula



wherein:

the -X^2-R^{15e} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 1; and

R^{15e} is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15f}, wherein:

a is 2 or 3;

b is 0; and

Y^2 is -C(O)-;

R^{15f} is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group; or

4) 2-[N,N-di(C_{1-4}alkyl)carbamoyl]ethyl- or 3-[N,N-di(C_{1-4}alkyl)carbamoyl]propyl-, wherein the C_{1-4}alkyl group is optionally substituted by 1 hydroxy group;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

9. A compound according to Claim 6 wherein:

R^5 is 2-[N,N-di(C_{1-4}alkyl)carbamoyl]ethyl-, or

3-[N,N-di(C_{1-4}alkyl)carbamoyl]propyl-,

wherein the C_{1-4}alkyl group is optionally substituted by 1 hydroxy group;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

10. A compound selected from:

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 5-(4-acetylpiperazin-l-yl)-5-oxopentanoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c}cyclohepten-3-yl 4-(4-acetylpiperazin-1-yl)-4-oxobutanoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-acetylpiperazin-1-ylmethyl)benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[3-(4-methylpiperazin-l-yl)propionylamino]benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-carbamoylpiperazin-1-ylmethyl)benzoatc;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5II-dibenzo[a,c]cyclohepten-3-yl N-acetylpiperidin-1-ylcarboxylate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-[N,N-di-(2-hydroxyethyl)carbamoyl]propanoate; and

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[N,N-di(2-hydroxyethyl)carbamoyl]butanoate;

and pharmaceutically-acceptable salts, solvates and pro-drugs thereof.

11. A pharmaceutical composition comprising a compound according to any one of claims 1 to 10 or pharmaceutically acceptable salt, solvate or pro-drug thereof, in association with a pharmaceutically acceptable carrier.

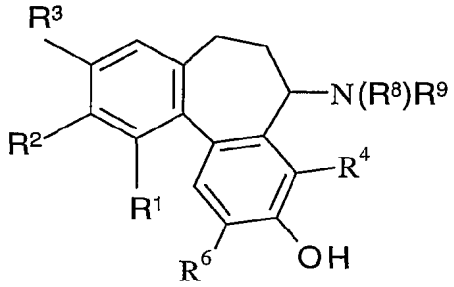
12. The use of a compound according any one of claims 1 to 10, or a pharmaceutically-acceptable salt, solvate or pro-drug thereof, in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm-blooded animal.

13. The use of a compound according to any one of claim 1 to 10 or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for

administration in divided doses for use in the production of a vascular damaging effect in a warm-blooded animal.

14. A process for preparing a compound of the formula (I), or a compound of the formula (I) wherein at least 1 functional group is protected, wherein R^1, R^2, R^3 R^4, R^5, R^6, R^7, R^8, R^9, R^{10} R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}, R^{21}, R^{22}, R^{23}, R^{24}, R^{25}, R^{26}, R^{27}, A, B, Q, X^1, X^2, Y^1, Y^2, Y^3, Y^4, Y^5, Y^7, p and r are as defined in claim 1, comprising:

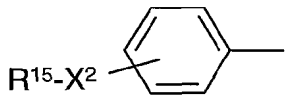
(a) reacting a compound of the formula (X):



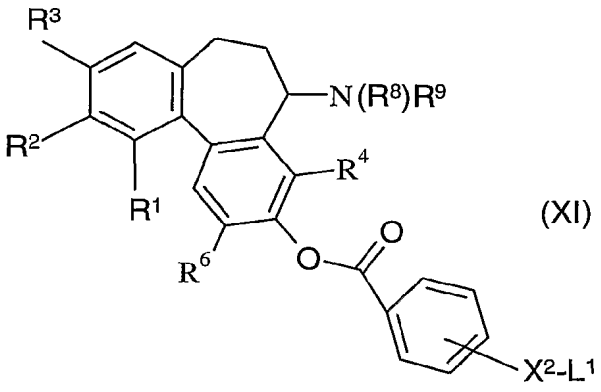
(X)

with a compound of the formula R^5-COOH or an activated derivative thereof;

(b) when R^5 is of the formula:



reacting a compound of the formula (XI):



(XI)

with R^{15} (wherein L^1 is a leaving group) ;

(c) introducing substituents onto a ring nitrogen atom in R^{12} or R^{15};

(d) converting one compound of the formula (I) into another compound of the formula (I);

(e) when a phosphoryloxy group is desired, reacting the corresponding hydroxy compound with a phosphoramidite;

wherein any functional groups are optionally protected.

and thereafter if necessary:

i) converting a compound of formula (I) into another compound of formula (I);

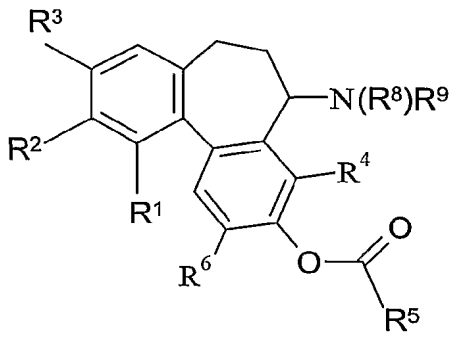
ii) removing any protecting groups;

iii) forming a pharmaceutically acceptable salt, solvate or pro-drug thereof.

AMENDED CLAIMS

[received by the International Bureau on 11 December 2001 (11.12.01);
original claims 1-14 replaced by amended claims 1-14 (14 pages)]

1. A compound of the formula I:



(I)

wherein:

R^1, R^2 and **R^3** are each independently hydroxy, phosphoryloxy ($-OPO_3H_2$), C_{1-4}alkoxy or an in vivo hydrolysable ester of hydroxy; with the proviso that at least two of R^1, R^2 and R^3 are C_{1-4}alkoxy;

R^4 and **R^6** are each independently selected from: hydrogen, nitro, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, fluoro, C_{1-4}alkoxy and C_{1-4}alkyl;

R^5 is selected from one of the following groups:

1) of the formula -A -X^1-Y^1 -B, wherein:

A is C_{1-4}alkylene or -$(CH_2)_p$-Q- (wherein **p** is 0, 1 or 2 and **Q** is phenylene or thienylene);

X^1 is -O-, -CO-, -C(O)O-, -CON(R^{10})-, -N(R^{10})-, -N(R^{10})CO-, N(R^{10})C(O)O-, -N(R^{10})CON(R^{11})-, -N(R^{10})SO_2-, -SO_2N(R^{10})- or OC(O)N(R^{10})- (wherein **R^{10}** is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl);

Y^1 is C_{1-3}alkylene;

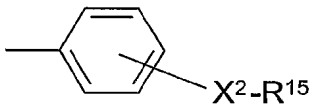
B is carboxy, sulpho, phosphoryloxy, hydroxy, amino, N-(C_{1-4}alkyl)amino, N,N-di (C_{1-3}alkyl)amino, -R^{12} or -NHC(R^{13})COOH; (wherein **R^{12}** is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S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

which heterocyclic group is optionally substituted by 1 or 2 substituents selected from: oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, aminoC_{1-4}alkyl, N,N-di(C_{1-4}alkyl)amino-C_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein **R^{14}** is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) containing 1 or 2 ring heteroatoms, selected independently from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from:

oxo, hydroxy, halogeno, C_{1-4}alkyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkoxy-C_{1-4}alkyl and C_{1-4}alkylsulphonylC_{1-4}alkyl));

R^{13} is an amino acid side chain;

2) of the formula:



wherein:

the phenyl ring is substituted by -**X^2**-**R^{15}** in the 3- or 4-position;

X^2 is -CO- or of the formula -$(CH_2)_r$- (wherein **r** is 0, 1, 2 or 3) and

R^{15} is a 5-6 membered saturated heterocyclic group (linked via a ring carbon or nitrogen atom) containing 1 or 2 ring heteroatoms selected from O, S and N, which heterocyclic group is optionally substituted by 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl, N,N-di-(C_{1-4}alkyl)carbamoyl, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, cyanoC_{1-3}alkyl, carbamoylC_{1-3}alkyl, carboxyC_{1-4}alkyl, C_{1-4}aminoalkyl, N,N-di(C_{1-4}alkyl)aminoC_{1-4}alkyl, C_{1-4}alkoxyC_{1-4}alkyl, C_{1-4}alkylsulphonylC_{1-4}alkyl and R^{14} (wherein **R^{14}** is as hereinabove defined); provided that the heterocyclic group (R^{15}) is substituted by at least one substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N,N-di(C_{1-4}alkyl)carbamoyl;

3) -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15}

(wherein **a** is 0, 1, 2, 3 or 4; **b** is 0, 1, 2, 3 or 4; $\mathbf{Y^2}$ is a direct bond, -O-, -C(O)-, -N(R^{16})-, -N(R^{16})C(O)- or -C(O)N(R^{16})- (wherein $\mathbf{R^{16}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and wherein 1 or 2 of the $(CH_2)_a$ or $(CH_2)_b$ groups are optionally substituted by 1 or 2 substituents selected from hydroxy and amino and $\mathbf{R^{15}}$ is as hereinabove defined; provided that when a is 0, then Y^2 is a single direct bond);

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-

(wherein the alkyl groups are independently optionally substituted by 1 or 2 substituents selected from: amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, hydroxyC_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkanoyl, carboxy, sulpho and phosphoryloxy);

provided that:

a) when A is C_{1-4}alkylene and X^1 is of the formula -CO-, -N(R^{10})-, -N(R^{10})CO- or -CON(R^{10})- then when B is R^{12}, $R^{12}$is defined hereinabove for R^{15} ;

b) when A is C_{1-4}alkylene and X^1 is of the formula -N(R^{10})CO-, -CON(R^{10})-, or -C(O)O-, then B is not carboxy;

c) when A is C_{1-4}alkylene and X^1 is -CONH- or -NHCO-, then B is not carboxy, hydroxy, phosphoryloxy, amino, N-C_{1-4}alkylamino or N,N-di-C_{1-4}alkylamino;

$\mathbf{R^8}$ is a group -Y^3R^{17}

(wherein $\mathbf{Y^3}$ is a direct bond, -C(O)-, -C(O)O-, -N(R^{18})-, -C(O)N(R^{18})-, -SO_2- or -SO_2NR^{18}- (wherein $\mathbf{R^{18}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and $\mathbf{R^{17}}$ is selected from one of the following 4 groups:

1) hydrogen, C_{1-4}alkyl, phenyl, C_{1-4}alkylY^4C_{1-4}alkyl (wherein $\mathbf{Y^4}$ is -C(O)-, -NR^{19}C(O)- or -C(O)NR^{19}- (wherein $\mathbf{R^{19}}$ is hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl));

[which alkyl, alkylY^4alkyl or phenyl group is optionally substituted by 1 or 2 substituents selected from: halogeno, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, hydroxy, carboxy, -CON(R^{23})R^{24} (wherein $\mathbf{R^{23}}$ **and** $\mathbf{R^{24}}$ are independently selected from hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl and C_{1-3}alkoxyC_{2-3}alkyl), C_{1-4}alkoxy, C_{1-4}alkoxycarbonylamino,

C_{1-4}alkanoyl, sulpho, phosphoryloxy, R^{12} (wherein $\mathbf{R^{12}}$ is as hereinabove defined), and a group $\mathbf{-Y^5R^{20}}$ [wherein $\mathbf{Y^5}$ is $-NR^{21}C(O)-$ or $-OC(O)-$ (wherein $\mathbf{R^{21}}$ represents hydrogen, C_{1-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl) and $\mathbf{R^{20}}$ is C_{1-4}alkyl or a group R^{22} (wherein $\mathbf{R^{22}}$ is a 5 or 6 membered aromatic heterocyclic group containing 1 to 4, inclusive, ring heteroatoms selected independently from O, N and S, which aromatic heterocyclic group is optionally substituted by 1 or 2 substituents selected from hydroxy, amino, C_{1-4}alkyl, aminoC_{1-4}alkyl, N-C_{1-4}alkylaminoC_{1-4}alkyl, N, N-di(C_{1-4}alkyl)aminoC_{1-4}alkyl, carboxy, $-CONR^{25}R^{26}$ and $-NR^{25}COR^{27}$ (wherein $\mathbf{R^{25}}$ **and** $\mathbf{R^{26}}$, which may be the same or different, are hydrogen, C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl and $\mathbf{R^{27}}$ is C_{1-3}alkyl, hydroxyC_{2-3}alkyl, aminoC_{2-3}alkyl or C_{1-3}alkoxyC_{2-3}alkyl))]];

2) R^{22} (wherein R^{22} is as hereinabove defined);

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

4) $R^{12}Y^7C_{1-4}$alkyl- (wherein R^{12} is as hereinabove defined and Y^7 is -C(O)-, $-NR^{23}C(O)-$, $-NR^{23}C(O)C_{1-4}$alkyl-, $-C(O)NR^{23}-$ or $-C(O)NR^{23}C_{1-4}$alkyl- (wherein R^{23} is as hereinabove defined)));

and $\mathbf{R^9}$ is hydrogen or C_{1-3}alkyl;

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

2. A compound according to claim 1 where R^1, R^2 and R^3 are all methoxy or a pharmaceutically-acceptable salt, solvate or pro-drug thereof.

3. A compound according to claim 1 wherein:

R^1 , R^2, and R^3 are all C_{1-4}alkoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, C_{1-3} alkoxy, and C_{1-3}alkyl;

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

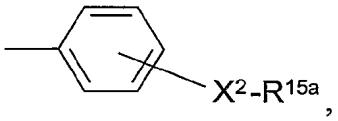
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, $-CON(R^{10})-$, $-N(R^{10})$ -, $-N(R^{10})CO-$ or $-OC(O)N(R^{10})-$;

B is carboxy sulpho, phosphoryloxy or of the formula $-R^{12}$ (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the $-X^2-R^{15a}$ substituent is in the 3, or 4-position of the phenyl ring;

X^2 is $-(CH_2)_r-$;

r is 0, 1 and 2; and

R^{15a} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula $-(CH_2)_a-Y^2-(CH_2)_b-R^{15b}$, wherein:

a is 2 or 3;

b is 0, 1, or 2; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; and

R^{15b} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanyl, carbamoyl, N-C_{1-4} alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino,

N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy; and

$\mathbf{R^8}$ is a group $-Y^3R^{17}$ (wherein Y^3 is -C(O)-, -C(O)O- or -C(O)NH-; and

$\mathbf{R^{17}}$ is selected from one of the following 4 groups:

1) hydrogen, C_{1-4}alkyl, phenyl or C_{1-4}alkylY^4C_{1-4}alkyl (wherein Y^4 is -NHCO- or -CONH-); [which alkyl, alkylY^4alkyl or phenyl group is optionally substituted by 1 or 2 substituents selected from: halogeno, amino, N-C_{1-4}alkylamino, N,N-di(C_{1-4}alkyl)amino, C_{1-4}alkoxy, C_{1-4}alkoxycarbonylamino, C_{1-4}alkanoyl, phosphoryloxy, R^{12a} (wherein R^{12a} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4}alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl), $-Y^5-R^{20}$ [wherein Y^5 is -NHCO-; and R^{20} is C_{1-4}alkyl or R^{22a} (wherein R^{22a} is imidazolyl, pyridyl, pyrimidyl, thiazolyl or pyrazinyl, each of which is optionally substituted by C_{1-4}alkyl)]];

2) R^{22a} (wherein R^{22a} is as hereinabove defined);

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

4) $R^{12a}Y^7C_{1-4}$alkyl- (wherein R^{12a} is as hereinabove defined and Y^7 is Y^7 is -NHC(O)- or -CONH-));

and $\mathbf{R^9}$ is hydrogen;

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

4. A compound according to claim 1 wherein:

R^1 , R^2, and R^3 are all methoxy;

R^4 and R^6 are independently selected from hydrogen, hydroxy, methoxy and methyl;

R^5 is selected from one of the following groups:

1) of the formula $-A-X^1-Y^1-B$, wherein:

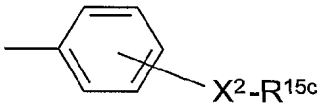
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, $-CON(R^{10})-$, $-N(R^{10})-$, $-N(R^{10})CO-$ or $-OC(O)N(R^{10})-$, wherein R^{10} is as defined in claim 1;

B is carboxy sulpho, phosphoryloxy or of the formula $-R^{12}$ (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the $-X^2-R^{15c}$ substituent is in the 3, or 4-position of the phenyl ring;

X^2 is $-(CH_2)_r-$;

r is 0, 1 and 2; and

R^{15c} is morpholinyl, piperazinyl or piperidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula $-(CH_2)_a-Y^2-(CH_2)_b-R^{15d}$, wherein:

a is 2 or 3;

b is 0 or 1; and

Y^2 is a single direct bond, -C(O)- or -NHC(O)-; and

R^{15d} is morpholinyl, piperazinyl or piperidinyl optionally substituted by 1 or 2 substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl; and substituted by at least 1 substituent selected from C_{2-4}alkanoyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino, N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy; and

$\mathbf{R^8}$ is a group $-Y^3R^{17}$ (wherein Y^3 is -C(O)- or -C(O)O-; and

$\mathbf{R^{17}}$ is selected from one of the following 4 groups:

1) C_{1-4}alkyl [which alkyl, group is optionally substituted by 1 or 2 substituents selected from: fluoro, chloro and bromo];

2) R^{22b} (wherein R^{22b} is imidazolyl, pyridyl, pyrimidyl, thiazolyl or pyrazinyl, each of which is optionally substituted by C_{1-4}alkyl);

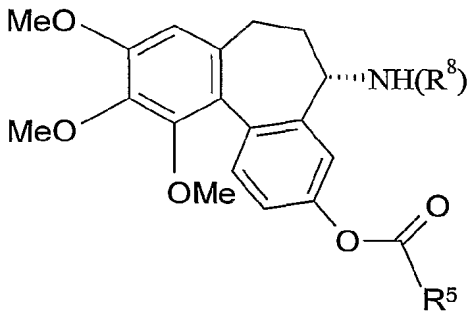
3) R^{22b} -C_{1-4}alkyl- (wherein R^{22b} is as hereinabove defined); or

4) $R^{12b}Y^7C_{1-4}$alkyl- (wherein R^{12a} is morpholinyl, piperidinyl or piperazinyl each of which is optionally substituted by methyl, ethyl, acetyl, propionyl, carbamoyl or 2-hydroxyethyl; and Y^7 is -NHC(O)- or -CONH-));

and $\mathbf{R^9}$ is hydrogen;

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

5. A compound of formula (II):

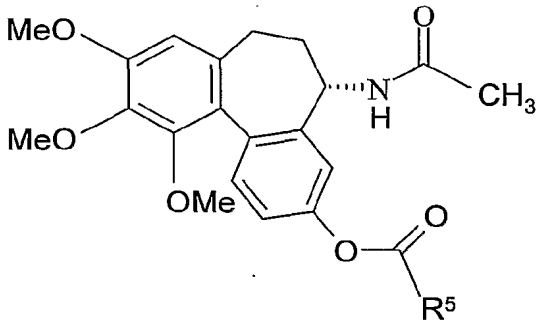


(II)

wherein R^5 and R^8 are as defined in claim 1;

or a pharmacetically-acceptable salt, solvate or pro-drug thereof.

6. A compound of the formula (III) wherein:



(III)

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

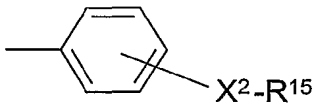
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, -CONH-, -NH-, -NHCO- or -OC(O)NH-;

B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the -X^2-R^{15} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 0, 1 and 2; and

R^{15} is morpholinyl, piperazinyl, piperidinyl or pyrrolidinyl optionally substituted as immediately hereinabove defined for R^{12}, and substituted by at least 1 substituent selected from C_{2-4}alkanyl, carbamoyl, N-C_{1-4}alkylcarbamoyl and N, N-di(C_{1-4}alkyl)carbamoyl;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15}, wherein:

a is 2 or 3;

b is 0, 1, or 2; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, N-methylamino, N-N-dimethylamino, hydroxy, methoxy, carboxy, sulpho and phosphoryloxy;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

7. A compound according to Claim 6 wherein:

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

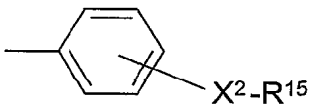
A is ethylene or phenylene;

Y^1 is C_{1-3}alkylene;

X^1 is -CO-, -NHCO-;

B is carboxy sulpho, phosphoryloxy or of the formula -R^{12} (wherein R^{12} is piperazinyl, morpholinyl or piperidinyl, each of which is linked via a ring carbon or nitrogen ring atom and each ring is optionally substituted by 1 or 2 of the substituents selected from C_{1-4} alkyl, C_{2-4}alkanoyl, carbamoyl, cyanoC_{1-3}alkyl, hydroxyC_{1-3}alkyl, carboxyC_{1-3}alkyl and aminoC_{1-3}alkyl);

2) of the formula



wherein:

the -X^2-R^{15} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 1 and 2; and

R^{15a} is as defined in claim 3;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15b}, wherein:

a is 2 or 3;

b is 0; and

Y^2 is a single direct bond, -C(O)-, -NHC(O)- or -C(O)NH-; and

R^{15b} is as defined in claim 3; or

4) N,N-di(C_{1-4}alkyl)carbamoylC_{1-4}alkyl-, wherein the alkyl group is optionally substituted by 1 or 2 substituents selected from amino, hydroxy and phosphoryloxy;

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

8. A compound according to Claim 6 wherein:

R^5 is selected from one of the following groups:

1) of the formula -A-X^1-Y^1-B, wherein:

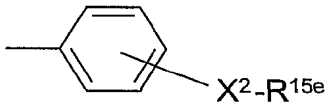
A is phenylene;

X^1 is -CO-, -NHCO-;

Y^1 is methylene or ethylene;

B is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group;

2) of the formula



wherein:

the -X^2-R^{15e} substituent is in the 3, or 4-position of the phenyl ring;

X^2 is -$(CH_2)_r$-;

r is 1; and

R^{15e} is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group;

3) of the formula -$(CH_2)_a$-Y^2-$(CH_2)_b$-R^{15f}, wherein:

a is 2 or 3;

b is 0; and

Y^2 is -C(O)-;

R^{15f} is piperazino or morpholinyl each of which is linked via a ring nitrogen atom and each ring is optionally substituted by 1 methyl or acetyl group; or

4) 2-[N,N-di(C_{1-4}alkyl)carbamoyl]ethyl- or 3-[N,N-di(C_{1-4}alkyl)carbamoyl]propyl-, wherein the C_{1-4}alkyl group is optionally substituted by 1 hydroxy group;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

9. A compound according to Claim 6 wherein:

R^5 is 2-[N,N-di(C_{1-4}alkyl)carbamoyl]ethyl-, or

3-[N,N-di(C_{1-4}alkyl)carbamoyl]propyl-,

wherein the C_{1-4}alkyl group is optionally substituted by 1 hydroxy group;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

10. A compound selected from:

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 5-(4-acetylpiperazin-1-yl)-5-oxopentanoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c}cyclohepten-3-yl 4-(4-acetylpiperazin-1-yl)-4-oxobutanoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-acetylpiperazin-1-ylmethyl)benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[3-(4-methylpiperazin-1-yl)propionylamino]benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-(4-carbamoylpiperazin-1-ylmethyl)benzoate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl N-acetylpiperidin-1-ylcarboxylate;

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 3-[N,N-di-(2-hydroxyethyl)carbamoyl]propanoate; and

(5S)-5-acetylamino-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-3-yl 4-[N,N-di(2-hydroxyethyl)carbamoyl]butanoate;

and pharmaceutically-acceptable salts, solvates and pro-drugs thereof.

11. A pharmaceutical composition comprising a compound according to any one of claims 1 to 10 or pharmaceutically acceptable salt, solvate or pro-drug thereof, in association with a pharmaceutically acceptable carrier.

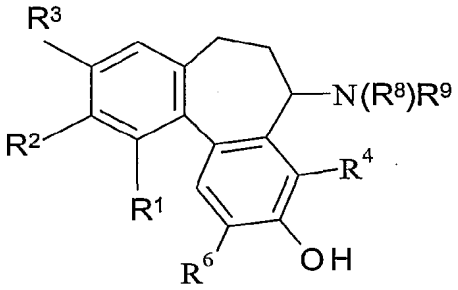
12. The use of a compound according any one of claims 1 to 10, or a pharmaceutically-acceptable salt, solvate or pro-drug thereof, in the manufacture of a medicament for use in the production of a vascular damaging effect in a warm-blooded animal.

13. The use of a compound according to any one of claim 1 to 10 or pharmaceutically-acceptable salt, solvate or pro-drug thereof in the manufacture of a medicament for

administration in divided doses for use in the production of a vascular damaging effect in a warm-blooded animal.

14. A process for preparing a compound of the formula (I), or a compound of the formula (I) wherein at least 1 functional group is protected, wherein R^1, R^2, R^3 R^4, R^5, R^6, R^7, R^8, R^9, R^{10} R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}, R^{21}, R^{22}, R^{23}, R^{24}, R^{25}, R^{26}, R^{27}, A, B, Q, X^1, X^2, Y^1, Y^2, Y^3, Y^4, Y^5, Y^7, p and r are as defined in claim 1, comprising:

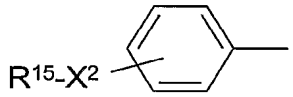
(a) reacting a compound of the formula (X):



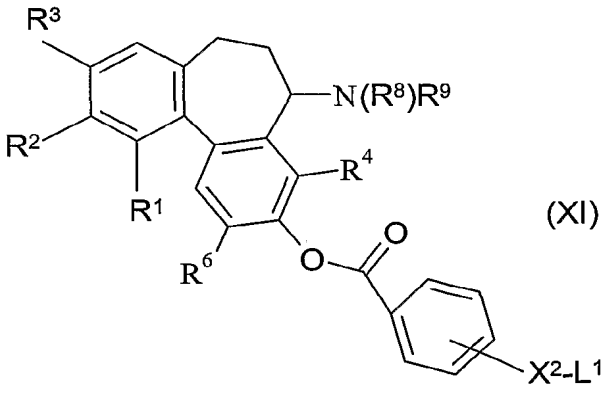
(X)

with a compound of the formula R^5-COOH or an activated derivative thereof;

(b) when R^5 is of the formula:



reacting a compound of the formula (XI):



(XI)

with R^{15} (wherein L^1 is a leaving group) ;

(c) introducing substituents onto a ring nitrogen atom in R^{12} or R^{15};

(d) converting one compound of the formula (I) into another compound of the formula (I);

(e) when a phosphoryloxy group is desired, reacting the corresponding hydroxy compound with a phosphoramidite;

wherein any functional groups are optionally protected.

and thereafter if necessary:

i) converting a compound of formula (I) into another compound of formula (I);

ii) removing any protecting groups;

iii) forming a pharmaceutically acceptable salt, solvate or pro-drug thereof.

INTERNATIONAL SEARCH REPORT

Int plication No
PCT/GB 01/02966

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D295/16 C07D295/18 C07D295/14 C07D211/62 C07C235/74
A61K31/495 A61K31/16 A61K31/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D C07C A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99 02166 A (ANGIOGENE PHARM LTD ;DOUGHERTY GRAEME (GB)) 21 January 1999 (1999-01-21) the whole document ---	1-14
A,P	WO 00 40529 A (ARNOULD JEAN CLAUDE ;ANGIOGENE PHARM LTD (GB); DAVIS PETER DAVID () 13 July 2000 (2000-07-13) the whole document ---	1-14

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 October 2001	17/10/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Frelon, D

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

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	BOYE O ET AL: "Synthesis of carbon-14 labeled electrophilic ligands of the colchicine binding site of tubulin: chloroacetates of demethylthiocolchicines and of N-acetylcolchinol, isothiocyanates of 9-deoxy-N-acetylcolchinol" JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS, SUSSEX, GB, vol. 33, no. 4, 1993, pages 293-299, XP002081866 ISSN: 0362-4803 example 5 ---	1-14
A	GIL-JONG KANG ET AL: "n-acetylcolchinol O-methyl ether and thiocolchicine, potent nalogs of colchicine modified in the C-ring" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 265, no. 18, 25 June 1990 (1990-06-25), pages 10255-10259, XP002081868 ISSN: 0021-9258 the whole document -----	1-14

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-14 relate to an extremely large number of possible compounds. In fact, the claims contain many options, variables, possible permutations and combinations.
"Prodrugs" are also claimed which are not defined so that it is impossible to determine which compounds are actually meant. Furthermore many brackets are opened but in some definitions they do not appear clearly closed.
Consequently a lack of clarity and conciseness within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. The search includes the compounds as defined by the general formula (I). Prodrugs were only searched as far as they are covered by the formula as such.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int lication No
PCT/GB 01/02966

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9902166	A	21-01-1999	AU	8231198 A	08-02-1999
			BR	9810681 A	15-08-2000
			CN	1262624 T	09-08-2000
			EP	1001785 A1	24-05-2000
			WO	9902166 A1	21-01-1999
			HU	0002493 A2	28-08-2001
			NO	20000077 A	07-01-2000
			PL	337926 A1	11-09-2000
			SK	132000 A3	11-12-2000
			TR	9903149 T2	21-09-2000
WO 0040529	A	13-07-2000	AU	1882300 A	24-07-2000
			WO	0040529 A1	13-07-2000

(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).

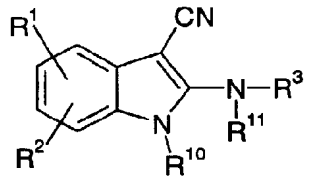
(74) Agent: **BRYANT, Tracey**; AstraZeneca, Global Intellectual Property, P.O. Box 272, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4GR (GB).

(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.

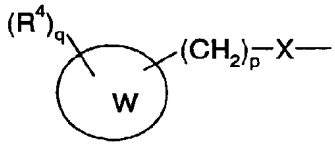
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian

[Continued on next page]

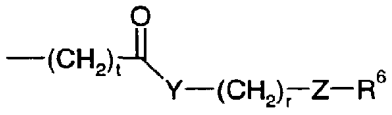
(54) Title: INDOLE DERIVATIVES WITH VASCULAR DAMAGING ACTIVITY



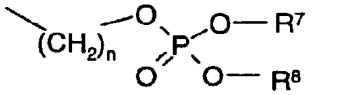
(I)



(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+-, -+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): 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).

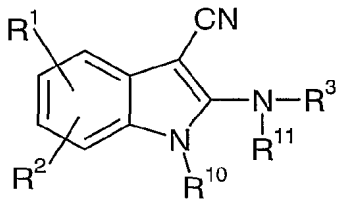
Several anticancer agents have been described which bind to tubulin, these include Taxol™ and Taxotere™ . However, these agents do not act as selective vascular targeting agents. For example, Taxol does not cause cell detachment in the HUVEC detachment assay, described herein. Other tubulin-binding agents 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] .

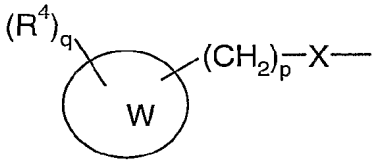
The compounds of the present in invention are tubulin binding agents that have similar activity to combretastatin and ZD6126 on proliferating endothelial cells. It can therefore be expected that the compounds of the present invention will have damaging effects on newly-formed vasculature, for example 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,

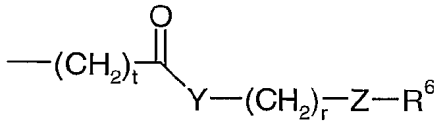
R^4 is independently selected from hydrogen, halogen, -OH, amino, alkanoylamino, —OPO_3H_2, or a hydrocarbyl group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified or two R^4 groups together form an optionally substituted cyclic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

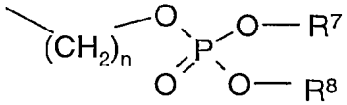
wherein Y is selected from —NH—, —O— or a bond;

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;

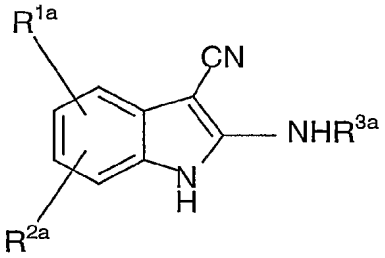
provided that:

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.

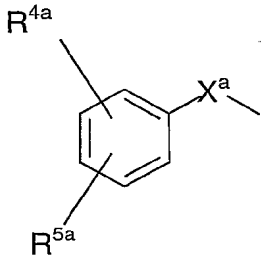
According to a further feature of the first aspect of the present invention there is provided a compound of the general Formula (Ia):



Formula (Ia)

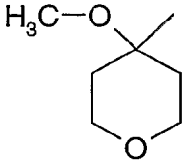
or a derivative thereof,

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);

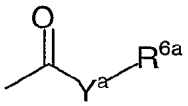


Formula (IIIa)

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—;

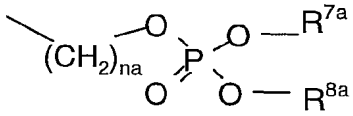
R^{3a} is either hydrogen or a group of Formula (IVa):



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.

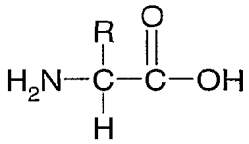
The term **"functional group"** refers to reactive groups such as halogen, cyano, nitro, oxo, $C(O)_yR^a$, OR^a, $S(O)_zR^a$, NR^bR^c, $OC(O)NR^bR^c$, $C(O)NR^bR^c$, $-NR^7C(O)_yR^6$, $-NR^aCONR^bR^c$, $-C=NOR^a$, $-N=CR^bR^c$, $S(O)_zNR^bR^c$, $C(S)_yR^a$, $C(S)OR^a$, $C(S)NR^bR^c$ or $-NR^bS(O)_zR^a$ 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.

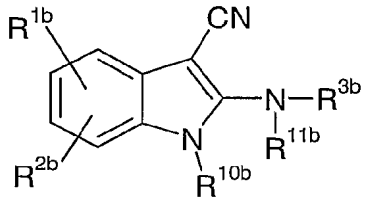
A suitable pharmaceutically-acceptable salt of a compound of the invention is, for example, an acid-addition salt of a compound of the invention which is sufficiently basic, for example, an acid-addition salt with, for example, an inorganic or organic acid, for example hydrochloric, hydrobromic, sulphuric, 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.

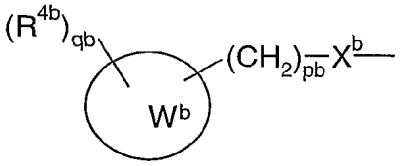
It will also be understood that certain compounds of the present invention may exist in solvated, for example hydrated, as well as unsolvated forms. It is to be understood that the present invention encompasses all such solvated forms which possess the property of the property of inhibiting and/or reversing 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,

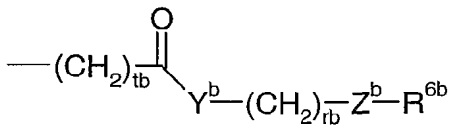
R^{4b} is independently selected from hydrogen, halogen, -OH, amino, alkanoylamino, —OPO_3H_2, or a hydrocarbyl group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified or two R^{4b} groups together form an optionally substituted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

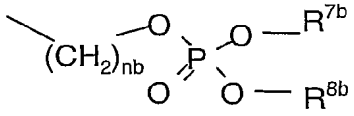
wherein Y^b is selected from —NH—, —O— or a bond;

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:

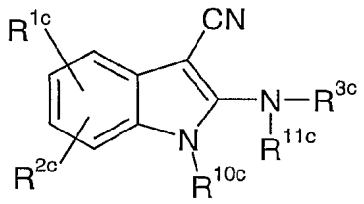
i) when R^{1b} is an unsubstituted phenylthio group (Ph-S-), R^{2b} is H, R^{10b} is H and R^{11b} is H then R^{3b} is neither H nor $-C(O)-O-CH_2CH_3$.

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.

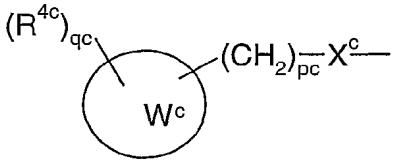
For each feature of the second aspect of the invention, the surgical, therapeutic or diagnostic method preferably involves the treatment of a human or animal patient to inhibit and/or reverse, and/or to alleviate a symptom of, angiogenesis and/or any disease state associated with angiogenesis. In other 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,

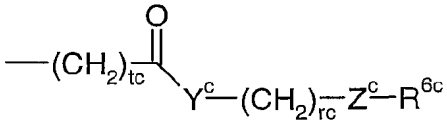
R^{4c} is independently selected from hydrogen, halogen, -OH, amino, alkanoylamino, —OPO_3H_2, or a hydrocarbyl group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified or two R^{4c} groups together form an optionally substituted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

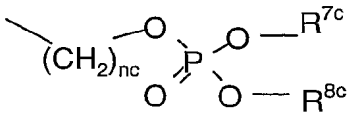
wherein Y^c is selected from —NH—, —O— or a bond;

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.

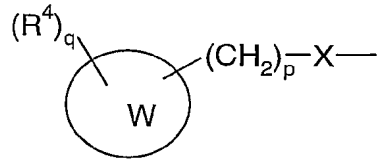
According to a further feature of the third aspect of the invention provides a method of treatment of a patient to inhibit and/or reverse, and/or to alleviate a symptom of, angiogenesis and/or any disease state associated with angiogenesis, the method involving administering to the patient a therapeutically (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.

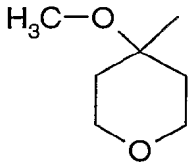
A preferred group of values for R^2 in each aspect of the invention is hydrogen, halogen, preferably fluoro or chloro, a lower alkyl group, preferably methyl, ethyl, propyl, isopropyl, butyl or *t*-butyl. More preferably R^2 is hydrogen, fluoro, chloro or methyl. Most preferably R^2 is hydrogen.

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 preferably 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),

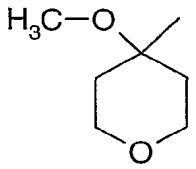


Formula (III)

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),



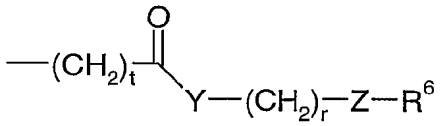
Formula (III)

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.

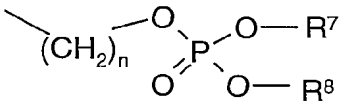
A preferred group of values for t in each aspect of the invention is 0 or 1. Preferably t is 0.

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,

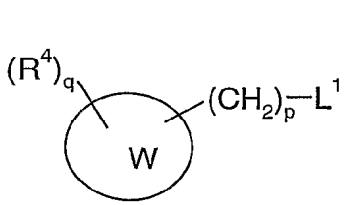
sulphate or sugar group, to enhance the bioavailability of the compound on administration to a patient.

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.

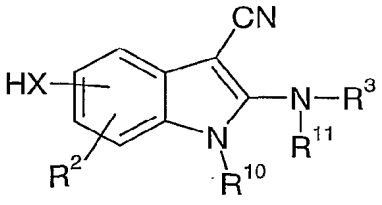
A compound of the invention or a pharmaceutically-acceptable salt, or solvate thereof, may be prepared by any process known to be applicable to the preparation of chemically related compounds. Such processes, when used to prepare a compound of the invention or a pharmaceutically-acceptable salt, or solvate 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),



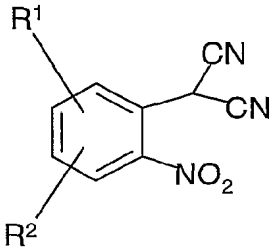
Formula (A)



Formula (B)

wherein L^1 is a leaving group;

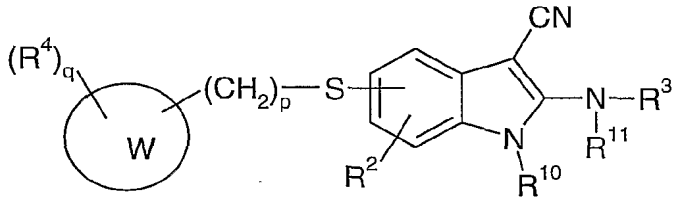
b) for compounds of Formula (I) in which R^3 is hydrogen and R^{11} is hydrogen may be prepared by reduction followed by ring closure of compounds of Formula (C):



Formula (C)

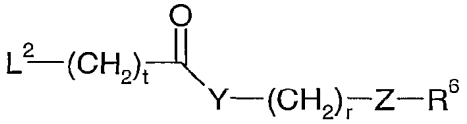
c) for compounds of Formula (I) wherein R^{10} is lower alkyl, reacting a compound of Formula (I) wherein R^{10} is hydrogen with a suitable alkylhalide,

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.

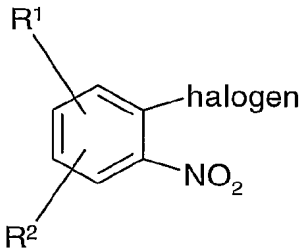
According to a further feature of the fourth aspect of the invention there is provided the processes a), b), c), d) and e) described above for the preparation of compounds of the Formula (I), or a salt, or solvate thereof.

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);

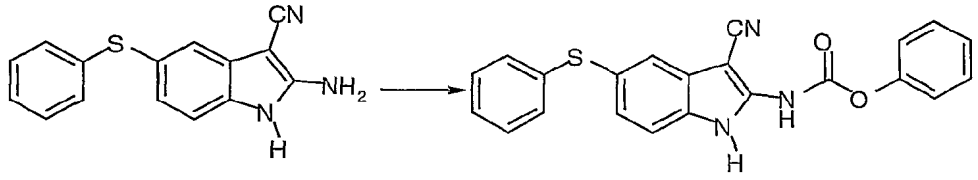
(viii) HP20SS resin (DIAION® HP20SS) was obtained from Mitsubishi Chemical America Inc.

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%

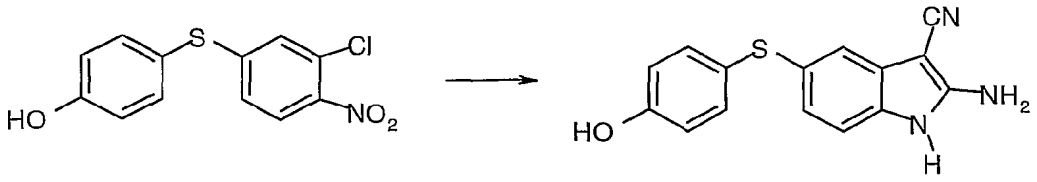
^{1}H NMR spectrum ($CDCl_3$) : 7.14-7.35 (m, 10H) ; 7.44 (t, 2H) ; 7.71 (d, 1H) ; 10.83 (s, 1H) ; 10.99 (br s, 1H).

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%.

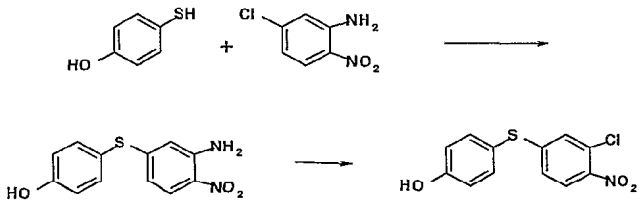
Further flash chromatography of a small sample eluting with increasingly polar mixtures of EtOAc / hexanes (60-70% EtOAc) gave colourless crystals, Melting Point 241-242°C.

^{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%

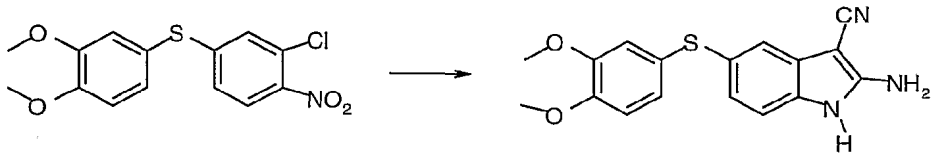
^{1}H NMR spectrum ($CDCl_3$) : 5.18 (s, 1H) ; 6.93 - 6.99 (m, 3H) ; 7.09 (d, 1H) ; 7.44 (d, 2H) ; 7.79 (d, 1H).

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):

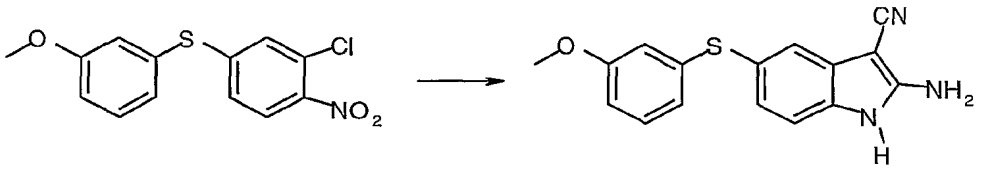
Yield : 56%

^{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).

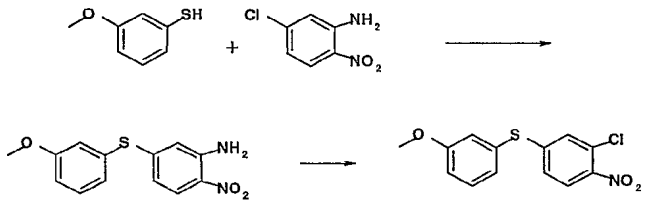
Yield : 19%

^{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:

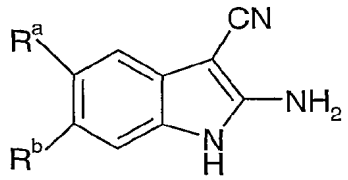
4-(3-Methoxyphenylsulphanyl)-2-chloro-nitrobenzene (2.27 g):

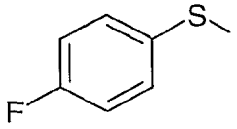
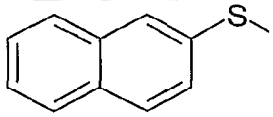
Yield : 53%

^{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).

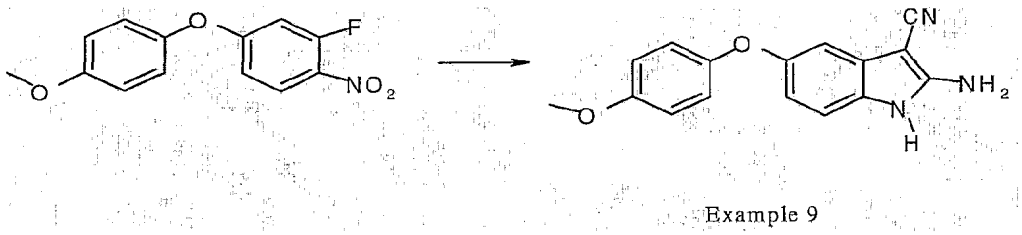
MS-EI : 315 $[M]^+$

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.

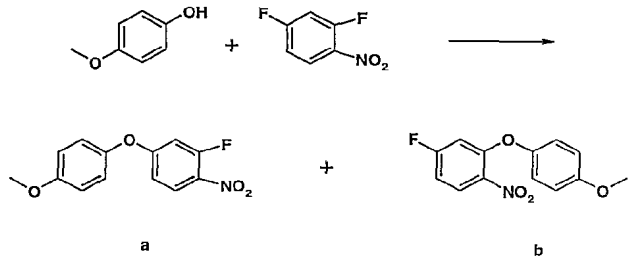
Yield : 21%

^{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).

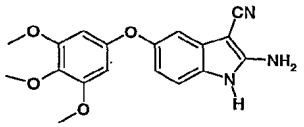
Yield : 50%

^{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).

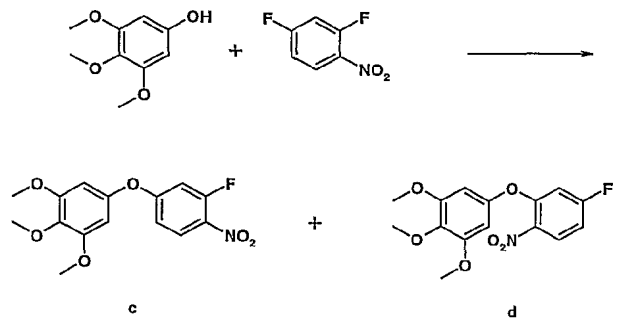
Yield : 18%

^{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).

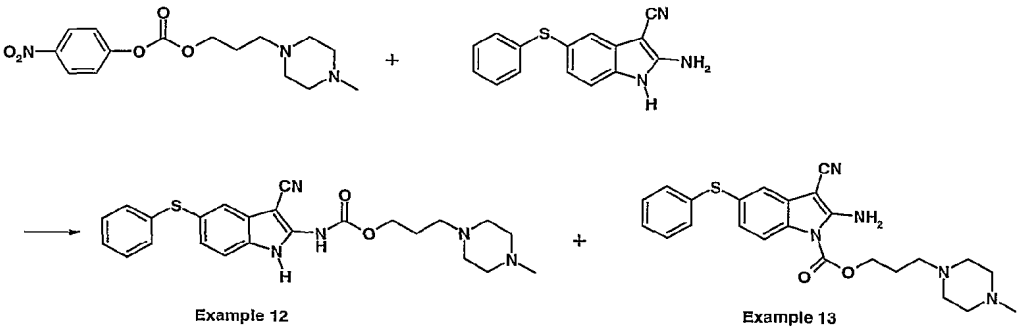
Yield : 83%

^{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%

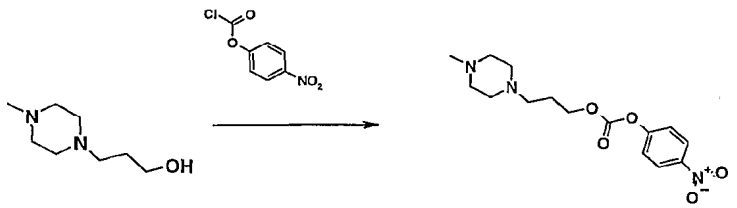
^{1}H NMR spectrum (DMSO d_6) : 1.81 (t, 2H) ; 2.15 (s, 3H) ; 2.2 - 2.25 (m, 10H) ; 4.21 (t, 2H) ; 7.16 - 7.25 (m, 4H) ; 7.3 (m, 2H) ; 7.46 (d, 1H) ; 7.55 (d, 1H) ; 11.2 (br s, 1H) ; 12.1 (br s, 1H).

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

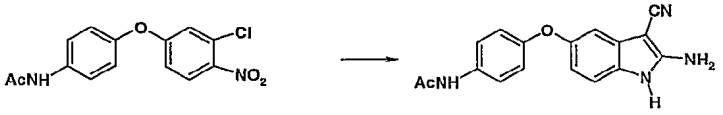
0°C. The mixture was stirred at ambient temperature for 3 hours, evaporated to dryness and triturated with Et_2O/aq. HCl (2N). The precipitate was filtered, washed with Et_2O and dried to give the dihydrochloride of 3-(4-methylpiperazin-1-yl)-propyl 4-nitrophenyl carbonate (1.22g).

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).

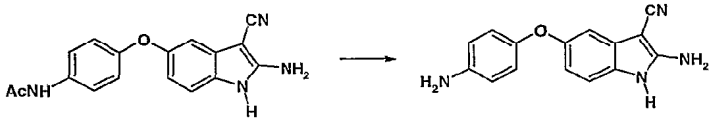
Yield : 72%

^{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%

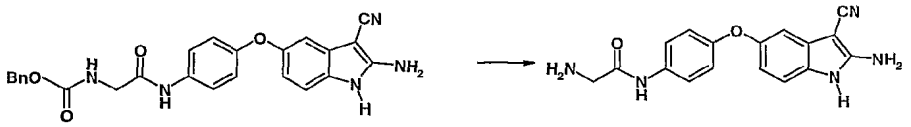
^{1}H NMR spectrum (DMSO d_6) : 4.89 (s, 2H) ; 6.55 (m, 4H) ; 6.73 (m, 4H) ; 7.06 (d, 1H) ; 10.61 (br s, 1H).

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%

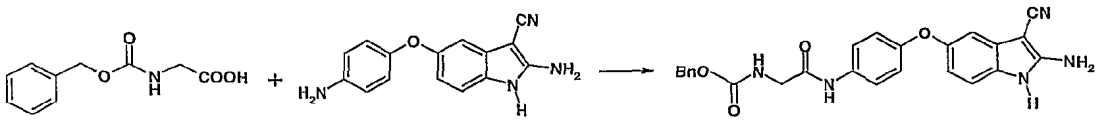
^{1}H NMR spectrum (DMSO d_6) : 2.03 (s, 2H) ; 3.24 (m, 2H) ; 6.59 (dd, 1H) ; 6.66 (d, 1H) ; 6.79 (s, 2H) ; 6.91 (d, 2H) ; 7.10 (d, 1H) ; 7.59 (d, 2H) ; 9.72 (br s, 1H) ; 10.69 (br s, 1H).

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).

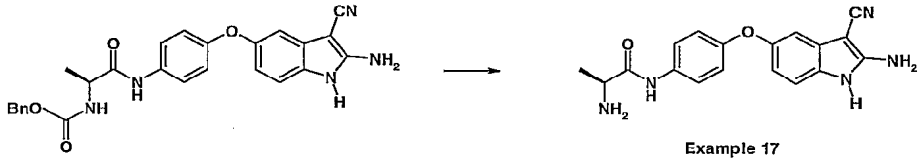
Yield : 83%

^{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-

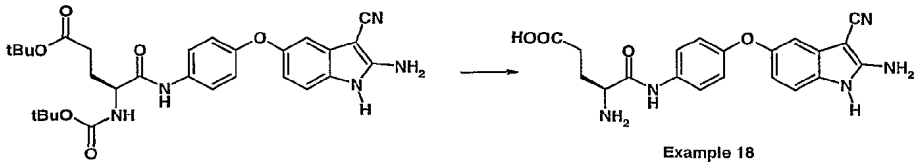
carbonitrile (Example 15)(400 mg ; 1.51 mmol) after purification by flash chromatography eluting with EtOAc/CH_2Cl_2 (50/50).

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).

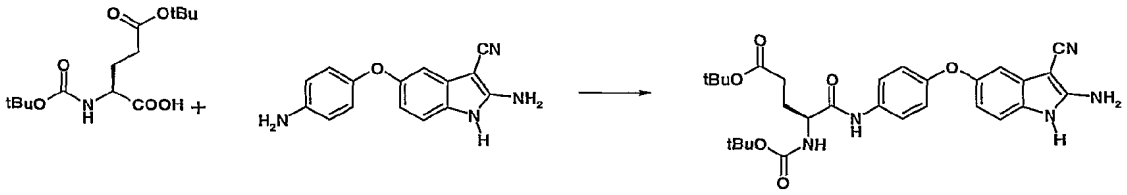
Yield : 100%

^{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).

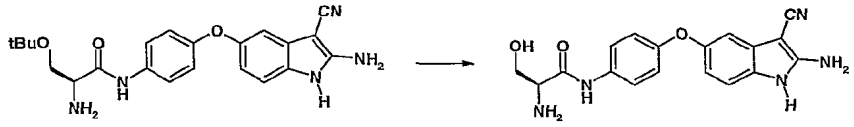
Yield : 91%

^{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.

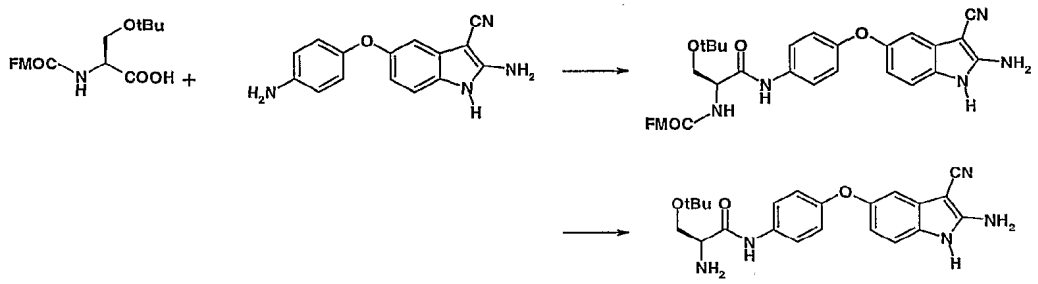
Yield : 73%

^{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).

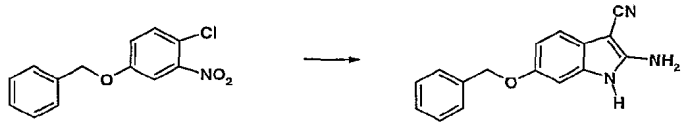
Yield : 94%

^{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).

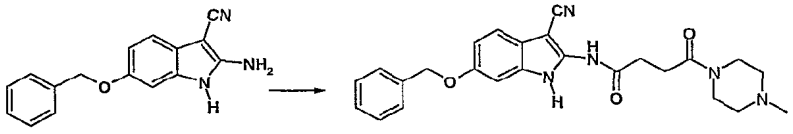
Yield : 94%

^{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%

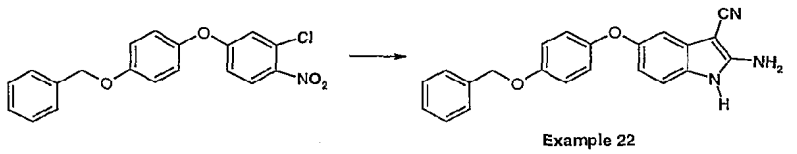
^{1}H NMR spectrum (DMSO d_6) : 2.18 (s, 3H) ; 2.22 (m, 2H) ; 2.31 (m, 2H) ; 2.6 - 2.75 (m, 4H) ; 3.4 - 3.5 (m, 4H) ; 5.08 (s, 2H) ; 6.68 (dd, 1H) ; 7.18 (d, 1H) ; 7.31 (d, 1H) ; 7.33 (t, 1H) ; 7.39 (t, 2H) ; 7.46 (d, 2H) ; 11.4 (br s, 1H) ; 11.9 (br s, 1H).

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

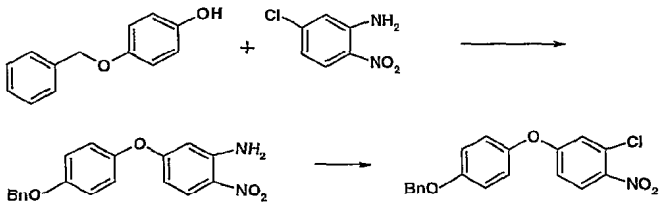
mmol) after purification by flash chromatography eluting with increasingly polar mixtures of CH_2Cl_2/EtOAc (0 to 80% EtOAc).

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).

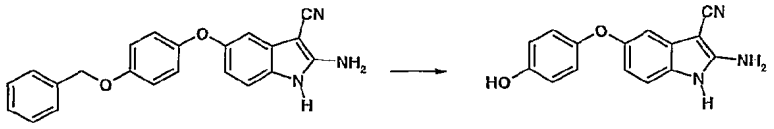
Yield : 20%

^{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%

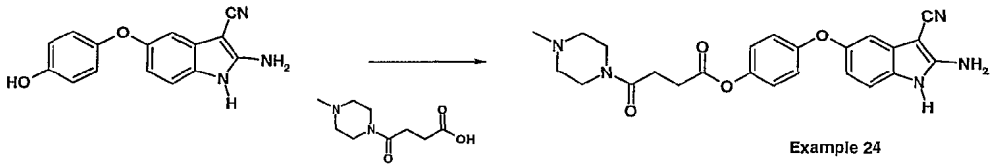
^{1}H NMR spectrum (DMSO d_6) : 6.54 (dd, 1H) ; 6.58 (d, 1H) ; 6.73 (d, 2H) ; 6.75 (s, 2H) ; 6.81 (d, 2H) ; 7.06 (d, 1H) 9.21 (br s , 1H) ; 10.64 (br s, 1H).

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%

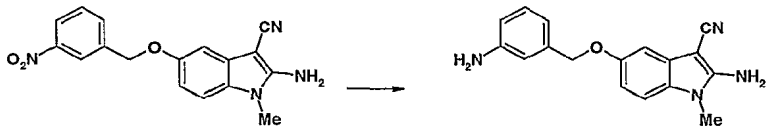
^{1}H NMR spectrum (DMSO d_6) : 2.17 (s, 3H) ; 2.23 (m, 2H) ; 2.30 (m, 2H) ; 2.72 (m, 4H) ; 3.45 (m, 4H) ; 6.61 (dd, 1H) ; 6.73 (d, 1H) ; 6.82 (s, 2H) ; 6.94 (d, 2H); 7.04 (d, 2H) ; 7.13 (d, 1H) ; 10.73 (s, 1H).

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).

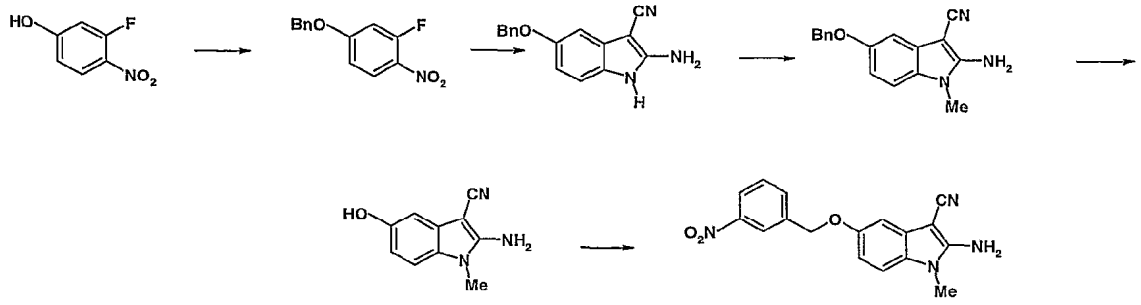
Yield : 91%

^{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).

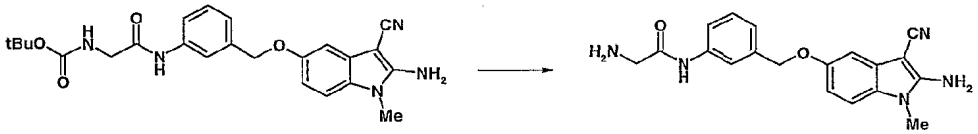
Yield : 56%

^{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%

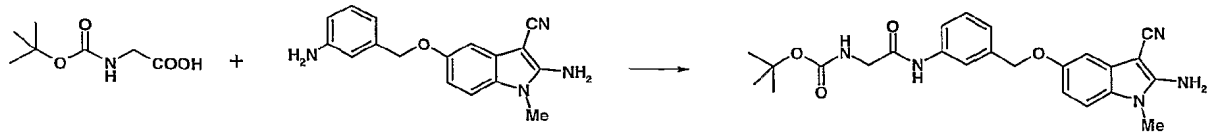
^{1}H NMR spectrum (DMSO d_6) : 3.47 (s, 3H) ; 3.80 (s, 2H) ; 5.10 (s, 2H) ; 6.65 (dd, 1H) ; 6.78 (d, 1H) ; 6.95 (br s, 2H) ; 7.10 (d, 1H) ; 7.18 (d, 1H) ; 7.36 (t, 1H) ; 7.58 (d, 1H) ; 7.68 (s, 1H) ; 8.14 (br s, 3H) ; 10.54 (s, 1H).

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).

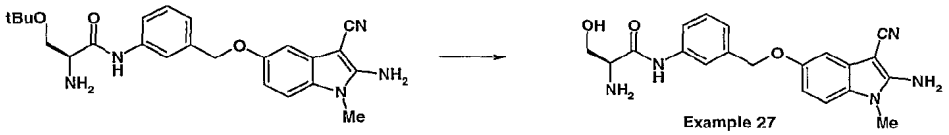
Yield : 80%

^{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).

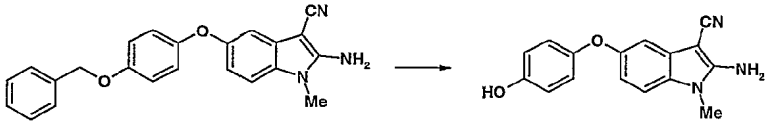
Yield : 42%

^{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

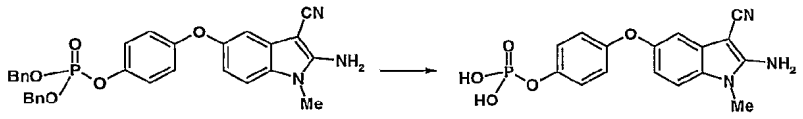
This was prepared using the general method of selective N-alkylation described for 5-benzyloxy-1*H*-indole-3-carbonitrile in Example 25, 5-(4-benzyloxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (1.77 g) was obtained as a white solid from 5-(4-benzyloxyphenloxy)-2-amino-1*H*-indole-3-carbonitrile (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

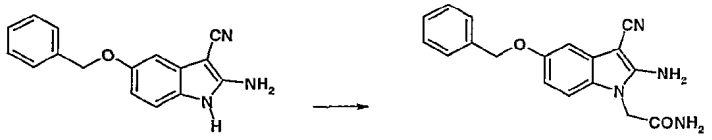
This was prepared as follows:- A solution of 5-(4-hydroxyphenoxy)-2-amino-1-methyl-1*H*-indole-3-carbonitrile (1.4 g ; 5 mmol) in acetonitrile (80 ml), CCl_4 (2.44 ml ; 25 mmol) and diisopropylethylamine (1.82 ml ; 10.5 mmol) was treated with a catalytic amount of DMAP (50 mg) and then cooled to 0°C. 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%

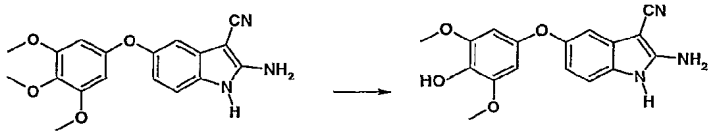
^{1}H NMR spectrum (DMSO d_6) : 4.61 (s, 2H) ; 5.11 (s, 2H) ; 6.65 (dd, 1H) ; 6.81 (d, 1H) ; 6.91 (s, 2H) ; 6.94 (d, 1H) ; 7.30 (s, 1H) ; 7.34 (d, 1H) ; 7.40 (m, 2H) ; 7.47 (m, 2H) ; 7.57 (s, 1H).

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).

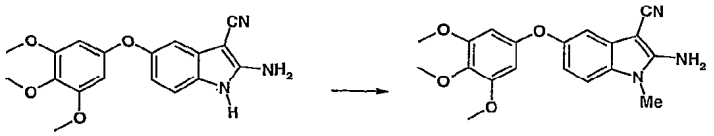
Yield : 100%

^{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%

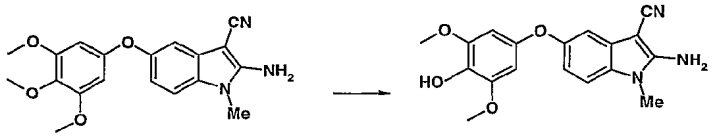
^{1}H NMR spectrum (DMSO d_6) : 3.52 (s, 3H) ; 3.64 (s, 3H) ; 3.69 (s, 6H) ; 6.28 (s, 2H) ; 6.70 (dd, 1H) ; 6.78 (d, 1H) ; 7.05 (s, 2H) ; 7.22 (d, 1H).

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%

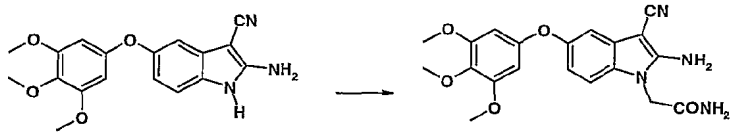
^{1}H NMR spectrum (DMSO d_6) : 3.51 (s, 3H) ; 3.69 (s, 6H) ; 6.30 (s, 2H) ; 6.66 (dd, 1H) ; 6.71 (d, 1H) ; 7.01 (s, 2H) ; 7.19 (d, 1H) ; 8.10 (s, 1H).

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%

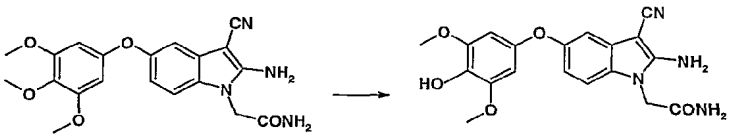
^1H NMR spectrum (DMSO d_6) : 3.64 (s, 3H) ; 3.70 (s, 6H) ; 4.66 (s, 2H) ; 6.31 (s, 2H) ; 6.68 (dd, 1H) ; 6.77 (d, 1H) ; 7.03 (d, 1H) ; 7.04 (s, 2H) ; 7.33 (s, 1H) ; 7.62 (s, 1H).

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.

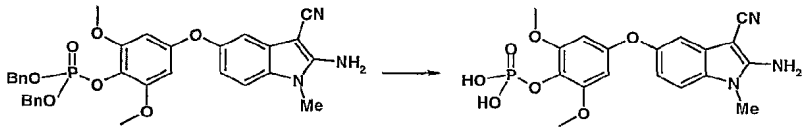
Yield : 67%

^{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).

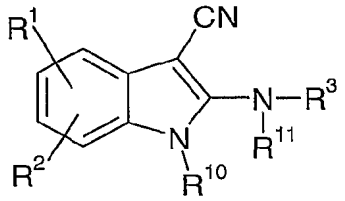
Yield : 44%

^{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 $[M-H]^-$

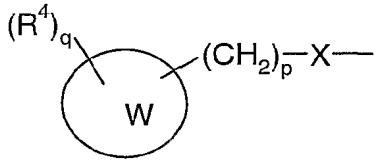
Claims

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,

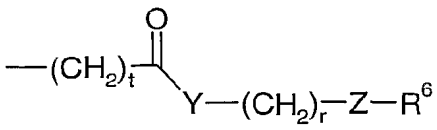
R^4 is independently selected from hydrogen, halogen, -OH, amino, alkanoylamino, —OPO_3H_2, or a hydrocarbyl group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified or two R^4 groups together form an optionally substituted cyclic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

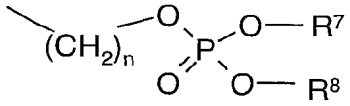
wherein Y is selected from —NH—, —O— or a bond;

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;

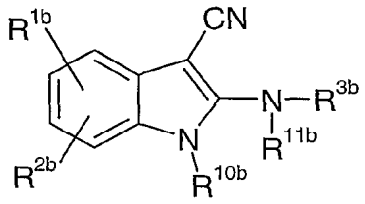
5-(3,4,5-trimethoxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile;

5-(3,4-dimethoxyphenylsulphanyl)-2-amino-1*H*-indole-3-carbonitrile; and

5-(3,4,5-trimethoxyphenoxy)-2-amino-1*H*-indole-3-carbonitrile;

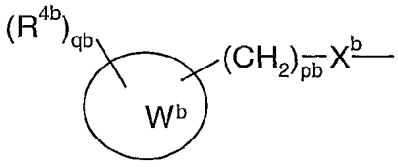
or salt or solvate thereof.

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,

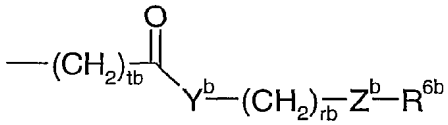
R^{4b} is independently selected from hydrogen, halogen, -OH, amino, alkanoylamino, —OPO_3H_2, or a hydrocarbyl group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified or two R^{4b} groups together form an optionally substituted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

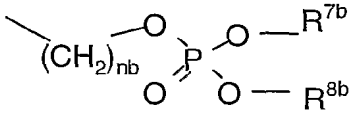
wherein Y^b is selected from —NH—, —O— or a bond;

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

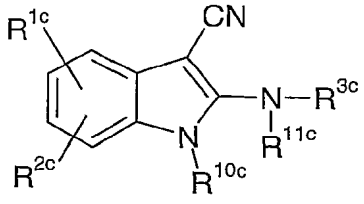
R^{11b} is hydrogen or lower alkyl;

or a salt or solvate thereof;

provided that:

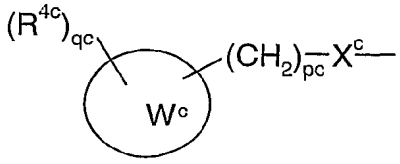
ii) when R^1 is an unsubstituted phenylthio group (Ph-S-), R^2 is H, R^{10} is H and R^{11} is H then R^3 is neither H nor $-C(O)-O-CH_2CH_3$.

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,

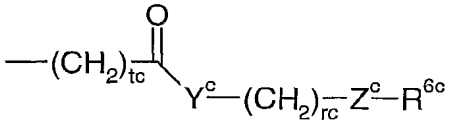
R^{4c} is independently selected from hydrogen, halogen, -OH, amino, alkanoylamino, —OPO_3H_2, or a hydrocarbyl group, wherein the amino group is optionally substituted by an amino acid residue and the hydroxy group is optionally esterified or two R^{4c} groups together form an optionally substituted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

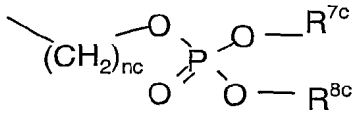
wherein Y^c is selected from —NH—, —O— or a bond;

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)

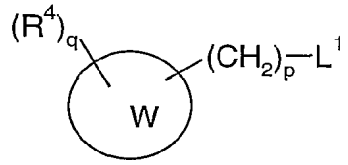
wherein nc is an integer of from 1 to 6, and;

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

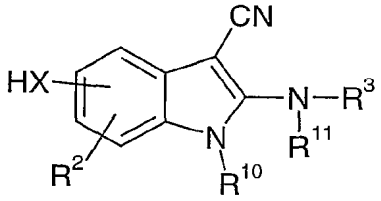
R^{11c} is hydrogen or lower alkyl;

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),



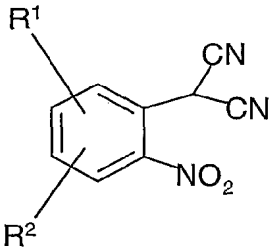
Formula (A)



Formula (B)

wherein L^1 is a leaving group;

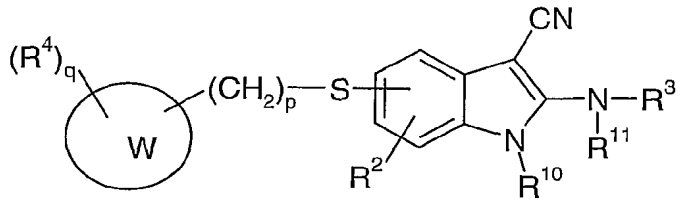
b) for compounds of Formula (I) in which R^3 is hydrogen and R^{11} is hydrogen may be prepared by reduction followed by ring closure of compounds of Formula (C):



Formula (C)

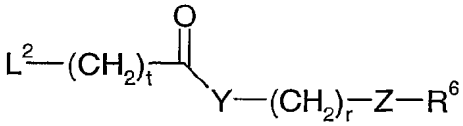
c) for compounds of Formula (I) wherein R^{10} is lower alkyl, reacting a compound of Formula (I) wherein R^{10} is hydrogen with a suitable alkylhalide,

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 : A61K 31/66, 31/165, 31/27, C07C 13/547 — A1

(11) International Publication Number: **WO 99/02166**

(43) International Publication Date: 21 January 1999 (21.01.99)

(21) International Application Number: PCT/GB98/01977

(22) International Filing Date: 6 July 1998 (06.07.98)

(30) Priority Data:
9714249.1 8 July 1997 (08.07.97) GB

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

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: DOUGHERTY, Graeme [GB/GB]; 4 Bonaly Grove, Edindurgh EH13 0QD (GB).

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

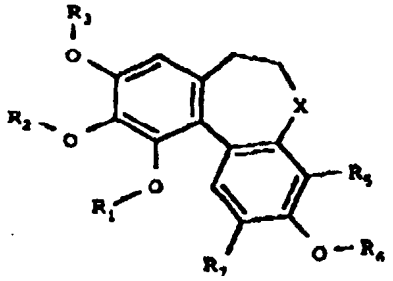
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, 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, 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: USE OF COLCHINOL DERIVATIVES AS VASCULAR DAMAGING AGENTS

(57) Abstract

Colchinol derivatives of formula (I) wherein R_1, R_2, R_3 and R_6 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, alkanoyl, PO_3H_2; X is carbonyl (CO), thiocarbonyl (CS), methylene (CH_2) or a group CHR_4; R_4 is OH, O–alkyl or NR_8R_9; R_5 and R_7 are each independently H, alkyl, halogen, hydroxy, alkoxy, nitro or amino; R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulfonyl, arylsulfonyl, aminosulfonyl, alkylaminosulfonyl, dialkylaminosulfonyl or arylaminosulfonyl; and R_9 is H, alkyl or cycloalkyl and the pharmaceutically acceptable salts, solvates, and hydrates thereof have been found to be useful for treatment of diseases involving angiogenesis. Some of these compounds are novel. Particularly preferred are those compounds in which R_6 is PO_3H_2.



(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.

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		

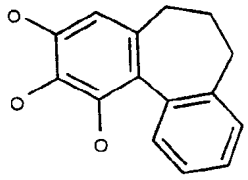
USE OF COLCHINOL DERIVATIVES AS VASCULAR DAMAGING AGENTS

This invention relates to vascular damaging agents and particularly to use in the preparation of agents for treatment of neovascularisation of a group of colchinol derivatives some of which are new compounds.

Formation of new vasculature by angiogenesis is a key pathological feature of several diseases (J Folkman, New England Journal of Medicine 333, 1757 (1995)). For example, for a solid tumour to grow it must develop its own blood supply upon which it depends critically for the provision of oxygen and nutrients; if this blood supply is mechanically shut off the tumour undergoes necrotic death. Neovascularisation is also a clinical feature of skin lesions in psoriasis, of the invasive pannus in the joints of rheumatoid arthritis patients and of atherosclerotic plaques. Retinal neovascularisation is pathological in macular degeneration and in diabetic retinopathy. In all these diseases reversal of neovascularisation by damaging the newly-formed vascular endothelium is expected to have a beneficial therapeutic effect.

Colchinol derivatives for example N-acetyl-colchinol are known. Anti-tumour effects have been noted on animal models (see for example - JNCI (Journal National Cancer Institute) Page 379-392 1952, Vol 13). However, the effect studied was that of gross damage (haemorrhage, softening and necrosis) and there is no suggestion of treatment of inappropriate angiogenesis by destruction of neovasculature.

A search of Chemical Abstracts (post 1955) based on the substructure

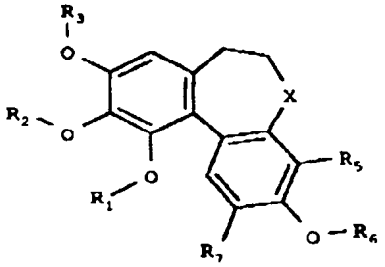


revealed a number of colchinol related structures.

To the extent that any of these compounds have been studied for anti-cancer activity it is because tubulin binding agents might be expected to be anti-mitotic and therefore to have a direct effect on tumour cells.

In the course of the work on the present invention, the issue of the relevance of tubulin-binding properties to possible effectiveness as anti-vascular agent was studied but no predictability was found. Thus docetaxel (Lancet, 344, 1267-1271, 1994), which is a tubulin-binding agent, had no vascular-damaging effects even when administered at its Maximum Tolerated Dose. Even when the present inventors tested some compounds structurally related to the present invention, the therapeutic window (ratio of MTD (Maximum tolerated dose) to MED (Minimum effective dose)) was found to be too small for potential clinical effectiveness.

According to the present invention there is provided the use of colchinol derivatives for the preparation of compositions for the treatment of diseases involving angiogenesis in which the colchinol derivative has the formula



I

wherein

R_1, R_2, R_3 and R_6 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, alkanoyl, PO_3H_2;

X is carbonyl (CO), thiocarbonyl (CS), methylene (CH_2) or a group CHR_4

R_4 is OH, O-alkyl or NR_8R_9;

R_5 and R_7 are each independently H, alkyl, halogen, hydroxy, alkoxy, nitro or amino;

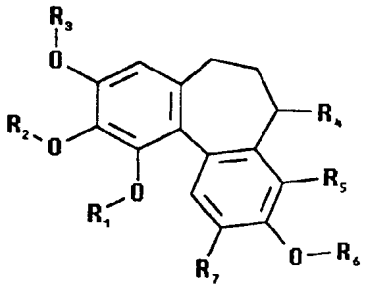
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;

and R_9 is H, alkyl or cycloalkyl

and the pharmaceutically acceptable salts, solvates, and hydrates thereof.

It is believed, though this is not limiting on the invention, that the use of compounds of the invention damages newly-formed vasculature, for example the vasculature of tumours, thus effectively reversing the process of angiogenesis as compared to known anti-angiogenic agents which tend to be less effective once the vasculature has formed.

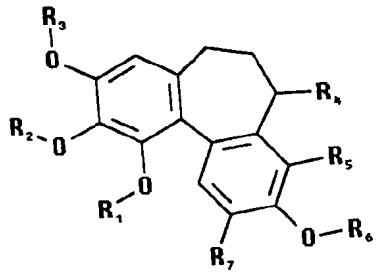
Certain of these compounds are novel. In one embodiment the novel compounds are those of formula I in which at least one of R_1, R_2, R_3, R_6 is PO_3H_2. In a particular preferred embodiment R_6 is PO_3H_2. Particularly preferred are compounds defined by the formula



II

wherein
R_1, R_2 and R_3 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, alkanoyl, or PO_3H_2;
R_6 is PO_3H_2;
R_4 is H or NR_8R_9;
R_5 and R_7 are each independently H, alkyl, halogen, alkoxy, nitro or amino;
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;
and R_9 is H, alkyl or cycloalkyl,
and the pharmaceutically acceptable salts, solvates and hydrates thereof.

In another aspect of the invention the novel compounds are of formula



IIA

wherein

R_1, R_2 and R_3 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, alkanoyl or PO_3H_2;

R_6 is H, optionally substituted alkyl, cycloalkyl, alkenyl, akynyl or PO_3H_2;

R_4 is H or NR_8R_9;

R_5 and R_7 are each independently H, alkyl, halogen, nitro or amino;

R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;

and R_9 is H, alkyl or cycloalkyl, with the proviso that, when R_1, R_2 and R_3 are all methyl groups and R_4 is hydrogen, acetylamino, acetylmethylamino, amino, methylamino or dimethylamino then R_6 is not hydrogen, methyl or hydroxyethyl, or acetoxyethyl,

and the pharmaceutically acceptable salts, solvates and hydrates thereof.

Preferred compounds used in the invention and of the invention are those in which R_1, R_2 and R_3 are alkyl and

those in which R_4 is acylamino.

As used herein the term "alkyl", (including any aliphatic structure chain related to alkyl) means a straight or branched-chain group containing from one to seven, preferably a maximum of four, carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, t-butyl and pentyl. Optional substituents which may be present on the alkyl groups include one or more substituents selected from halogen, amino, monoalkylamino, dialkylamino, hydroxy, alkoxy, alkylthio, alkylsulphonyl, acylamino, alkoxycarbonylamino, alkanoyl, acyloxy, carboxyl, sulphate or phosphate groups. Examples of alkoxy groups are methoxy, ethoxy, propoxy, isopropoxy, butoxy and t-butoxy. The term "halogen" means fluorine, chlorine, bromine or iodine.

An alkenyl group is an olefinic group containing from two to seven carbon atoms for example methylene, ethylene, n-propylene, i-propylene, n-butylene, i-butylene, s-butylene and t-butylene. An alkynyl group is of a group of 2-7 carbon atoms for example ethynyl, propynyl or butynyl group.

The term aryl alone or in combination means an unsubstituted phenyl group or a phenyl group carrying one or more, preferably one to three, substituents examples of which are halogen, alkyl, haloalkyl, hydroxy, nitro, cyano, amino and alkoxy. A haloalkyl group can carry one or more halogen atoms with the examples of such groups being trifluoromethyl and dichloromethyl.

The term heteroaryl is defined herein as a mono- or bi-cyclic aromatic group containing one to four heteroatoms selected in any combination from N, S or O atoms and a maximum of 9 carbon atoms. Examples of heteroaryl groups include pyridyl, pyrimidyl, furyl, thienyl, pyrrolyl,

pyrazolyl, indolyl, benzofuryl, benzothienyl, benzothiazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, imidazolyl, triazolyl, quinolyl and isoquinolyl groups.

The term aralkyl is defined herein as an alkyl group, as previously defined, in which one of the hydrogen atoms is replaced by an aryl or heteroaryl group as defined herein.

Where one or more functional groups in compounds of formulae I, II, IIA are sufficiently basic or acidic the formation of salts is possible. Suitable salts include pharmaceutically acceptable salts for example acid addition salts including hydrochlorides, hrdrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates and tartrates, salts derived from inorganic bases including alkali metal salts such as sodium or potassium salts, alkaline earth metal salts such as magnesium or calcium salts, and salts derived from organic amines such as morpholine, piperidine or dimethylamine salts.

Those skilled in the art will recognise that compounds of formulae I, II, IIA may exist as stereoisomers and/or geometrical isomers and accordingly the present invention includes all such isomers and mixtures thereof.

One useful group of compounds includes those in which R_1, R_2 and R_3 are each alkyl.

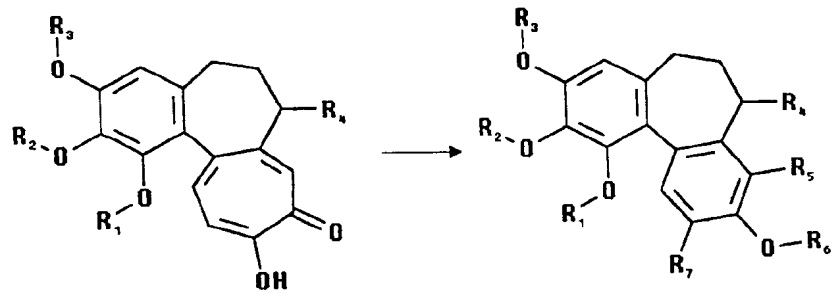
Another useful group of compounds includes those in which R_1, R_2 and R_3 are each alkyl and R_5 and R_7 are each hydrogen. A particularly useful subset of this group includes compounds in which R_1, R_2 and R_3 are each methyl and R_6 is hydrogen, alkyl or PO_3H_2.

Particularly useful compounds according to the invention include:

N-Acetylcolchinol-O-phosphate and its salts, solvates and hydrates.

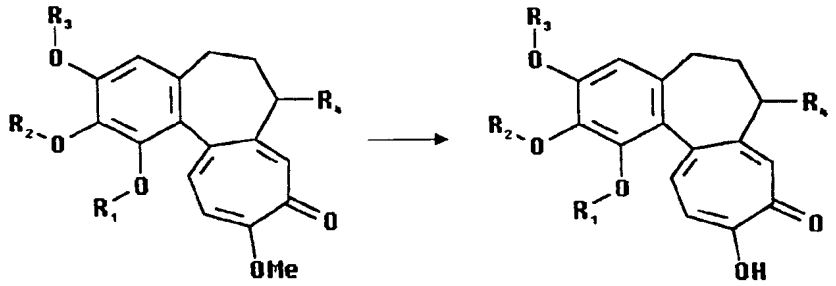
Compounds of formulae I, II or IIA may be prepared by a number of processes as generally described below and more specifically in the Examples hereinafter. In the following process description the symbols R_1, R_2, R_3, R_4, R_5, R_6 and R_7 when used in the formulae depicted are to be understood to represent those groups described above in relation to formulae I, II or IIA unless otherwise indicated. In the schemes described below it may be necessary to employ protecting groups which are then removed during the final stages of the synthesis. The appropriate use of such protecting groups and processes for their removal will be readily apparent to those skilled in the art.

Thus according to a further aspect of the invention compounds of formulae II or IIA in which R_5, R_6 and R_7 are each hydrogen may be prepared by treatment of a compound of formula (2) with alkaline hydrogen peroxide. The reaction may be conveniently performed in aqueous sodium hydroxide solution in the absence or presence of a cosolvent such as an alcohol, for example ethanol, at a temperature in the range for example 0- 100°C preferably at or near to 60°C.



(2) II or IIA

Intermediates of formulae (2) may be prepared by acid hydrolysis of compounds of formulae (3); The reaction is conveniently carried out in an aqueous acid such as hydrochloric acid at an elevated temperature, for example at or near 100°C.



(3) (2)

Compounds of formula (3) are either known or can be prepared from colchicine by conventional procedures.

Compounds of formulae I, II or IIA may also be prepared from other compounds of formulae I, II or IIA, by chemical modification. Examples of such chemical modifications that may be applied are standard alkylation, arylation, heteroarylation, acylation, thioacylation, sulphonylation, sulphation, phosphorylation, aromatic halogenation and coupling reactions. These reactions may be used to add new substituents or to modify existing substituents. Alternatively, existing substituents in compounds of formulae I, II or IIA may be modified by, for example oxidation, reduction, elimination, hydrolysis or other cleavage reaction to yield other compounds of formulae I, II or IIA.

Thus for example a compound of formulae II or IIA containing an amino group may be acylated on the amino group by treatment with, for example, an acyl halide or anhydride in the presence of a base, for example a

tertiary amine base such as triethylamine, in for example, a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In another general example of an interconversion process an amino group in a compound of formulae II or IIA may be sulphonylated by treatment with, for example, an alkyl or aryl sulphonyl chloride or an alkyl or aryl sulphonic anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for example a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In a further general example a compound of formulae II or IIA containing a hydroxy group can be converted into the corresponding dihydrogenphosphate ester by treatment with for example di-tert-butyl diethylphosphoramidite in the presence of a suitable catalyst for example tetrazole. In a solvent such as an ether solvent for example tetrahydrofuran at a temperature in the range -40 to 40°C, conveniently at or near room temperature, followed by treatment with an oxidising agent for example 3-chloroperoxy benzoic acid at a temperature in the range -78°C to 40°C preferably -40 to -10°C. The resulting intermediate phosphate triester is treated with an acid for example trifluoroacetic acid in a solvent such as a chlorinated solvent e.g. dichloromethane at a temperature in the range -30 to 40°C conveniently at or near 0°C to give the compound of formula (2) containing a dihydrogenphosphate ester.

In a further general example a compound of formula (2) containing an amide can be hydrolysed by treatment with

for example an acid such as hydrochloric acid in a solvent such as an alcohol, for example methanol at an elevated temperature conveniently at the reflux temperature.

In another general example an O-alkyl group may be cleaved to the corresponding alcohol (OH) by reaction with boron tribromide in a solvent such as a chlorinated solvent e.g. dichloromethane at a low temperature e.g. around -78°C.

In a further general example compounds of formulae II or IIA may be alkylated by reaction with a suitable alkylating agent such as an alkyl halide, an alkyl toluenesulphonate, an alkyl methanesulphonate or an alkyl triflate. The alkylation reaction can be carried out in the presence of a base for example an inorganic base such as a carbonate e.g. caesium or potassium carbonate, a hydride such as sodium hydride or an alkoxide such as potassium t-butoxide in a suitable solvent such as an aprotic solvent e.g. dimethylformamide or an ether solvent such as tetrahydrofuran at a temperature of around -10 to 80°C.

Preparation of a compound of formulae II or IIA as a single enantiomer or, where appropriate, diastereomer may be effected by synthesis from an enantiomerically pure starting material or intermediate or by resolution of the final product in a conventional manner.

Acid addition salts of the compounds of formulae II or IIA are prepared in a conventional manner by treating a solution or suspension of the free base II or IIA with about one equivalent of a pharmaceutically acceptable acid. Salts of compounds of formulae I, II or IIA derived from inorganic or organic bases are prepared in a conventional manner by treating a solution or suspension

of the free acid I, II or IIA with about one equivalent of a pharmaceutically acceptable organic or inorganic base. Alternatively both acid addition salts and salts derived from bases may be prepared by treatment of the parent compound with the appropriate ion-exchange resin in a standard fashion. Conventional concentration and recrystallisation techniques are employed in isolating the salts.

Compounds according to the invention are able to destroy tumour vasculature and vasculature that has been newly formed while leaving unaffected normal, mature vasculature. The ability of the compounds to act in this way may be determined by the tests described in the Examples hereinafter.

The compounds according to the invention are thus of particular use in the prophylaxis and treatment of cancers involving solid tumours and in the prophylaxis and treatment of diseases where inappropriate angiogenesis occurs such as diabetic retinopathy, psoriasis, rheumatoid arthritis, atherosclerosis and macular degeneration.

The compounds of the invention may be administered as a sole therapy or in combination with other treatments. For the treatment of solid tumours compounds of the invention may be administered in combination with radiotherapy or in combination with other anti-tumour substances for example those selected from mitotic inhibitors, for example vinblastine, paclitaxel and docetaxel; alkylating agents, for example cisplatin, carboplatin and cyclophosphamide, antimetabolites, for example 5-fluorouracil, cytosine arabinoside and hydroxyurea; intercalating agents for example adriamycin and bleomycin; enzymes, for example aspariginase; topoisomerase inhibitors for example etoposide, topotecan

and irinotecan; thymidylate synthase inhibitors for example raltitrexed; biological response modifiers for example interferon; antibodies for example edrecolomab; and anti-hormones for example tamoxifen. Such combination treatment may involve simultaneous or sequential application of the individual components of the treatment.

For the prophylaxis and treatment of disease the compounds according to the invention may be administered as pharmaceutical compositions selected with regard to the intended route of administration and standard pharmaceutical practice. Such pharmaceutical compositions may take a form suitable for oral, buccal, nasal, topical, rectal or parenteral administration and may be prepared in a conventional manner using conventional excipients. For example for oral administration the pharmaceutical compositions may take the form of tablets or capsules. For nasal administration or administration by inhalation the compounds may be conveniently delivered as a powder or in solution. Topical administration may be as an ointment or cream and rectal administration may be as a suppository. For parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) the composition may take the form of, for example, a sterile solution, suspension or emulsion.

The dose of a compound of the invention required for the prophylaxis or treatment of a particular condition will vary depending on the compound chosen, the route of administration, the form and severity of the condition and whether the compound is to be administered alone or in combination with another drug. Thus the precise dose will be determined by the administering physician but in general daily dosages may be in the range 0.001 to 100mg/kg preferably 0.1 to 50mg/kg.

BIOLOGICAL ACTIVITY

The following tests were used to demonstrate the activity and selectivity of compounds according to the invention.

Activity against tumour vasculature measured by radioactive tracer.

The following experiment demonstrates the ability of the compounds to damage selectively tumour vasculature.

Subcutaneous CaNT tumours were initiated by injecting 0.05ml of a crude tumour cell suspension, approximately 10^6 cells, under the skin overlying the rear dorsum of 12-16 week-old mice. The animals were selected for treatment after approximately 3-4 weeks, when their tumours reached a geometric mean diameter of 5.5-6.5mm. Compounds were dissolved in sterile saline and injected intraperitoneally in a volume of 0.1 ml per 10 g body weight. Tumour perfusion was measured 6 hours after intraperitoneal administration in tumour, kidney, liver, skin muscle, gut and brain by the ^{86}RbCI extraction technique (Sapirstein, Amer J Physiol, 193, 161-168, 1958). Tissue radioactivity measured 1 minute after an intravenous injection of ^{86}RbCI was used to calculate relative blood flow as a proportion of cardiac output (Hill and Denekamp, Brit J Radiol, 55, 905-913, 1982). Five animals were used in control and treated groups. Results were expressed as a percentage of the blood flow in the corresponding tissues in vehicle treated animals.

Activity against tumour vasculature measured by fluorescent dye.

The following experiment further demonstrates the ability of the compounds to damage tumour vasculature.

Tumour functional vascular volume in CaNT tumour-bearing mice was measured using the florescent dye Hoechst 33342

according to the method of Smith *et al* (Brit J Cancer 57, 247-253, 1988). Five animals were used in control and treated groups. The fluorescent dye was dissolved in saline at 6.25 mg/ml and injected intravenously at 10 mg/kg 6 hours after intraperitoneal drug treatment. One minute later, animals were killed and tumours excised and frozen; 10μm sections were cut at 3 different levels and observed under UV illumination using an Olympus microscope equipped with epifluorescence. Blood vessels were identified by their fluorescent outlines and vascular volume was quantified using a point scoring system based on that described by Chalkley, (J Natl Cancer Inst, 4, 47-53, 1943). All estimates were based on counting a minimum of 100 fields from sections cut at the 3 different levels. Compounds of the invention reduced tumour functional vascular volume by greater than 20% at doses of 50mg/kg or below.

The following non-limiting Examples illustrate the invention. In the Examples all ^{1}Hnmr were run at 300MHz unless otherwise specified. Column chromatography was performed on silica gel. All temperatures are in °C. The following abbreviations are used: THF - tetrahydrofuran; DMSO - dimethylsulphoxide; MCPBA - 3-chloroperoxybenzoic acid.

EXAMPLE 1

N-Acetylcolchinol-O-phosphate

A solution of N-acetylcolchinol (260mg, 0.76 mmol) in anhydrous THF (2ml) under an atmosphere of nitrogen was treated with di-t-butyl diethylphosphoramidite (189mg, 0.75mmol) and 1(H)-tetrazole (0.14g, 1.99mmol) and the solution stirred at 20° for 0.5h. The solution was cooled to -40° and a solution of 85% MCPBA (202mg, 0.99mmol) in anhydrous dichloromethane (2ml) at such a rate that the temperature remained below -10°. The solution was allowed to warm to room temperature, diethyl

ether (30ml) was added and the resulting solution washed successively with 10% aqueous sodium metabisulphite (two 25ml portions), 5% aqueous sodium bicarbonate (two 25ml portions), 5% aqueous citric acid (30ml), 5% aqueous sodium bicarbonate, and brine. The organic solution was concentrated under reduced pressure and the residue subjected to column chromatography to afford a white foam (170mg) containing N-acetylcolchinol-O-di-*t*-butylphosphate which was redissolved in dichloromethane (5ml), cooled to 0° and treated with trifluoroacetic acid (0.5ml).

The solution was allowed to warm to room temperature and stir 1 hr before being concentrated under reduced pressure and triturated with ether to give the title compound (110mg) as a white solid m.p. 233-235°. δH (d6-DMSO) 8.38(d,1H, J=8Hz), 7.27(d, 1H,J=7Hz), 7.12(d, 1H, J=8Hz), 7.10(s,1H), 6.77 (s, 1H), 4.48 (m, 1H), 3.81 (s, 3H), 3.76 (s, 3H), 3.49 (s, 3H), 2.5 (signal partially obscured by DMSO peak), 1.9-2.2 (m, 2H), 1.86 (s, 3H).

The activity of the phosphate compound was measured by the radioactive tracer assay described above: the compound of this Example gave a 65% decrease in tumour blood flow at a dose of 125mg/kg with no significant reduction in blood flow in skin, muscle, liver, kidney, gut or heart.

The phosphate compound was compared with the parent N-acetylcolchinol for the maximum tolerated dose (MTD) (no deaths in three animals), minimum effective dose (MED) measured by the fluorescent dye technique already described and the therapeutic window (MTD/MED).

	MTD mg/Kg body weight	MED mg/Kg body weight	Therapeutic Window (MTD/MED)
N-acetyl colchinol	125	30	4
N-acetyl colchinol-O-phosphate	750	50	15

Though the phosphate had a slightly higher MED the "window" was significantly greater. This was unexpected. The phosphate also gave greater solubility.

For comparison the "therapeutic windows" for colchicine (the closest structure to the present compounds and docetaxel (a tubulin-binding drug, marketed as "Taxotere", which has no vascular-damaging activity) and these data are presented in the following table:

Table 1 - Therapeutic windows of other tubulin-binding agents (by fluorescent dye technique)

Compound	MED (mg/kg body weight)	MTD (mg/kg body weight)	MTD/MED
Docetaxel	>30(No effect at 30)	30	<1
Colchicine	2.5	5	2

EXAMPLE 2

N-Ethylcolchinol

A solution of N-acetylcolchinol (500mg, 1.4mmol) in THF (15ml) was added dropwise over 15 minutes to a suspension of lithium aluminum hydride (106mg, 2.74mmol) in THF (10ml) with ice-bath cooling. The mixture was heated at reflux for 15h, allowed to cool and treated with further lithium aluminium hydride (53mg, 1.4mmol) before heating at reflux for a further 3h. The mixture was cooled (ice bath) and water (10ml) added dropwise before extraction with three portions of ethyl acetate. The combined,

dried (MgSO4) extracts were concentrated under reduced pressure to a green gum which, on trituration with ether, gave the title compound as a light-green solid. m.p. 185°C (dec.), m/e 343 (M+). Anal. Calculated for $C_{20}H_{25}NO_4$ H_2O: C, 66.46; H, 7.53; N, 3.88. Found: C,66.50; H, 7.17; N, 3.79.

EXAMPLE 3

N-Benzyloxycarbonylcolchinol

A solution of colchinol (625mg, 1.98mmol) in dry pyridine (10ml) was treated dropwise with benzylchloroformate (0.566ml, 3.97 mmol) and the mixture stirred 16h. Solvent was removed under reduced pressure, water added and the resulting mixture extracted with three portions of chloroform. The combined, dried ($MgSO_4$) extracts were concentrated under reduced pressure to a dark brown gum which was subjected to column chromatography on silica gel eluting with 50% ethyl acetate/petroleum ether. The resultant orange gum was crystallised from ether/petroleum ether to give the title compound (346mg) as a pale yellow solid. m.p. 79-81°C, m/e 449 (M+). Anal. Calculated for $C_{26}H_{27}NO_6$ $0.33H_20$; C, 68.57; H, 6.07; N, 3.08. Found: C,68.71; H, 6.18; N, 2.91.

EXAMPLE 4

N-(Phenylcarbamoyl) colchinol

A solution of colchinol (400mg, 1.27mmol) in dry pyridine (10ml) was treated dropwise with phenyl isocyanate (0.151ml, 1.39mmol) and the mixture stirred for 18h before heating at reflux for 2h. Solvent was removed under reduced pressure, water added and the resulting mixture extracted with three portions of chloroform. The combined, dried ($MgSO_4$) extracts were concentrated under reduced pressure to a dark brown gum which was subjected to column chromatography on silica gel eluting with 35% ethyl acetate/petroleum ether. The resultant gum was crystallised from ether/petroleum ether to give the title

compound (261mg) as a pale orange solid. m.p. 145-146°C, m/e 434 (M+).

EXAMPLE 5

N-Mesylcolchinol

A solution of N,O-dimesylcolchinol (234mg, 0.5mmol) in methanol (8ml) was treated with sodium hydroxide (40mg, 1mmol) and the mixture heated at reflux for 3h. Solvent was removed under reduced pressure and water (5ml) added. The solution was rendered neutral by the addition of 1M hydrochloric acid and extracted with three portions of dichloromethane. The combined, dried ($MgSO_4$) extracts were concentrated under reduced pressure to give the title compound (123mg) as a pink solid. m.p. 234-236°C, m/e 393 (M+).

The N,O-dimesylcolchinol used as starting material was prepared as follows: A solution of colchinol (500mg, 1.6mmol) in dry pyridine (15ml) was treated with mesyl chloride (0.135ml, 1.7mmol) and the mixture stirred at room temperature 36h. A further portion of mesyl chloride (0.135ml, 1.7mmol) was added and stirring continued 16h. Solvent was removed under reduced pressure and water (5ml) added. The solution was extracted with three portions of chloroform and the combined, dried ($MgSO_4$) extracts were concentrated under reduced pressure to give a brown gum which was subjected to column chromatography on silica gel eluting with ethyl acetate to give N,O-dimesylcolchinol (292mg) as a light orange solid.

EXAMPLE 6

N-Dimethylsulphamoylcolchinol

A solution of colchinol (50mg, 0.16mmol) in dry acetonitrile (3ml) and triethylamine (0.022ml, 0.16mmol) was treated with dimethylsulphamoyl chloride and the mixture stirred for 30 minutes before heating at reflux

for 15h. Solvent was removed under reduced pressure, water added and the resulting mixture extracted with three portions of chloroform. The combined, dried (Na_2SO_4) extracts were concentrated under reduced pressure to a dark brown gum which was subjected to column chromatography on silica gel eluting with ethyl acetate to give the title compound (46mg) as a pale orange gum which solidified. m.p. 82-85°C m/e 422 (M+).

EXAMPLE 7

N-Acetyl-O-methoxycarbonylmethylcolchinol

A solution of N-acetylcolchinol (500mg, 1.4mmol) in dry DMF (5ml) at 0°C was treated with methylbromoacetate (322mg, 2.1mmol) and sodium hydride (84mg of a 60% suspension in oil, 2.1mmol) and the mixture stirred for 30 minutes. Water (50ml) was added and the mixture extracted with four portions of ethyl acetate. The combined extracts were washed successively with four portions of water and two portions of saturated aqueous sodium chloride solution, dried ($MgSO_4$) and solvent removed under reduced pressure to give the title compound (280mg) as a white solid m.p. 82-83°C. m/e 429 2 (M+). Anal. Calculated for $C_{23}H_{27}NO_7$ $0.33H_20$; C, 63.45; H, 6.40; N, 3.22. Found: C, 63.53; H, 6.29; N, 3.17.

EXAMPLE 8

N-Acetyl-O-carboxymethylcolchinol

A solution of N-acetyl-O-methoxycarbonylmethylcolchinol (140mg, 0.33mmol) in acetonitrile (5ml) was treated with aqueous potassium hydroxide solution (1.0M, 5ml) and the mixture heated at 80°C for 30 minutes. The cooled mixture was adjusted to pH3 by addition of 2M hydrochloric acid and extracted with four portions of ethyl acetate. The combined extracts were washed with two portions of saturated aqueous sodium chloride solution, dried ($MgSO_4$) and concentrated under reduced

pressure. Addition of acetone (2ml) and hexane (1ml) produced the title compound (58mg) as a white solid m.p. 220-221°C. m/e 415.3 (M+). Anal. Calculated for $C_{22}H_{25}NO_7$ $0.33H_2O$; C,62.71; H, 6.14; N, 3.32. Found: C, 62.63; H, 6.02; N, 3.26.

EXAMPLE 9

N-Acetyl-O-cyclopentylcolchinol

A solution of N-acetylcolchinol (200mg, 0.56mmol) in dry DMF (2ml) at 0°C was treated with sodium hydride (33mg of a 60% suspension in oil, 0.84mmol) followed by cyclopentyl bromide (125mg, 0.84mmol) and the mixture stirred 1h. A further portion of sodium hydride (17mg of a 60% suspension in oil, 0.42mmol) and of cyclopentyl bromide (63mg, 0.42mmol) and the mixture stirred overnight at room temperature. Water (10ml) was added and the mixture extracted with four portions of ethyl acetate. The combined extracts were washed with two portions of saturated aqueous sodium chloride solution, dried ($MgSO_4$) and concentrated under reduced pressure. The title compound (160mg) was obtained as a white solid m.p. 89-94°C. m/e 425.3 (M+). Anal. Calculated for $C_{25}H_{31}NO_5$: C,70.54; H, 7.35; N, 3.29. Found: C, 70.55; H, 7.35; N, 3.25.

EXAMPLE 10

N-Acetyl-10-nitrocolchinol

A solution of N-acetylcolchinol (100mg, 0.27mmol) in glacial acetic acid (20ml) was treated slowly with 20ml of a solution of concentrated nitric acid (0.34ml) in acetic acid (100ml) keeping the temperature at about 12°C. The mixture was stirred at room temperature for 18h, a further 1ml of the nitric acid/acetic acid solution added and stirring continued for 2h. The mixture was poured onto ice and extracted with three portions of ethyl acetate. The combined extracts were washed with two portions of saturated aqueous sodium

cloride solution, dried ($MgSO_4$) and concentrated under reduced pressure. Purification on silica gel eluting with ethyl acetate gave the title compound (50mg) as a pale yellow solid m.p. 117-8°C. m/e 401.9 (M+) Anal. Calculated for $C_{20}H_{22}N_2O_7$ $0.33H_2O$; C, 58.82; H, 5.56; N, 6.86. Found: C, 58.87; H, 5.66; N, 6.55.

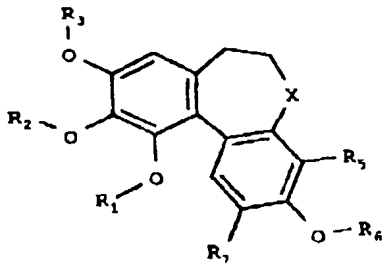
EXAMPLE 11

The activity against tumour vasculature was measured by the fluorescent dye technique described above for the compounds of Examples 1-10 administered at 50mg/kg and N-acetylcolchinol

Compound of Example	% decrease in vascular volume
1	89
2	38
3	43
4	37
5	38
6	30
7	12
8	49
9	59
10	28
N-acetylcolchinol	78

CLAIMS:

1. The use of colchinol derivatives for the preparation of compositions for the treatment of diseases involving angiogenesis in which the colchinol derivative has the formula



I

wherein

R_1, R_2, R_3 and R_6 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, alkanoyl, PO_3H_2;
X is carbonyl (CO), thiocarbonyl (CS), methylene (CH_2) or a group CHR_4
R_4 is OH, O-alkyl or NR_8R_9;
R_5 and R_7 are each independently H, alkyl, halogen, hydroxy, alkoxy, nitro or amino;
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;
and R_9 is H, alkyl or cycloalkyl
and the pharmaceutically acceptable salts, solvates, and hydrates thereof.

2. A use according to claim 1 wherein at least one of R_1, R_2, R_3 and R_6 is PO_3H_2.

3. A use according to claim 2 wherein R_6 is PO_3H_2.

4. A use according to any one of claims 1 to 3 in which R_1, R_2 and R_3 are alkyl.

5. A use according to any one of claims 1 to 4 in which R_4 is acylamino.

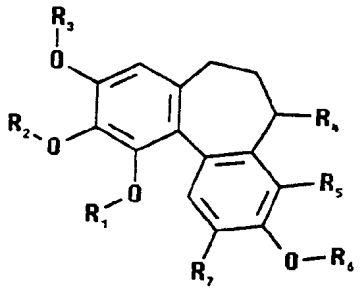
6. A use according to claim 1 in which R_6 is PO_3H, R_1, R_2 and R_3 are alkyl and R_4 is acylamino.

7. Compounds of formula I in which at least one of R_1, R_2, R_3 and R_6 is PO_3H_2.

8. A compound according to claim 7 in which R_6 is PO_3H_2.

9. A compound according to claim 8 in which R_1, R_2 and R_3 are alkyl and R_4 is acylamino.

10. A compound according to claim 7 of formula



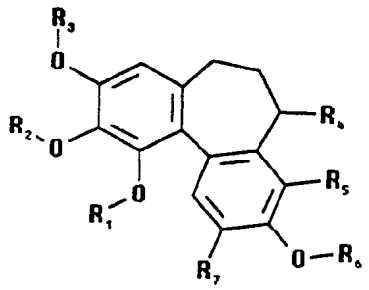
II

wherein

R_1, R_2 and R_3 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, alkanoyl, or PO_3H_2;

R_6 is PO_3H_2;
R_4 is H or NR_8R_9;
R_5 and R_7 are each independently H, alkyl, halogen, alkoxy, nitro or amino;
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;
and R_9 is H, alkyl or cycloalkyl,
and the pharmaceutically acceptable salts, solvates and hydrates thereof.

11. A compound of the formula



IIA

wherein
R_1, R_2 and R_3 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, alkanoyl or PO_3H_2;
R_6 is H, optionally substituted alkyl, cycloalkyl, alkenyl, akynyl or PO_3H_2;
R_4 is H or NR_8R_9;
R_5 and R_7 are each independently H, alkyl, halogen, nitro or amino;
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl,

aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylamnosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;

and R_9 is H, alkyl or cycloalkyl, with the proviso that, when R_1, R_2 and R_3 are all methyl groups and R_4 is hydrogen, acetylamino, acetylmethylamino, amino, methylamino or dimethylamino then R_6 is not hydrogen, methyl, hydroxyethyl, or acetoxyethyl,

and the pharmaceutically acceptable salts, solvates and hydrates thereof.

12. A compound according to claim 11 in which R_1, R_2 and R_3 are alkyl and R_4 is acylamino.

INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/GB 98/01977

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/66 A61K31/165 A61K31/27 C07C13/547

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	O. BOYE ET AL.: "Synthesis of carbon-14 labeled electrophilic ligands of the colchicine binding site of tubulin: chloroacetates of demethylthiocolchicines and of N-acetylcolchinol, isothiocyanates of 9-deoxy-N-acetylcolchinol." J. LABELLED COMPD. RADIOPHARM., vol. 33, no. 4, 1993, pages 293-299, XP002081866 ---	
A	R. BRECHT ET AL.: "Dihydrocolchicine 8,12-endoperoxide. a novel starting material for convenient syntheses of the allocolchicinoids N-acetylcolchinol O-methyl ether and androbiphenyline." LIEBIGS ANN., no. 11, 1997, pages 2275-2279, XP002081867 --- -/--	

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 October 1998	11/11/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Klaver, T

1

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

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/01977

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	GIL-JONG KANG ET AL: "n-acetylcolchinol O-methyl ether and thiocolchicine, potent nalogs of colchicine modified in the C-ring" J. BIOL. CHEM., vol. 265, no. 18, 1990, pages 10255-10259, XP002081868 ---	
A	DATABASE WPI Week 6800 Derwent Publications Ltd., London, GB; AN 66-13831f XP002081869 & JP 39 019635 B (SANKYO CO.) see abstract ---	
A	DATABASE WPI Week 6800 Derwent Publications Ltd., London, GB; AN 66-13830f XP002081870 & JP 39 019634 B (SANKYO CO.) see abstract -----	

1

Patents JP2017503030

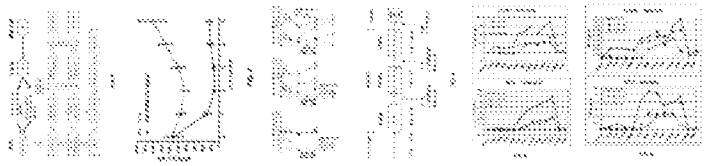
CRF1 receptor antagonist for the treatment of congenital adrenocortical hyperplasia

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: ディミトリィー、グリゴリアディス、,ディミトリィー、グリゴリアディス、

Worldwide applications

2015 CA *MX* JP WO AU EP NZ CA KR RU SG 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 JP6585625B2
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 mineralcorticoid 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 5, 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): 315-21 (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): 8566-59 (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 2000/059888, 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 2011/092290, 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-19567470, 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)" 518-531, 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" (IN0 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
TW477767B	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
US6531475B1	1998-11-12	2003-03-11	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
FR2796388B3	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
US7276526B2	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	エミスフェア・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
TWI370820B	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 (pxdicl) 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
WO2009144632A1	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	斯恩蒂斯有限公司	Adjustable rod assembly
US8551996B2	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
CA2936974A1	2014-01-21	2015-07-30	Neurocrine Biosciences, Inc.	Crf1 receptor antagonists for the treatment of congenital adrenal hyperplasia
WO2016065177A1	2014-10-22	2016-04-28	The Trustees Of The University Of Pennsylvania	Method of treating depression and other stress related disorders

EP3277836B1	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 corcosteroids and/or tnf inhibitors and uses thereof
WO2018219804A1	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 solic 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
KR20220052927A *	2019-07-19	2022-04-28	스프루스 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	美商紐羅克里生物科學有限公司	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
JP6585625B2	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	2008-02-13	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

AU2020318970A1	2022-03-03	Methods of treating congenital adrenal hyperplasia
EP1842543A1	2007-10-10	Pharmaceutical composition coprising an aldosterone synthase inhibitor and a mineralcorticoid receptor antagonist
Charmandari et al	2002	Treatment with flutamide decreases cortisol clearance: implications for therapy in congenital adrenal hyperplasia
CA2446506C	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	0	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
JP2019043466A	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
JP2019043466A	2019-03-11	CRF1 receptor antagonist for the treatment of congenital adrenal hyperplasia

Legal Events

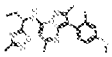
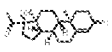
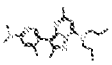
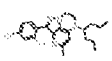
Date	Code	Title	Description
2018-01-19	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20180119
2018-01-19	A621	Written request for application examination	**Free format text**: JAPANESE INTERMEDIATE CODE: A621 **Effective date**: 20180119
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

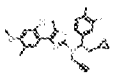
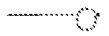
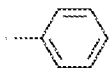
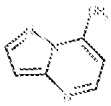
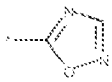
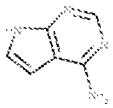
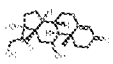
2019-09-12	A61	First payment of annual fees (during grant procedure)	**Free format text:** JAPANESE INTERMEDIATE CODE: A61 **Effective date:** 20190905
2019-09-13	R150	Certificate of patent or registration of utility model	**Ref document number:** 6585625 **Country of ref document:** JP **Free format text:** JAPANESE INTERMEDIATE CODE: R150
2022-08-24	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
YNR048W		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　WO2015/112642
(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ＣＲＦ１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。

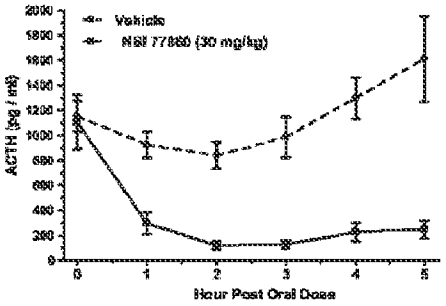


FIG. 2

【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－ｅ］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％低減する工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、CRF_1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。

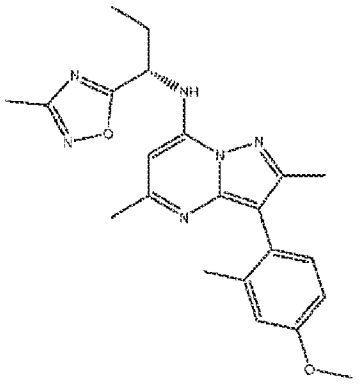
【００４８】

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Ｉ」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。

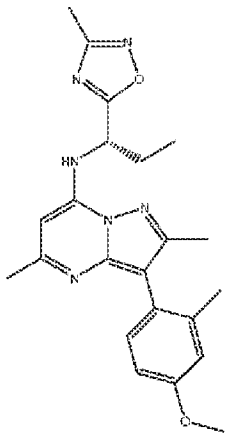
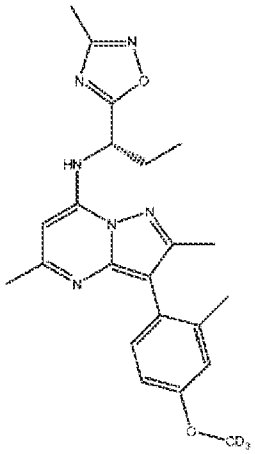
【００５３】

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。

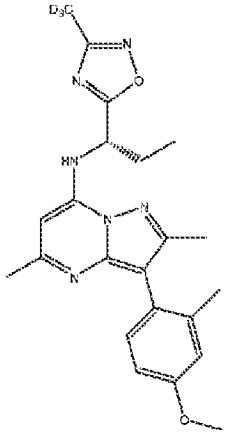
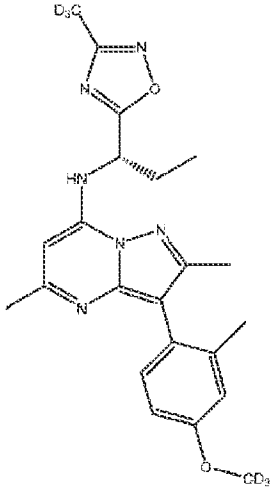
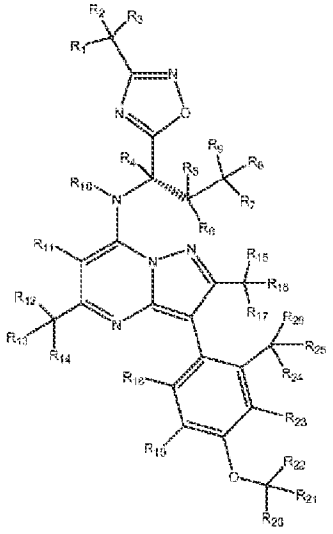
【００５４】

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。

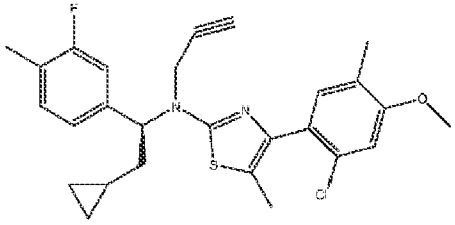
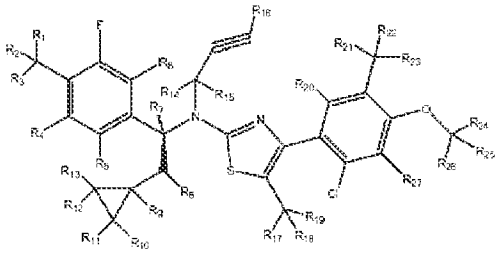
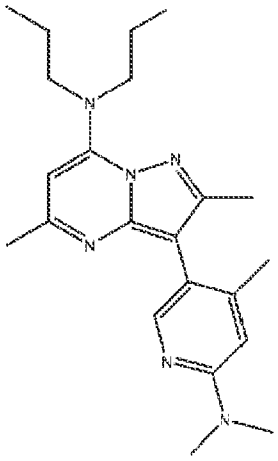
【化２－１】

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

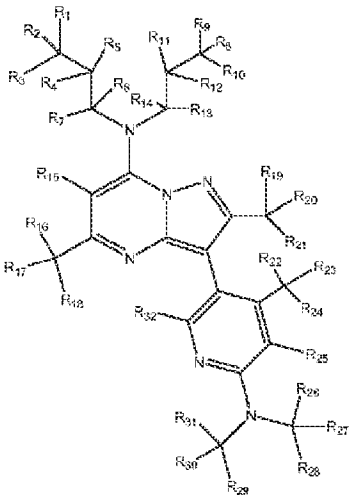
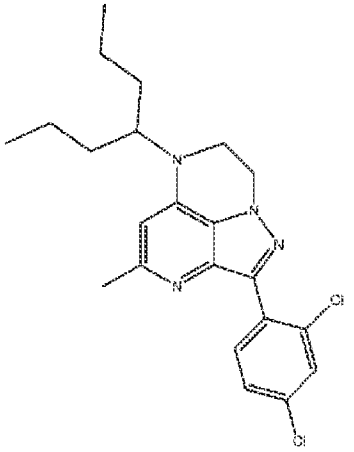
【化２－２】

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		

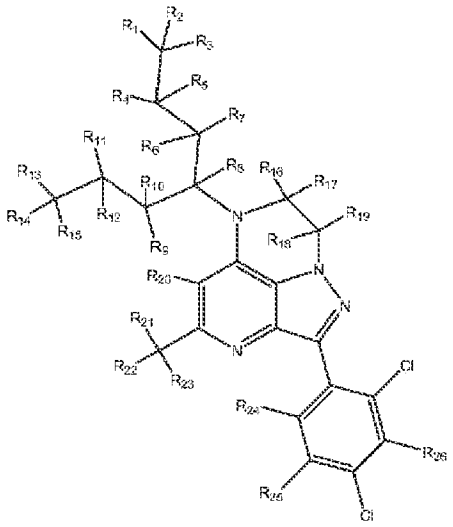
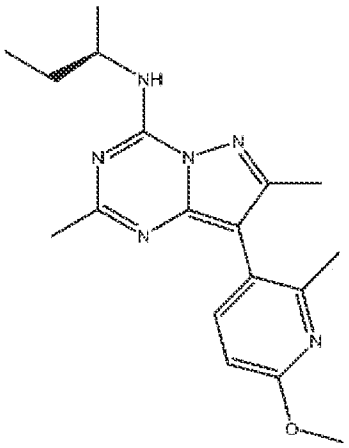
【化２－３】

化合物	構造	化学名
	R_1–R_{26}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

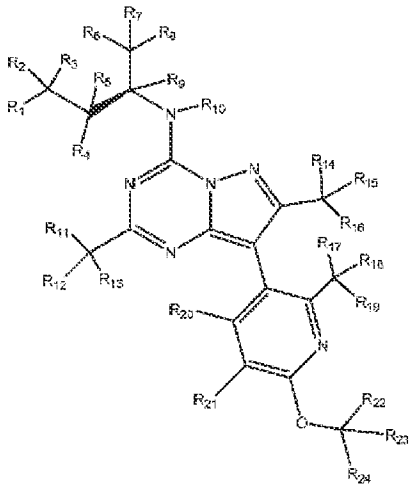
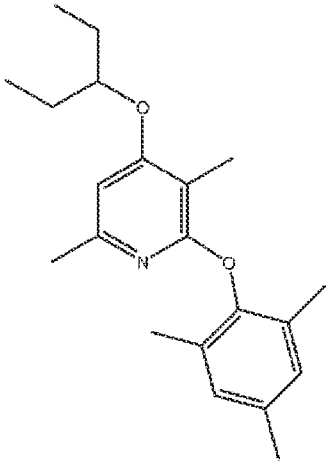
【化２－４】

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

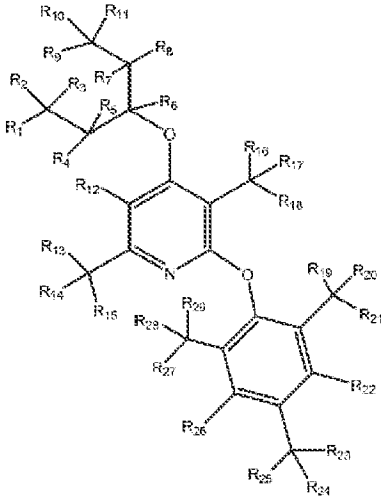
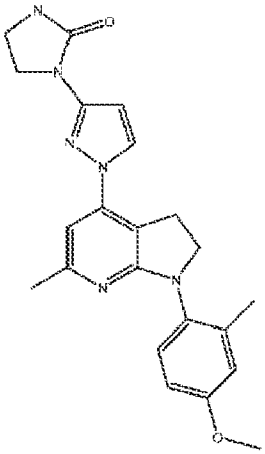
【化２－５】

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

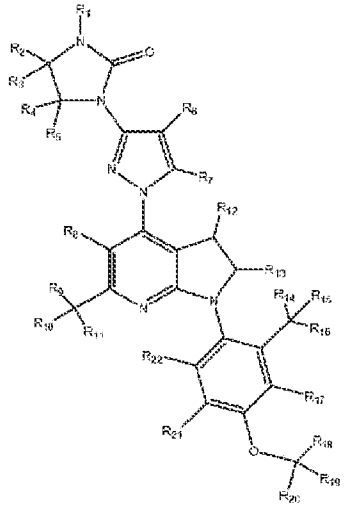
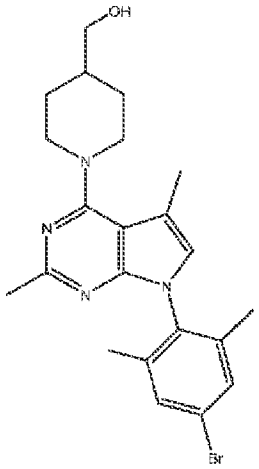
【化２－６】

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

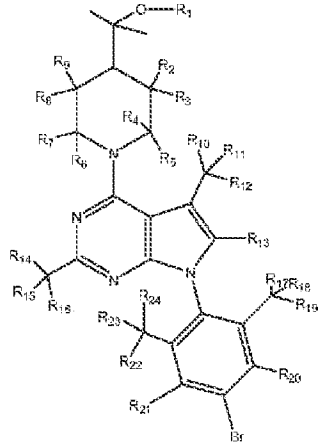
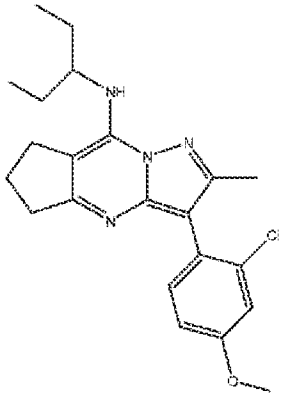
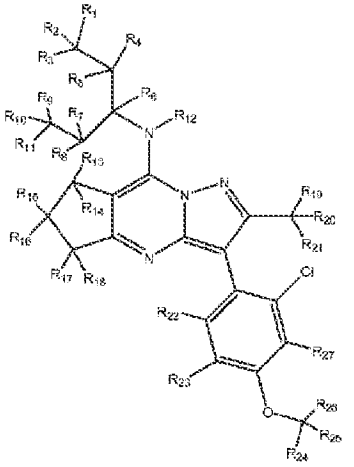
【化２－７】

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		

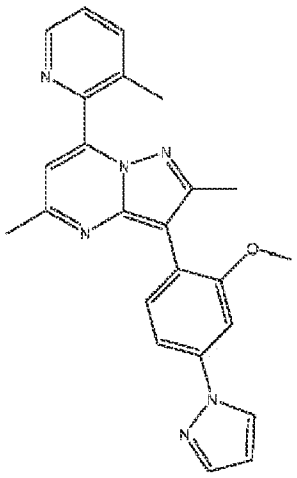
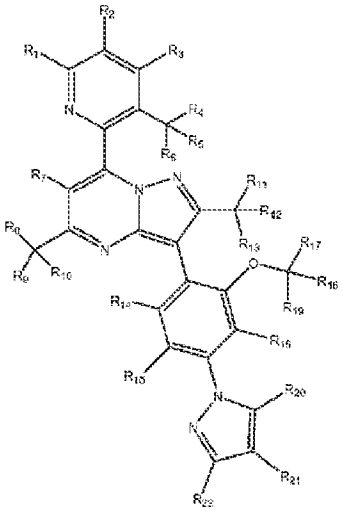
【化２－８】

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］チロシン－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１、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】

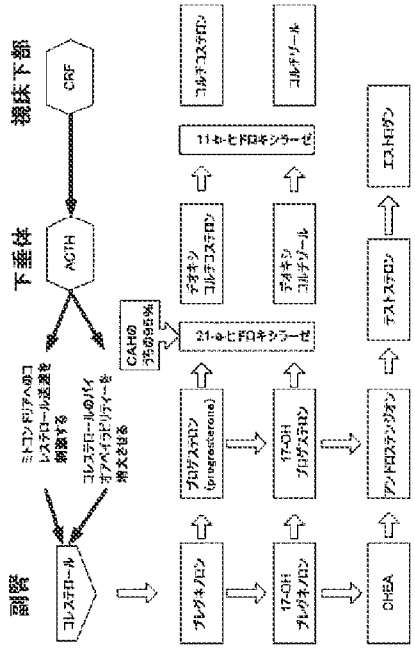


FIG. 1

【図２】

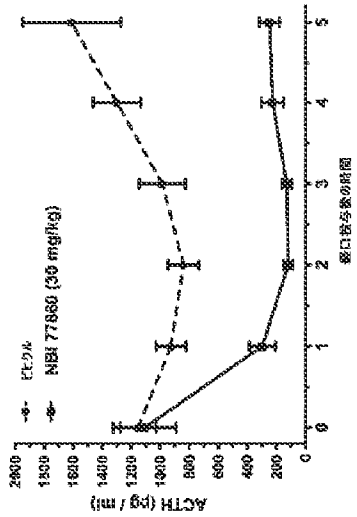


FIG. 2

【図３】

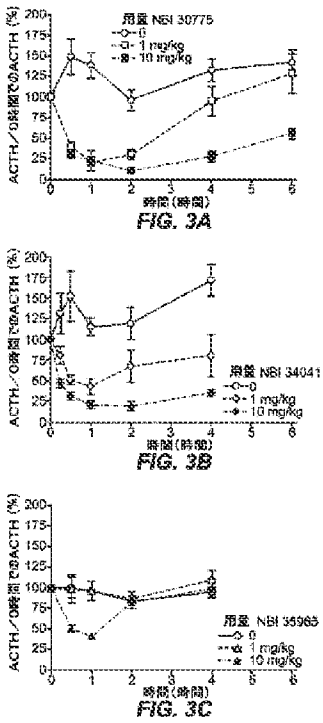


FIG. 3A

FIG. 3B

FIG. 3C

【図４】

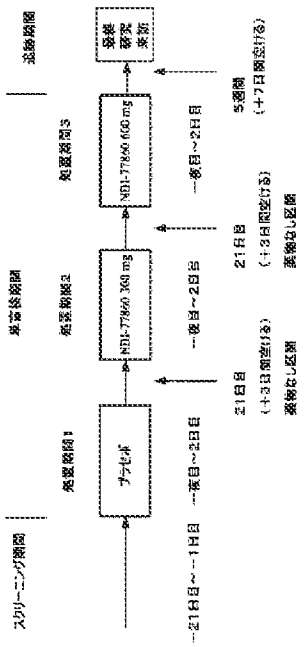


FIG. 4

【図５】

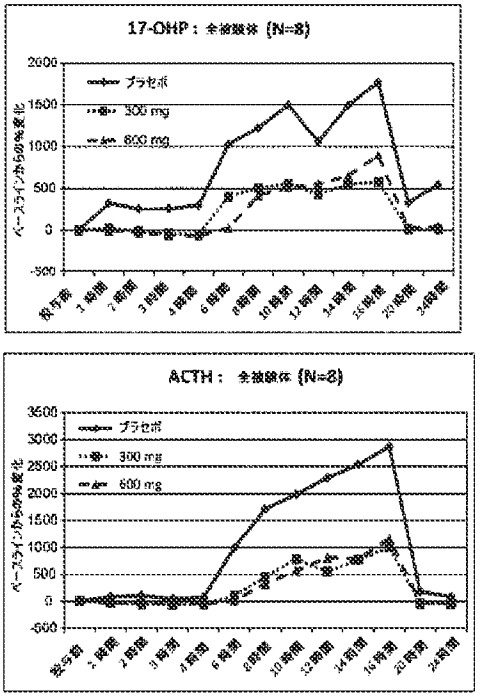


FIG. 5

【図６】

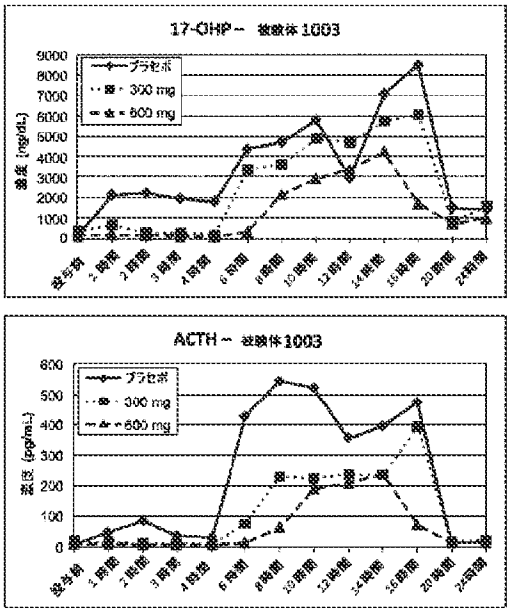


FIG. 6

【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, XP082738177, 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, XP000651739, 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 062886 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 101516887 A	26-08-2009
		CR 10682 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

Bibliographic data

Title:	Thiazolc pyrazolopyrimidincs as crf1 rcccptor antagonists
Pub/Pat no:	CN101516887A
Pub/Issue Date:	2009-08-26
Inventor(s):	ZHAOGEN CHEN[US]| HAMDOUCHI HAMDOUCHI CHAFIQ [US]| JAMES HEMBRE ERIK[US]| ARTHUR HIPSKIND PHILIP[US]| KENNETH MYERS JASON[US]| TAKAKO TAKAKUWA[US]| LEE TOTH JAMES[US] |CHEN ZHAOGEN |HAMDOUCHI CHAFIQ HAMDOUCHI |HEMBRE ERIK JAMES |HIPSKIND PHILIP ARTHUR |MYERS JASON KENNETH |TAKAKUWA TAKAKO |TOTH JAMES LEE
Applicant(s):	LILLY CO ELI[US]
Classification:	A61K31/505AI; A61P25/22AI; A61P25/24AI; A61P25/30AI; A61P29/00AI; C07D487/04AI
Application number:	CN20078035145 2007-09-17
Priority number:	WO2007US78605 20070917; US20060826264P 20060920;

Abstract of CN101516887A

The present invention relates to compounds of Formula (I), pharmaceutical compositions thereof, and use thereof as corticotropin releasing factor 1 (CRF1) receptor antagonists in the treatment of psychiatric and neuroendocrine disorders, neurological diseases, and metabolic syndrome.

[19] 中华人民共和国国家知识产权局

[12] 发明专利申请公布说明书

[21] 申请号 200780035145.3

[51] Int. Cl.
C07D 487/04 (2006.01)
A61P 25/22 (2006.01)
A61K 31/505 (2006.01)
A61P 25/24 (2006.01)
A61P 29/00 (2006.01)
A61P 25/30 (2006.01)

[43] 公开日 2009 年 8 月 26 日

[11] 公开号 CN 101516887A

[22] 申请日 2007.9.17
[21] 申请号 200780035145.3
[30] 优先权
[32] 2006. 9.20 [33] US [31] 60/826,264
[86] 国际申请 PCT/US2007/078605 2007.9.17
[87] 国际公布 WO2008/036579 英 2008.3.27
[85] 进入国家阶段日期 2009.3.20
[71] 申请人 伊莱利利公司
地址 美国印第安纳州
[72] 发明人 Z·陈 C·H·哈姆杜迟
E·J·亨布雷
P·A·希普斯金德
J·K·迈尔斯 高赤贵子
J·L·托特

[74] 专利代理机构 北京市中咨律师事务所
代理人 黄革生 贾士聪

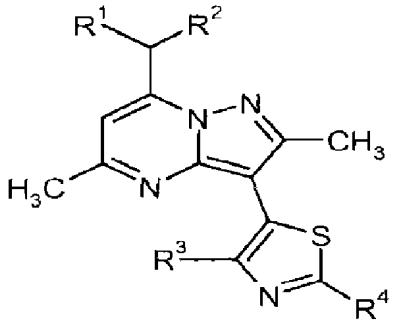
权利要求书 3 页 说明书 49 页

[54] 发明名称

作为 CRF1 受体拮抗剂的噻唑吡唑并嘧啶类

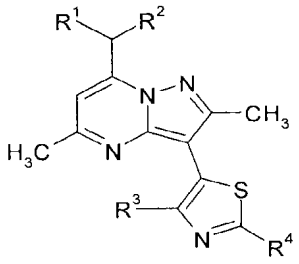
[57] 摘要

本发明涉及式(I)化合物、其药学上可接受的盐以及其作为促肾上腺皮质激素释放因子 1(CRF1)受体拮抗剂在治疗精神病学和神经内分泌障碍、神经病学疾病和代谢综合征中的用途。



I

1. 式 I 化合物或其药学上可接受的盐:

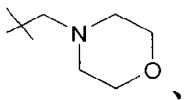
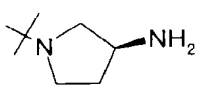
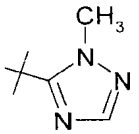
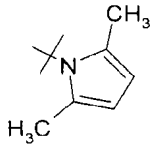


式 I

其中:

R^1 和 R^2 独立地是乙基或正丙基;

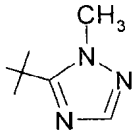
R^3 是氢、Cl、Br、甲基、三氟甲基或甲氧基;

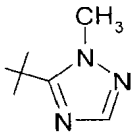
R^4 是氢、Br、R^aR^bN-、甲氧基甲基、正丁基、乙酰氨基、吡啶-4-基、吗啉-4-基、、、或;

R^a 和 R^b 独立地是氢、C_1-C_3 烷基、$H_2NCH_2CH_2$-、$(CH_3)_3COC(O)NHCH_2CH_2$-或 $CH_3CH_2CH_2NHCH_2CH_2$-。

2. 根据权利要求 1 所述的化合物或其药学上可接受的盐,其中 R^3 是 Cl、Br、甲基或三氟甲基。

3. 根据权利要求 1 或 2 所述的化合物或其药学上可接受的盐,其中 R^3 是 Cl 或 Br。

4. 根据权利要求 1 至 3 中任意一项所述的化合物或其药学上可接受的盐,其中 R^4 是 R^aR^bN-、吡啶-4-基、吗啉-4-基或。

5. 根据权利要求 1 至 4 中任意一项所述的化合物或其药学上可接受的盐,其中 R^4 是吗啉-4-基或。

6. 根据权利要求 1 至 4 中任意一项所述的化合物或其药学上可接受的盐，其中 R^4 是 R^aR^bN-，R^a 和 R^b 独立地是 C_1-C_3 烷基。

7. 根据权利要求 1 所述的化合物或其药学上可接受的盐，其是 3-[4-溴-2-(2-甲基-2*H*-[1,2,4]三唑-3-基)-噻唑-5-基]-2,5-二甲基-7-(1-丙基-丁基)-吡唑并[1,5-*a*]嘧啶。

8. 根据权利要求 1 所述的化合物或其药学上可接受的盐，其是 3-(4-溴-2-(2-甲基-2H-[1,2,4]三唑-3-基)-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶。

9. 根据权利要求 1 所述的化合物或其药学上可接受的盐，其是 3-(4-氯-2-吗啉-4-基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶。

10. 一种药物组合物，其包含：权利要求 1 至 9 中任意一项所述的化合物或其药学上可接受的盐和药学上可接受的赋形剂。

11. 一种治疗患者的抑郁、焦虑、酒精或物质滥用、肥胖、高血压、代谢综合征、肠易激惹综合征、癫痫、中风、睡眠障碍、变态反应、偏头痛、经前期综合征、不孕、性功能障碍、先天性肾上腺增生、库欣病、早产、应激诱导的胃溃疡、炎性障碍、垂体肿瘤或异位垂体源肿瘤、慢性疲劳综合征、纤维肌痛、内脏痛或多发性硬化的方法，其包括：给需要其的患者施用治疗有效量的权利要求 1 至 9 中任意一项所述的化合物或其药学上可接受的盐。

12. 一种治疗患者的焦虑或抑郁的方法，其包括：给需要其的患者施用治疗有效量的权利要求 1 至 9 中任意一项所述的化合物或其药学上可接受的盐。

13. 一种治疗患者的酒精或物质滥用的方法，其包括：给需要其的患者施用治疗有效量的权利要求 1 至 9 中任意一项所述的化合物或其药学上可接受的盐。

14. 根据权利要求 1 至 9 中任意一项所述的化合物或其药学上可接受的盐，其用在治疗中。

15. 根据权利要求 1 至 9 中任意一项所述的化合物或其药学上可接受

的盐，其用于治疗抑郁、焦虑、酒精或物质滥用、肥胖、高血压、代谢综合征、肠易激惹综合征、癫痫、中风、睡眠障碍、变态反应、偏头痛、经前期综合征、不孕、性功能障碍、先天性肾上腺增生、库欣病、早产、应激诱导的胃溃疡、炎性障碍、垂体肿瘤或异位垂体源肿瘤、慢性疲劳综合征、纤维肌痛、内脏痛或多发性硬化。

16. 根据权利要求 **1** 至 **9** 中任意一项所述的化合物或其药学上可接受的盐，其用于治疗焦虑或抑郁。

17. 根据权利要求 **1** 至 **9** 中任意一项所述的化合物或其药学上可接受的盐，其用于治疗酒精或物质滥用。

作为 CRF1 受体拮抗剂的噻唑吡唑并嘧啶类

技术领域

本发明涉及新的噻唑吡唑并嘧啶化合物、其药物组合物以及其作为 CRF1 受体拮抗剂在治疗精神病学和神经内分泌障碍、神经病学疾病和代谢综合征中的用途。

背景技术

促肾上腺皮质激素释放因子(CRF)是一种 41 个氨基酸的肽，其是垂体前叶分泌阿黑皮素原(POMC)衍生肽的主要生理调节物。除其对垂体的内分泌作用外，CRF 的免疫组织化学定位还证明，该激素在中枢神经组织中具有广泛的下丘脑外(extrahypothalamic)分布并且产生与其在脑中的神经递质或神经调节物作用相一致的广谱自律、电生理和行为作用。还有迹象表明，CRF 在整合免疫系统对生理学、心理学和免疫学应激物的响应方面具有重要作用。

已经表明在精神病学障碍和包括抑郁和焦虑在内的神经病学疾病以及下面的疾病中涉及 CRF：阿尔茨海默病、亨廷顿舞蹈病、进行性核上性麻痹、肌萎缩性侧索硬化、帕金森病、癫痫、偏头痛、酒精和物质滥用以及相关的戒断症状、肥胖、代谢综合征、先天性肾上腺增生、库欣病、高血压、中风、肠易激惹综合征、应激诱导的胃溃疡、经前期综合征、性功能障碍、早产、炎性障碍、变态反应、多发性硬化、内脏痛、睡眠障碍、垂体肿瘤或异位垂体源肿瘤(ectopic pituitary-derived tumors)、慢性疲劳综合征和纤维肌痛。

已经鉴定了 CRF 受体亚型 CRF1 和 CRF2，其在脑中的分布不均，从而表明可能的功能多样性。例如，广泛分布的脑 CRF1 受体强烈参与与环境应激物接触时伴随的情绪性。显然是 CRF1 而不是 CRF2 受体介导选择

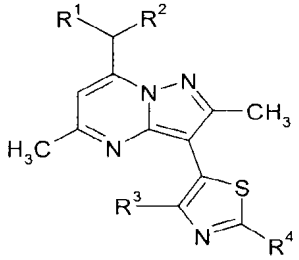
致焦虑样(select anxiogenic like)行为。更离散的间隔/下丘脑(hypothalmic)分布和供替代选择的内源性配体的可用性表明 CRF2 受体具有不同的功能。例如，据报道，一种相对于 CRF1 受体而言对 CRF2 具有优先亲合性的新 CRF-族神经肽在不产生用选择性 CRF1 激动作用所观察到的行为活化特性的情况下抑制食欲。在另一些情况中，CRF2 激动作用产生与 CRF1 拮抗剂或 CRF1 基因缺失所报道的那些作用相似的作用。例如，虽然已经提出用 CRF2 激动剂作为抗肥胖药，但是 CRF1 拮抗剂对肥胖而言也可能是一种重要的治疗。

在 WO 94/13676、WO 97/29109、WO 98/08847 和 WO 98/03510 中描述了可用作 CRF 拮抗剂的某些吡咯并[2,3-*d*]嘧啶类、吡咯并[3,2-*d*]嘧啶类、吡唑并[1,5-*a*]嘧啶类、1,2,3-三唑并[4,5-*b*]吡啶类和吡唑并[1,5-*a*]-1,3,5-三嗪类。

本发明提供了可用作 CRF1 受体拮抗剂的新的噻唑吡唑并嘧啶类。由于上述原因，希望发现对于精神病学和神经内分泌障碍、神经病学疾病和代谢综合征的治疗而言为可能有价值的治疗剂的新的有效的选择性 CRF1 拮抗剂。此外，由于大多数可商购获得的 CNS 和心血管药物表现出不良的生物利用度和药动学性质，还希望发现相对于已知 CRF 拮抗剂如 CP154526 和 NBI30775 而言具有良好生物利用度和药动学性质的新化合物。

发明概述

在一个实施方案中，本发明提供了式 I 化合物或其药学上可接受的盐，

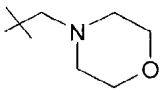
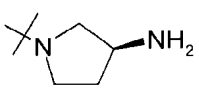
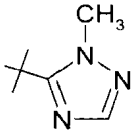
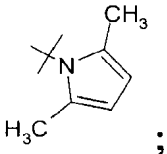


式 I

其中：

R^1和R^2独立地是乙基或正丙基;

R^3是氢、Cl、Br、甲基、三氟甲基或甲氧基;

R^4是氢、Br、R^aR^bN-、甲氧基甲基、正丁基、乙酰氨基、吡啶-4-基、吗啉-4-基、、、或;

R^a和R^b独立地是氢、C_1-C_3烷基、$H_2NCH_2CH_2$-、$(CH_3)_3COC(O)NHCH_2CH_2$-或$CH_3CH_2CH_2NHCH_2CH_2$-。

在另一个实施方案中，本发明提供了一种药物组合物，其包含：式I化合物或其药学上可接受的盐和药学上可接受的赋形剂。

在另一个实施方案中，本发明涉及一种治疗患者的抑郁、焦虑、酒精或物质滥用、肥胖、高血压、代谢综合征、肠易激惹综合征、癫痫、中风、睡眠障碍、变态反应、偏头痛、经前期综合征、不孕、性功能障碍、先天性肾上腺增生、库欣病、早产、应激诱导的胃溃疡、炎性障碍、垂体肿瘤或异位垂体源肿瘤、慢性疲劳综合征、纤维肌痛、内脏痛或多发性硬化的方法，其包括：给需要其的患者施用治疗有效量的式I化合物或其药学上可接受的盐。

在另一个实施方案中，本发明涉及式I化合物或其药学上可接受的盐在制备药物中的用途，所述药物用于治疗抑郁、焦虑、酒精或物质滥用、肥胖、高血压、代谢综合征、肠易激惹综合征、癫痫、中风、睡眠障碍、变态反应、偏头痛、经前期综合征、不孕、性功能障碍、先天性肾上腺增生、库欣病、早产、应激诱导的胃溃疡、炎性障碍、垂体肿瘤或异位垂体源肿瘤、慢性疲劳综合征、纤维肌痛、内脏痛或多发性硬化。

在另一个实施方案中，本发明涉及用于治疗抑郁、焦虑、酒精或物质滥用、肥胖、高血压、代谢综合征、肠易激惹综合征、癫痫、中风、睡眠障碍、变态反应、偏头痛、经前期综合征、不孕、性功能障碍、先天性肾上腺增生、库欣病、早产、应激诱导的胃溃疡、炎性障碍、垂体肿瘤或异位垂体源肿瘤、慢性疲劳综合征、纤维肌痛、内脏痛或多发性硬化的式I

化合物或其药学上可接受的盐。

在另一个实施方案中，本发明涉及用于治疗的式 I 化合物或其药学上可接受的盐。

发明详述

如上文和整个本申请的说明书中所用的那样，除非另有说明，否则下面的术语应被理解为具有下列含义:

“烷基”是指在链中具有 1 至 5 个碳原子的饱和脂族烃基，其可以是直链的或支链的。

“药学上可接受的赋形剂”是指用于增进制剂特性的药学上可接受的配制载体、溶液或添加剂。该类赋形剂必需与制剂中的其它成分相容并且对其接受者无害，它们是本领域技术人员所众所周知的(参见例如 ***Remingtons Pharmaceutical Sciences***, 第 19 版, **Mack Publishing Company, 1995**)。

“药学上可接受的盐”是指本发明的化合物相对无毒的无机和有机酸加成盐和碱加成盐。这些盐可以在化合物的最后分离和纯化中原位制备。具体而言，酸加成盐可通过分别将纯化的游离碱形式的化合物与适宜的有机酸或无机酸反应并分离由此形成的盐来制备(参见例如 ***Remingtons Pharmaceutical Sciences***, 第 19 版, **Mack Publishing Company, 1995**)。

“治疗有效量”或“有效量”是指将在组织、系统、动物或人中引起研究者、兽医、医生或其它临床医师所寻求的生物学或医学响应或者所需的治疗作用的本发明式 I 化合物或包含本发明式 I 化合物的药物组合物的量。

术语“治疗”等包括减缓和逆转障碍的进展。这些术语还包括即使障碍或病症实际上未被消除和即使障碍或病症的进展本身未被减缓或逆转但是缓解、改善、减弱、消除或减轻了障碍或病症的一种或多种症状。术语“治疗”以及类似术语还包括防止性(例如，预防性)治疗和姑息性治疗。疾病的预防表现为疾病的发作被拖延或延迟。

分子结构中的符号“——”表示该特定取代基的连接位置。

当任何变量在任何组分或在式 I 中出现一次以上时，其定义在每次出

现时都是彼此独立的。而且，只有当取代基和/或变量的组合产生稳定的化合物时，该类组合才是允许的。在选择本发明的化合物时，本领域普通技术人员将意识到各种取代基、即 R^1、R^2 等是根据化学结构连接性的众所周知的原则来进行选择的。

按照本公开物中所用的标准命名法，首先描述指定侧链的末端部分，然后描述朝向连接点的毗邻官能团。例如，芳基羰基氨基烷基取代基与芳基-C(O)-NH-烷基-等同。

本发明包括下列实施方案，它们可以被进一步组合：

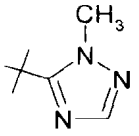
(a) 式 I 化合物，其中 R^1 和 R^2 是乙基；

(b) 式 I 化合物，其中 R^1 和 R^2 是正丙基；

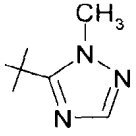
(c) 式 I 化合物，其中 R^3 是 Cl、Br、甲基或三氟甲基；

(d) 式 I 化合物，其中 R^3 是 Cl；

(e) 式 I 化合物，其中 R^3 是 Br；

(f) 式 I 化合物，其中 R^4 是 R^aR^bN-、吡啶-4-基、吗啉-4-基或 ；

(g) 式 I 化合物，其中 R^4 是吗啉-4-基；

(h) 式 I 化合物，其中 R^4 是 ；

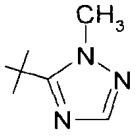
(i) 式 I 化合物，其中 R^a 和 R^b 独立地是 C_1-C_3 烷基；

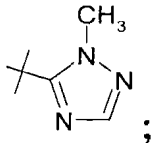
(j) 式 I 化合物，其中 R^1 和 R^2 是乙基，R^3 是 Cl，R^4 是吗啉-4-基；

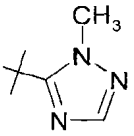
(k) 式 I 化合物，其中 R^1 和 R^2 是正丙基，R^3 是 Cl，R^4 是吗啉-4-基；

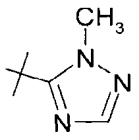
(l) 式 I 化合物，其中 R^1 和 R^2 是乙基，R^3 是 Br，R^4 是吗啉-4-基；

(m) 式 I 化合物，其中 R^1 和 R^2 是正丙基，R^3 是 Br，R^4 是吗啉-4-基；

(n) 式 I 化合物，其中 R^1 和 R^2 是乙基，R^3 是 Cl，R^4 是 ；

(o) 式 I 化合物，其中 R^1 和 R^2 是正丙基，R^3 是 Cl，R^4 是 ；

(p) 式 I 化合物，其中 R^1 和 R^2 是乙基，R^3 是 Br，R^4 是 ；

(q) 式 I 化合物，其中 R^1 和 R^2 是正丙基，R^3 是 Br，R^4 是 ；

(r) 式 I 化合物或其药学上可接受的盐用于治疗抑郁或焦虑的用途;

(s) 式 I 化合物或其药学上可接受的盐用于治疗酒精或物质滥用的用途;

(t) 式 I 化合物或其药学上可接受的盐用于治疗酒精或物质滥用以及相关的戒断症状的用途;

(u) 式 I 化合物或其药学上可接受的盐，对于 CRF1 结合而言，其表现出≤ 500 nM 的 Ki 值;

(v) 式 I 化合物或其药学上可接受的盐，对于 CRF1 结合而言，其表现出≤ 50 nM 的 Ki 值;

(w) 式 I 化合物或其药学上可接受的盐，对于 CRF1 结合而言，其表现出≤ 5 nM 的 Ki 值;

(x) 式 I 化合物或其药学上可接受的盐，对于 CRF1 结合而言，其表现出≤ 500 nM 的 Ki 值，并且相对于 CRF2 而言选择性与 CRF1 结合(即，更低的 Ki);

(y) 式 I 化合物或其药学上可接受的盐，对于 CRF1 结合而言，其表现出≤ 50 nM 的 Ki 值，并且相对于 CRF2 而言选择性与 CRF1 结合(即，更低的 Ki);

(z) 式 I 化合物或其药学上可接受的盐，对于 CRF1 结合而言，其表现出≤ 5 nM 的 Ki 值，并且相对于 CRF2 而言选择性与 CRF1 结合(即，更低的 Ki); 和/或

(aa) 相对于一些已知的 CRF 拮抗剂(例如，CP154526 和 NBI30775)

而言，具有优良的生物利用度和药动学性质的具体例举的化合物，如实施例 15。

本发明的化合物优选被配制为通过各种途径施用的药物组合物的形式。该类组合物优选用于口服施用。该类药物组合物和制备该类药物组合物的方法在本领域中是众所周知的(参见例如 ***Remington: The Science and Practice of Pharmacy***, A. Gennaro 等人编辑，第 19 版, Mack Publishing Co., 1995)。

式 I 化合物一般在宽的剂量范围内都有效。例如，每天的剂量通常落在约 0.0001 至约 30 mg/kg 体重范围内。在一些情况中，低于上述范围下限的剂量水平可能就远远足够了，而在另一些情况中，可以使用更大的剂量而不会导致任何有害的副作用，因此，上述剂量范围并非旨在以任何方式对本发明的范围进行限制。应当清楚的是，实际施用的化合物的量将由医师根据相关的情况来确定，所述情况包括所治疗的病症、所选择的施用途径、被施用的实际化合物或化合物组合、各患者的年龄、体重和响应情况以及患者的症状的严重程度。

式 I 化合物是 CRF-1 拮抗剂并且因此可用于治疗能通过降低 CRF1 受体的品质(tone)或兴奋性(stimulation)来治疗的病症。已经表明促肾上腺皮质激素释放因子(CRF)(一种 41 个氨基酸的肽，其是垂体前叶分泌阿黑皮素原(POMC)衍生肽的主要生理调节物)[J. Rivier 等人, ***Proc. Natl. Acad. Sci (USA)*** 80:4851 (1983); W. Vale 等人, ***Science*** 213:1394 (1981)]与许多医学病症有关。例如，除其对垂体的内分泌作用外，CRF 的免疫组织化学定位还证明，该激素在中枢神经组织中具有广泛的下丘脑外分布并且产生与其在脑中的神经递质或神经调节物作用相一致的广谱自律、电生理和行为作用[W. Vale 等人, ***Rec. Prog. Horm. Res.*** 39:245 (1983); G. F. Koob, ***Persp. Behav. Med.*** 2:39 (1985); E. B. De Souza 等人, ***J. Neurosci.*** 5:3189 (1985)]。还有迹象表明，CRF 在整合免疫系统对生理学、心理学和免疫学应激物的响应方面具有重要作用[参见例如 J. E. Blalock, ***Physiological Reviews*** 69:1 (1989); J. E. Morley, ***Life Sci.*** 41:527 (1987)]。

已经表明在精神病学障碍和包括抑郁和焦虑在内的神经病学疾病中涉及 CRF[D. M. Nielsen, *Life Sci.* 78:909-919; H. E. Kunzel 等人, *J. Psychiatr. Res.* 37:525-533; D. R. Gehlert 等人, *Eur. J. Pharmacol.* 509:145-153]。认为 CRF 还参与下列疾病的病因学和病理生理学：阿尔茨海默病、亨廷顿舞蹈病、进行性核上性麻痹和肌萎缩性侧索硬化，因为这些疾病涉及中枢神经系统中 CRF 神经元功能障碍[关于综述，参见：E. B. De Souze, *Hosp. Practice* 23:59 (1988)]。已经表明长期施用 CRF 产生多巴胺系统损害，这表明其在帕金森病中起作用[E. Izzo 等人, *Pharmacol. Biochem. Behav.* 81:701-708 (2005)]。其中有 CRF 参与的其它神经病学障碍包括癫痫[T. Z. Baram 等人, *Brain Res.* 770:89-95 (1997)]和偏头痛[T. C. Theoharides 等人, *Endocrinology* 136:5745-5750(1995)]。已经表明 CRF 参与酒精和物质滥用以及相关的戒断症状[D. H. Overstreet 等人, *Pharmacol. Biochem. Behav.* 77:405-413; Y. Shaham 等人, *Psychopharmacology (Berl)* 137: 184-190]。此外，有迹象表明 CRF 在各种内分泌障碍和心血管疾病如肥胖[E. Timofeeva 和 D. Richard, *Neuroendocrinology* 66:327-340 (1997)]、代谢综合征[A. M. Ward 等人, *Metabolism* 53:720-726(2004)]、先天性肾上腺增生[D. P. Merke 和 G. B. Cutler Jr., *Endocrinol. Metab. Clin. North Am.* 30:121-135(2001)]、库欣病[M. Labeur 等人, *Curr. Drug Targets Immune Endocr. Metabol. Disord.* 4:335-342(2004)]，高血压[R. J. Briscoe 等人，*Brain Res.* 881:204-207(2000)]和中风[S. L. Stevens 等人, *J. Cereb. Blood Flow Metab.* 23:1151-1159(2003)]中起作用。已经表明胃紊乱如肠易激惹综合征[Y. Tache 等人, *Eur J. Surg.* Suppl:16-22(2002)]和应激诱导的胃溃疡[K. E. Gabry 等人, *Mol. Psychiatry* 7:474-483, 433(2002)]与 CRF 有关。此外，还表明 CRF 在女性健康的各个领域例如经前期综合征[F. Facchinetti 等人, *Psychosom. Med.* 56:418-422(1994)]、不孕[L. Ghizzoni 等人，*Endocrinology* 138:4806-4811(1997)]、性功能障碍[J. E. Jones 等人, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 283:R591-597(2002)]和早产[P. D. Wadhwa 等人, *Am. J. Obstet. Gynecol.* 191:1063-1069(2004)]中起作用。还有迹象表明 CRF 在

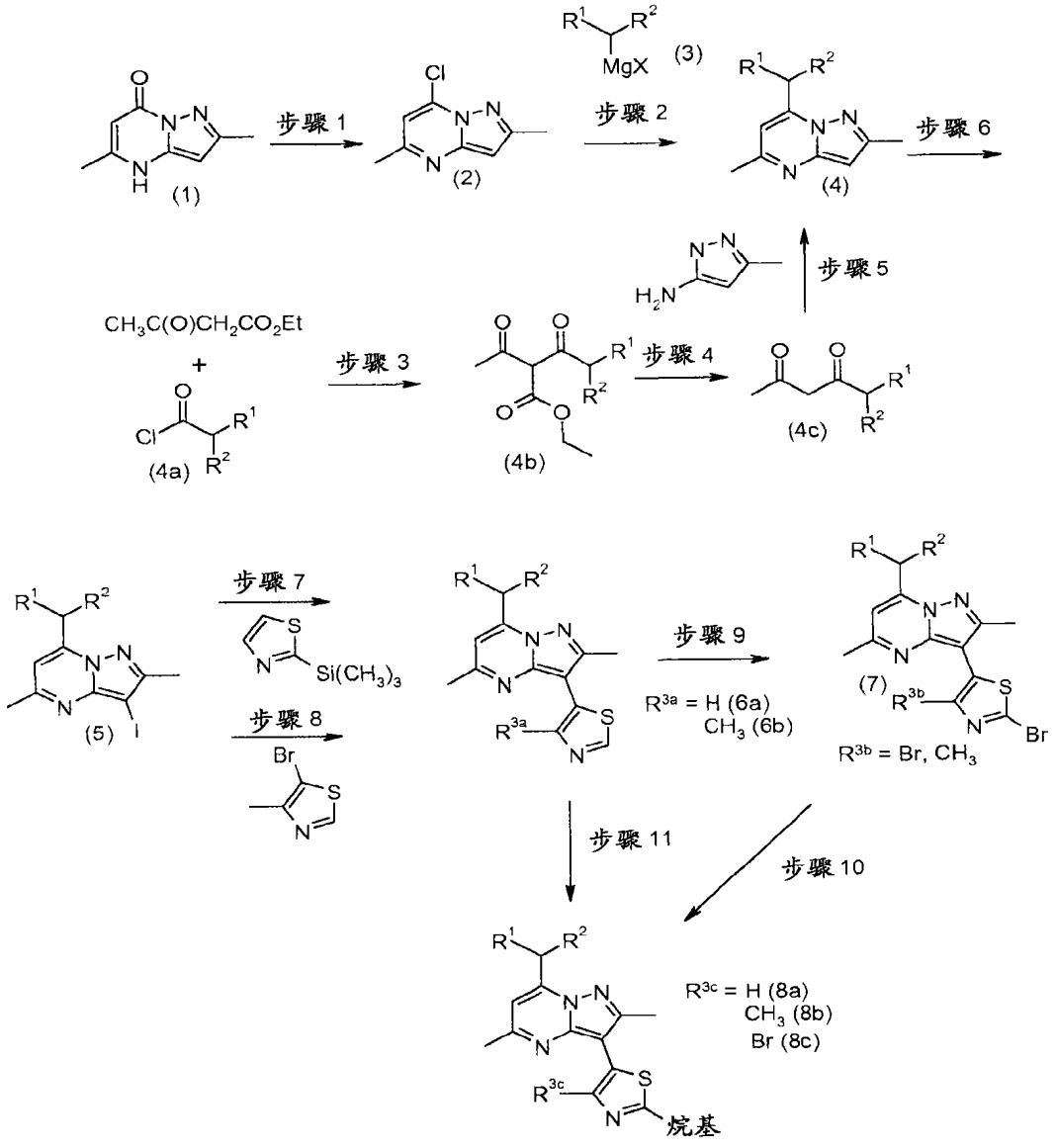
免疫系统中具有重要作用，表明其有治疗炎性障碍[A. Gravanis 和 A. N. Margioris, *Curr. Med. Chem.* 12:1503-1512(2005)]、变态反应[L. K. Singh 等人, *Brain Behav. Immun.* 13:225-239(1999)]、多发性硬化和其它自身免疫性障碍[C. Benou 等人, *J. Immunol.* 174:5407-5413(2005)]的潜能。除前述疾病外，还表明在内脏痛[M. Nijsen 等人，*Neurogastroenterol. Motil.* 17:423-432(2005)]、睡眠障碍[T. M. Buckley 和 A. F. Schatzberg, *J. Clin. Endocrinol. Metab.* 90:3106-3114(2005)]、垂体肿瘤或异位垂体源肿瘤[K. D. Dieterich 等人, *J. Clin. Endocrinol. Metab.* 83: 3327-3331 (1998)]、慢性疲劳综合征和纤维肌痛[G. Neeck 和 L. J. Crofford, *Rheum. Dis. Clin. North Am.* 26:989-1002(2000)]中也涉及 CRF。

已经鉴定了 CRF 受体亚型 CRF1 和 CRF2，其在脑中的分布不均[D. T. Chalmers 等人, *TIPS* 17:166-72(1996)]，从而表明其可能的功能多样性[S. C. Heinrichs 等人, *Regul. Peptides* 71:15(1997)]。例如，广泛分布的脑 CRF1 受体强烈参与与环境应激物接触时伴随的情绪性[G. Liebsch 等人, *Regul. Peptides* 59: 229-39(1995); D. W. Schulz, *PNAS* 93: 10477-82(1996)]。显然，是 CRF1 而不是 CRF2 受体介导选择致焦虑样行为[Heinrichs 等人, 1997]。更离散的间隔/下丘脑分布[D. T. Chalmers 等人, *J. Neurosci.* 15(10): 6340-50 (1995)]和供替代选择的内源性配体的可用性[J. Vaughan 等人, *Nature* 378: 287-92(1995)]表明 CRF2 受体具有不同的功能角色[Heinrichs 等人, 1997]。例如，据报道，一种相对于 CRF1 受体而言对 CRF2 具有优先亲合性的新 CRF-族神经肽在不产生用选择性 CRF1 激动作用所观察到的行为活化特性的情况下抑制食欲(H. Tezval 等人, *PNAS* 101(25): 9468-9473 (2004)]。在一些情况中，CRF2 激动作用产生与 CRF1 拮抗剂或 CRF1 基因缺失所报道的那些作用相似的作用[S. C. Heinrichs, *Trends in Pharmacological Sciences* 20(8):311-5(1999)]。例如，虽然已经提出用 CRF2 激动剂作为抗肥胖药，但是 CRF1 拮抗剂对肥胖而言也可能是一种重要的治疗[C. Contoreggi 等人, *Neuroendocrinology* 80(2):111-23(2004)]。

制备本发明的化合物

本发明的所有化合物都可以化学制备，例如，通过按照下面所述的合成途径来制备。但是，下面的讨论并非旨在以任何方式对本发明的范围进行限制。例如，所述各途径的特定合成步骤可以以不同方式组合，或者与来自不同流程图的步骤联合，从而制备另外的式 I 化合物。各步骤的产物可以用常规方法来回收，所述方法包括萃取、蒸发、沉淀、色谱法、过滤、研磨、结晶等。在下面的流程图中，除非另有说明，否则所有取代基均如前面所定义，适宜的试剂在本领域中是众所周知和可理解的。

流程图 1



可以按照流程图 1 中所述的反应来形成式(6a,b)、(7)或 8(a-c)的化合物。一种适宜的式(6a,b)、(7)或(8a-c)的化合物是其中基团定义如下的化合物：R^1 和 R^2 如式 I 中所定义，R^{3a} = H 或 CH_3，R^{3b} = Br 或 CH_3，R^{3c} = H、CH_3 或 Br。

在流程图 1 的步骤 1 中，用氯氧化磷(phosphorous oxychloride)和二甲基苯胺在惰性溶剂如甲苯中在溶剂的回流温度下将式(1)的吡唑并[1,5-a]嘧啶-7-酮转化成 7-氯-2,5-二甲基-吡唑并[1,5-a]嘧啶。

在步骤 2 中，使式(3)的格氏试剂(X = Cl 或 Br)与式(2)的氯化物在惰性溶剂如甲苯中在回流温度下反应，得到式(4)的 7-烷基吡唑并嘧啶。

或者，可以如步骤 3、4 和 5 中所示的那样获得式(4)的 7-烷基吡唑并嘧啶。在步骤 3 中，将乙酰乙酸乙酯用式(4a)的酰卤在存在氯化镁的情况下进行酰化，得到式(4b)的二酮基-酯。将式(4b)的二酮基-酯在 Krapcho 条件下脱羧，得到式(4c)的二酮。例如，将(4b)在二甲基亚砜中在约 130 至 170℃的温度下加热以进行脱羧。在步骤 5 中，将式(4c)的二酮与 3-氨基-5-甲基吡唑在质子溶剂如甲醇、乙醇或乙酸中环化，得到式(4)的 7-烷基吡唑并嘧啶。优选的条件在约 0 至 60℃的温度下使用乙酸。

在步骤 6 中，用过量的 N-碘琥珀酰亚胺在乙腈中将式(4)的吡唑并嘧啶官能化成式(5)的碘代吡唑并嘧啶。

在流程图 1 的步骤 7 或 8 中，使式(5)的碘代吡唑并嘧啶与噻唑卤化锌在 Negishi 交叉偶联反应中反应，得到式(6a)或(6b)的噻唑基吡唑并嘧啶(Jensen, J.; Skjaerbaek, N.; Vedso, P. *Synthesis* 2001, 128)。所述噻唑卤化锌是用本领域技术人员众所周知的方法产生的。例如，在步骤 7 中，将 2-三甲基硅烷基噻唑用正丁基锂、仲丁基锂或叔丁基锂处理，然后用 $ZnCl_2$ 进行锂-锌交换。将有机锌试剂与式(5)的碘代吡唑并嘧啶在存在钯催化剂例如二氯[1,1'-双(二苯基-膦基)二茂铁]钯(II)二氯甲烷的情况下在惰性溶剂如 THF 中在回流温度下偶联约 12 至 36 小时，得到式(6a)的噻唑吡唑并嘧啶。

或者，在步骤 8 中，用 5-溴-4-甲基噻唑和锌金属产生噻唑溴化锌并基本如步骤 7 中所述的那样将其用于 Negishi 交叉偶联中，得到式(6b)的噻唑

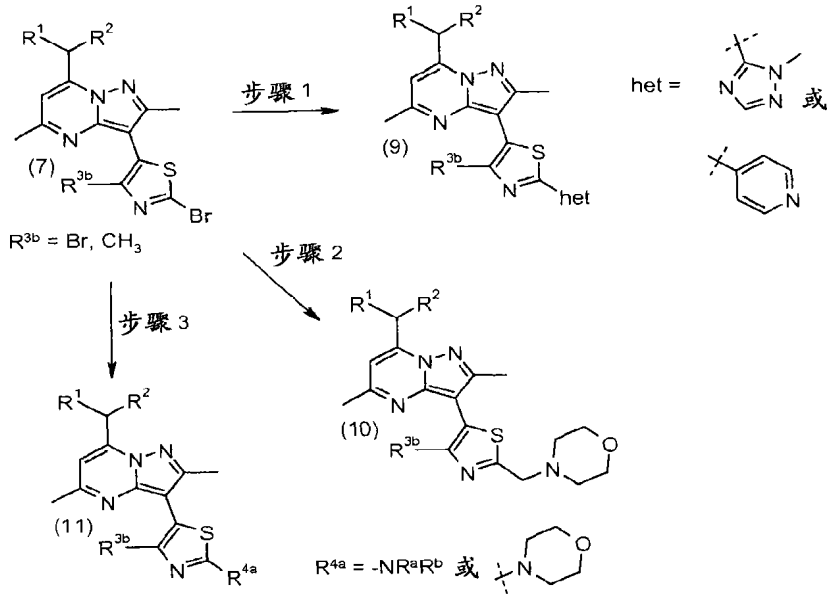
吡唑并嘧啶。

在流程图 1 的步骤 9 中，将式(6a,b)的噻唑溴化，得到式(7)的溴代或二溴代噻唑，其中 R^{3b} = Br 或 CH_3。根据 R^{3a} 是 CH_3 还是 H，将噻唑分别用 1 或 2 eq N-溴琥珀酰亚胺溴化。

式(8a-c)的烷基噻唑是在步骤 11 中由式(6a,b)的噻唑获得的或者在步骤 10 中由式(7)的溴代噻唑获得的。在步骤 10 中，用正丁基锂、仲丁基锂或叔丁基锂进行卤素-锂交换，得到噻唑锂试剂，随后使其与亲电子试剂如烷基卤如碘甲基甲基醚或碘丁烷反应。在步骤 11 中，通过用正丁基锂、仲丁基锂或叔丁基锂去质子化和随后与亲电子试剂如碘甲基甲基醚或碘丁烷反应来形成噻唑锂试剂。

本领域技术人员应当意识到，可以容易地对噻唑环系进行官能化，可以容易地制备噻唑中间体如 2-三甲基硅烷基-噻唑(Dondoni, A.等人 *J. Org. Chem.* 1988, *53*, 1748)。5-溴-4-甲基噻唑是通过用溴在乙酸中对 4-甲基噻唑进行溴化来获得的(Collins, I. J.等人, WO2003093252, 2003 年 11 月 13 日)。通过使乙酰乙酸乙酯与 5-甲基-2*H*-吡唑-3-基胺在回流的乙酸中进行缩合可容易地制得 2,5-二甲基-4*H*-吡唑并[1,5-a]嘧啶-7-酮(1)。

流程图 2



可以按照流程图 2 中所述的反应来形成式(9)、(10)或(11)的化合物。一

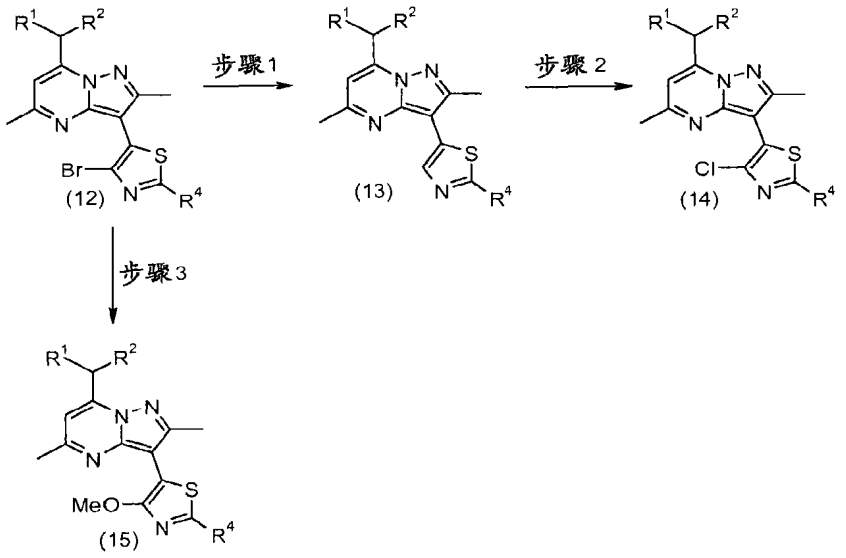
种适宜的式(9)、(10)或(11)的化合物是其中基团定义如下的化合物：R^1、R^2、R^a和R^b如式I中所定义，R^{3b} = Br或CH_3，R^{4a} = $-NR^aR^b$或-N-吗啉基，“het”具有所述的定义。

在步骤1中，使式(7)的溴代噻唑与杂环锌试剂在Negishi交叉偶联反应中偶联，得到式(9)的噻唑杂环。例如，将1-甲基-1,2,4-三唑在约-80至-65℃下用正丁基锂、仲丁基锂或叔丁基锂处理、然后用氯化锌处理，并且在原位与式(7)的溴代噻唑反应。该反应优先在惰性溶剂如THF中在存在钯催化剂如二氯[1,1'-双(二苯基-膦基)二茂铁]钯(II)二氯甲烷或四(三苯基膦)钯(0)的情况下进行。将反应温热至回流温度。或者，杂环锌试剂由卤代杂环如4-碘吡啶和锌金属形成。

在流程图2的步骤2中，中间体2-甲酰基噻唑是通过用正丁基锂、仲丁基锂或叔丁基锂进行卤素-锂交换、然后与N-甲酰基吗啉反应来形成的。使甲酰基噻唑在存在有机胺如吗啉的情况下进行还原胺化，得到式(10)的吗啉基甲基噻唑。还原胺化在本领域中是众所周知的，通常使用无机硼氢化物试剂如硼氢化钠或氰基硼氢化钠。优选的条件在惰性溶剂如二氯甲烷或THF中使用三乙酰氧基硼氢化钠。

在步骤3中，用式$-NR^aR^b$的胺或用吗啉使式(7)的溴代噻唑进行置换反应，得到式(11)的氨基噻唑。该反应在惰性溶剂如THF或二噁烷中用无机碱如碳酸铯在70至110℃下进行。

流程图3

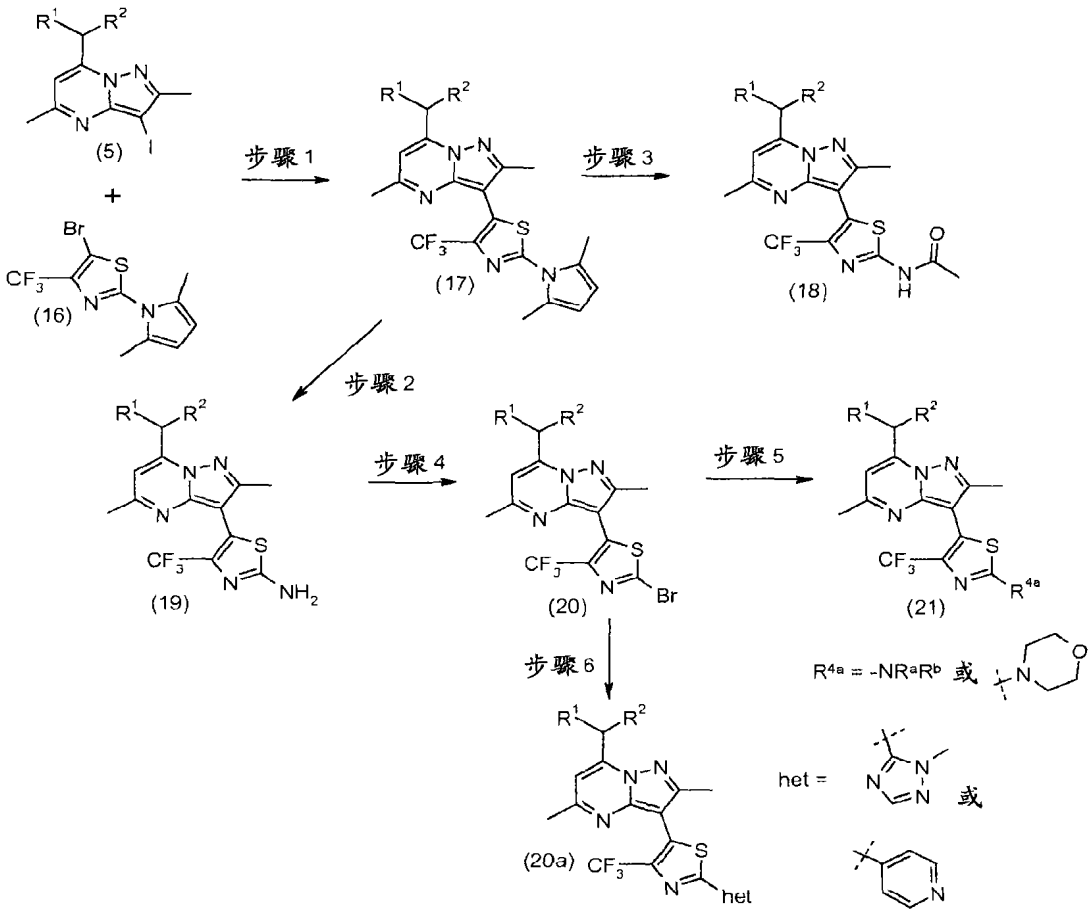


可以按照流程图 3 中所述的反应来形成式(13)、(14)或(15)的化合物。一种适宜的式(13)、(14)或(15)的化合物是其中 R^1、R^2 和 R^4 如式 I 中所定义的化合物。

本领域技术人员应当意识到，可以容易地将 4-溴噻唑如式(12)的 4-溴噻唑处理成具有其它官能团。例如，在步骤 1 中，可以用氯化亚铜将该溴化物脱卤素化，得到式(13)的噻唑，随后用 N-氯琥珀酰亚胺将其氯化，得到式(14)的 4-氯噻唑。

在流程图 3 的步骤 3 中，在存在处于甲醇中的碘化亚铜(I)的情况下在约 100 至 120℃下在惰性溶剂如二甲基甲酰胺中用甲醇钠对式(12)的 4-溴噻唑进行置换，得到式(15)的 4-甲氧基噻唑。

流程图 4



可以按照流程图 4 中所述的反应来形成式(17)、(18)、(19)、(20)、(20a)或(21)的化合物。一种适宜的式(17)、(18)、(19)、(20)、(20a)或(21)的化合物是其中基团定义如下的化合物：R^1、R^2、R^a和 R^b如式 I 中所定义，R^{4a}是-NR^aR^b或-N-吗啉基，“het”具有所述的定义。

在流程图 4 的步骤 1 中，使式(5)的碘代吡唑并嘧啶和式(6)的 5-溴噻唑进行 Negishi 交叉偶联，形成式(17)的二甲基吡咯基噻唑。例如，将式(16)的 5-溴噻唑在约-80 至-65℃下用正丁基锂、仲丁基锂或叔丁基锂处理、然后用氯化锌处理。将有机锌试剂在原位与式(5)的碘代吡唑并嘧啶反应。该偶联反应优先在惰性溶剂如 THF 中在回流温度下在存在钯催化剂如双(三-叔丁基膦)钯(0)的情况下进行。

在步骤 2 中，将式(17)的二甲基吡咯基噻唑去保护，得到式(19)的氨基噻唑。将二甲基吡咯用羟胺在乙酸中在约 60 至 100℃的温度下处理约 4 至

8 小时，优选约 6 小时。在步骤 3 中，为了形成式(18)的噻唑乙酰胺，使用与步骤 2 中所用条件相同的条件，不同的是反应持续约 72 小时。

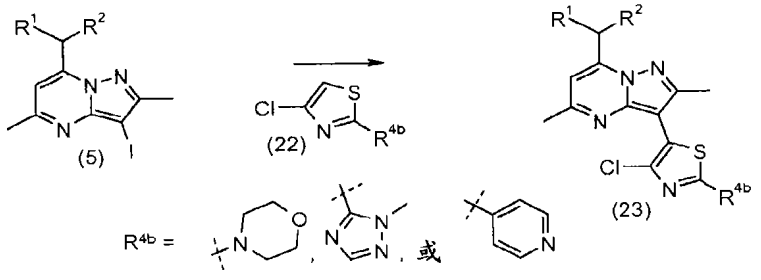
在流程图 4 的步骤 4 中，用改进的 Sandmeyer 反应将式(19)的 2-氨基噻唑转化成式(20)的 2-溴噻唑。优选的条件在约 60 至 80℃的温度下在乙腈中使用亚硝酸烷基酯如亚硝酸叔丁酯和溴化铜(II)。

在步骤 5 中，用式-NR^aR^b 的胺或用吗啉使式(20)的 2-溴噻唑进行置换反应，得到式(21)的氨基噻唑。该反应在惰性溶剂如甲醇、THF 或二噁烷中进行，或者在不使用溶剂的情况下进行，使用过量的胺，反应温度为约 70 至 110℃。或者，用反应胺和过量的三乙胺或无机碱如碳酸铯进行该反应。在式(21)的氨基噻唑的合成中，也考虑各种去保护步骤，如除去氨基甲酸叔丁酯(BOC)，因为在-NR^aR^b 携带式 I 中所定义的其它胺官能团的情况下，为了进行上述反应，这样的步骤可能是需要的或有益的。适宜的保护基团的选择和使用在本领域中是众所周知的和可理解的(参见例如 ***Protecting Groups in Organic Synthesis***, Theodora Greene (Wiley-Interscience))。

在流程图 4 的步骤 6 中，以与流程图 2 的步骤 1 中所述方式相似的方式使式(20)的 2-溴噻唑与杂环锌试剂在 Negishi 交叉偶联反应中偶联，得到式(20a)的噻唑杂环。

本领域技术人员应当意识到，式(16)的官能化的噻唑可用本领域中已知的方式来制备。例如，将硫脲与溴代酮如 3-溴-1,1,1-三氟丙-2-酮环化，得到 4-三氟甲基-噻唑-2-基胺。随后用己烷-2,5-二酮对该胺进行溴化和保护，得到(16)。

流程图 5

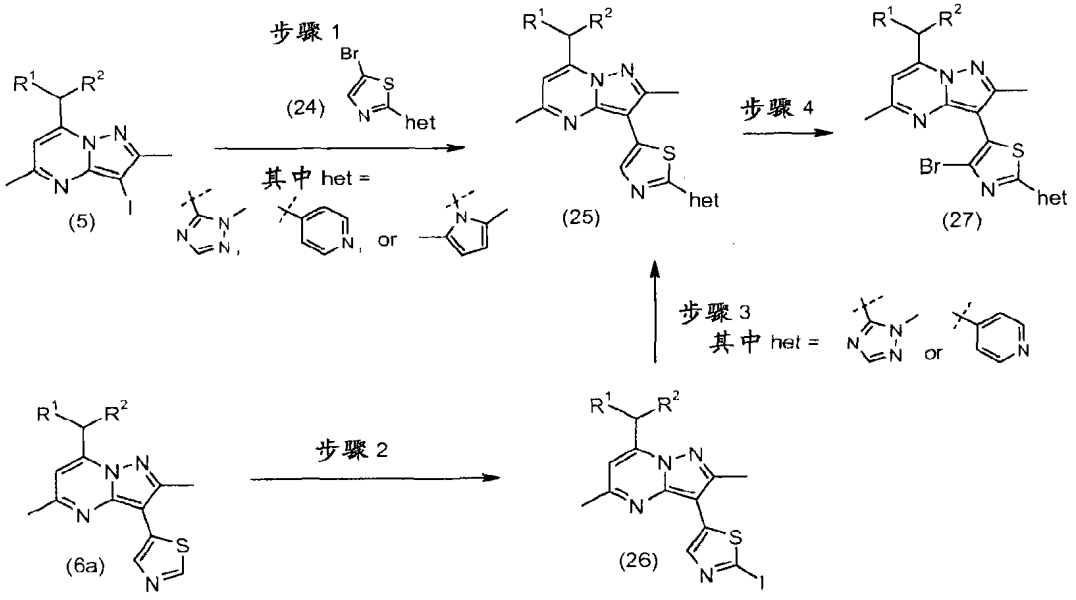


可以按照流程图 5 中所述的反应来形成式(23)的化合物。一种适宜的式(23)的化合物是其中基团定义如下的化合物：R^1 和 R^2 如式 I 中所定义，R^{4b} 具有所述定义。

使式(5)的碘代吡唑并嘧啶与例如式(22)的 4-氯-2-吗啉代-噻唑进行交叉偶联，形成式(23)的吡唑并嘧啶噻唑。例如，将所述反应物在存在碘化铜、醋酸钯和三苯基膦的情况下用碱如碳酸铯偶联。该偶联反应优先在惰性溶剂如 DMF 中在约 100 - 150℃下进行 4-24 小时。

本领域技术人员应当意识到，式(22)的官能化的噻唑可用本领域中已知的方式来制备。例如，可以使 2,4-二氯噻唑与吗啉反应，得到式(22)的 2-吗啉代-噻唑。也可以将 2,4-二氯噻唑耗竭性溴化，得到 2,5-二溴-4-氯噻唑。随后用正丁基锂在 THF 中在-90℃下进行溴-锂交换并用水淬灭，得到 2-溴-4-氯噻唑[*J. Chem. Soc. Perkin Trans I: Org Bioorg. Chem.* (1972-1999), (2):215-219(1992)]。可以用杂环锌试剂使 2-溴-4-氯噻唑进行 Negishi 交叉偶联反应，得到噻唑-2-基三唑或吡啶。

流程图 6



可以按照流程图 6 中所述的反应来形成式(27)的化合物。一种适宜的式(27)的化合物是其中基团定义如下的化合物：R^1 和 R^2 如式 I 中所定义，"het"分别具有步骤 1 或步骤 3 中所述的定义。

在流程图 6 的步骤 1 中，通过使式(5)的碘代吡唑并嘧啶与式(24)的溴代噻唑反应获得杂环噻唑，例如式(25)的杂环噻唑。该反应在存在溴化 N-丁基铵和碱如醋酸钾的情况下用钯催化剂如具有三(2,4-二-叔丁基-苯基)-磷烷(phosphane)的醋酸钯在惰性溶剂如 N-甲基吡咯烷酮中在约 100-150℃的温度下进行。

或者，在步骤 2 中，将式(6a)的噻唑基吡唑并嘧啶碘化，得到式(26)的 2-碘噻唑。将该噻唑用二异丙基氨基锂在-70 至-80℃的温度下处理约 1 小时，然后用 N-碘琥珀酰亚胺在大约相同的温度下在惰性溶剂如 THF 中处理。然后进行步骤 3，在该步骤中，用与流程图 2 的步骤 1 中所述条件相似的 Negishi 交叉偶联条件形成式(25)的三唑基或 4-吡啶基噻唑。

在流程图 6 的步骤 4 中，将式(25)的噻唑基吡唑并嘧啶溴化，得到式(27)的溴代噻唑。该溴化是用 N-溴琥珀酰亚胺在存在少量乙酸的情况下在惰性溶剂如乙腈中进行的。

可以如流程图 4 的步骤 2、3、4 和 5 中所述的那样进一步对其中 het = 2,5-二甲基-吡咯-1-基的式(27)的化合物进行修饰，从而获得本发明的化合物。

本领域技术人员应当意识到，可以用本领域中已知的方法容易地制备式(24)的杂环噻唑类。例如，可以用 1,1-二甲氧基-N,N-二甲基-甲胺将噻唑-2-甲酰胺环化成三唑，然后用 N-甲基-肼处理，获得三唑基噻唑，随后可以用 N-溴琥珀酰亚胺将其溴化。根据文献操作可得到 4-噻唑-2-基-吡啶，可将其溴化，得到 4-(5-溴-噻唑-2-基)-吡啶。5-溴-2-(2,5-二甲基-吡咯-1-基)-噻唑可以通过使 2-氨基-5-溴噻唑与己烷-2,5-二酮反应来容易地获得。

本文所用的“TLC”是指薄层色谱；“HPLC”是指高效液相色谱；“LC/MS”是指液相色谱/质谱；“GC/MS”是指气相色谱/质谱”；“HR-ToF’是指高分辨率的飞行时间(high resolution time-of-flight)；“APCI”是指大气压化学电离；“δ”是指从四甲基硅烷向低磁场的百万分数；“THF”是指四氢呋喃；“EtOAc”是指乙酸乙酯；“MeOH”是指甲醇；“EtOH”是指乙醇；“DMF”是指二甲基甲酰胺。

实施例

无需进一步加工，认为本领域技术人员可以用前面的描述来充分实施本发明。用下面的制备例和实施例来对本发明进行进一步详细说明。它们旨在进行举例说明，而不是要以任何方式对本发明进行限制。其中的试剂和起始材料可容易地获得或者可被本领域普通技术人员容易地合成。实施例 1-35 提供了代表性的化合物并说明了其制备。实施例 A-D 说明了可用于测定本发明化合物的生物学性质的各种生物学测定法。本领域技术人员将迅速意识到实施例中所述的操作的适宜变型。本发明的化合物的名称是用 **ChemDraw Ultra® 7.0.1** 版提供的。盐是以游离碱加共轭酸的形式来进行命名的。

制备例 1

2,5-二甲基-4H-吡唑并[1,5-*a*]嘧啶-7-酮

将乙酰乙酸乙酯(128 g, 0.98 mol)滴加到 5-甲基-2*H*-吡唑-3-基胺(100 g, 0.95 mol 的乙酸溶液(500 mL)中，同时保持其温度为 25-28℃。将混合物在回流下加热 10 小时，然后将其冷却至室温。将反应加入到被冷却至 5℃的叔-丁基甲基醚(5 L)中，同时保持其温度低于 10℃。将其在 5℃下搅拌 1 小时，过滤。将所得物质真空干燥过夜，得到白色固体(158 g, 96%)。

制备例 2

7-氯-2,5-二甲基-吡唑并[1,5-*a*]嘧啶

向 2,5-二甲基-4*H*-吡唑并[1,5-*a*]嘧啶-7-酮(10.0 g, 61.3 mmol)在甲苯(150 mL)中的混悬液中加入 *N*,*N*-二甲基苯胺(9.7 mL, 76.7 mmol)。向这种白色混悬液中滴加三氯氧化磷(phosphorus oxychloride)(11.2 mL, 122.6 mmol)。将其在惰性气氛下回流 3 小时，冷却至室温并将反应减压浓缩成棕色油状物。将该油状物溶解于乙酸乙酯(250 mL)中并用 1.0 N NaOH 碱化。分离出有机相，用乙酸乙酯对碱性水相进行萃取(2 × 100 mL)。将有机相合并，用无水硫酸镁干燥，过滤，减压浓缩，得到棕色固体。将该物质

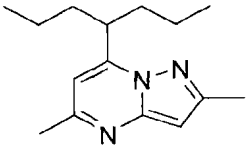
用快速色谱进行纯化，用 80%己烷/20%(30% THF/己烷)至 0%己烷/100%(30% THF/己烷)以 20%的增量逐步梯度洗脱，得到浅绿色固体(6.65 g, 59%)。ES/MS m/z (^{35}Cl) 182.3 $(M+1)^+$。

另一种操作：

将 2,5-二甲基吡唑并[1,5-*a*]嘧啶-7-酮(20 g, 122 mmol)加入到 1,4-二噁烷(60 mL)中。将混合物在 22℃下搅拌 10 分钟，然后加入 N,N-二乙基苯胺(20.8 mL, 128 mmol)。将其再搅拌 5 分钟，然后历经 15 分钟加入三氯氧化磷(11.7 mL, 126 mmol)。将混合物在 22℃下搅拌 15 分钟，然后历经 35 分钟加热至 80-85℃并将反应在这一温度下保持 1.5 小时。将被冷却的反应混合物缓慢加入到被冷却至 0-5℃的磷酸氢二钾(106.7 g, 612.82 mmol)在水(325 mL)中的溶液中，在加入期间保持温度低于 5℃。将混合物在 22℃下进行搅拌，然后加入甲基-叔-丁基醚(150 mL)。分离有机层，用甲基-叔-丁基醚对水层进行萃取(2 × 100 mL)。将有机部分合并，用硫酸钠干燥，过滤，蒸发掉溶剂。用硅胶色谱进行纯化，用己烷/乙酸乙酯(2/1)进行洗脱，得到黄色固体形式的标题化合物(20.7 g, 88%)。ES/MS m/z (^{35}Cl) 182 $(M+1)^+$。

制备例 3

7-(1-丙基-丁基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶



向一个配有回流冷凝器的烘箱干燥的烧瓶中加入无水 THF(40 mL)、碘(催化量)、镁条(1.92 g, 78.9 mmol)和 4-溴庚烷(9.4 mL, 52 mmol)。将反应在油浴中加热至回流。当格氏反应开始时，反应温度迅速升高。将反应在 90℃下再搅拌 4 小时，冷却至室温。使镁金属沉降(也可以进行离心)，在正氩气压下通过套管将格氏试剂加入到装有 7-氯-2,5-二甲基吡唑并[1,5-*a*]嘧啶(4.80 g, 26.3 mmol)(位于无水甲苯(20 mL)中)的烧瓶中。将反应

在惰性气氛下回流过夜。将反应冷却至室温并用水淬灭。用乙酸乙酯(150 mL)稀释，然后加入水(100 mL)和饱和氯化铵(50 mL)。将水相分离出来并用二氯甲烷(75 mL)萃取。将有机相合并，用无水硫酸镁干燥，过滤，减压浓缩。将所得残余物用快速色谱进行纯化，用 80%己烷/20%(20%乙酸乙酯/己烷)至 0%己烷/100%(20%乙酸乙酯/己烷)以 20%的增量逐步梯度洗脱，得到黄色晶体(3.08 g, 48%)。ES/MS m/z 246.3 $(M+1)^+$。

另一种操作:

将镁屑(3.5 g, 144 mmol)和催化量的碘(100 mg)在 THF(100 mL)中的混合物在氮气气氛下加热至 65℃。加入几滴纯 4-溴庚烷并将混合物加热至反应开始。然后，历经在 2 小时加入 4-溴庚烷(17.6 mL, 94.9 mmol)在 THF(42 mL)中的溶液，同时保持温度为 65-70℃。将混合物再回流 1 小时，然后将反应冷却至 22℃。在氮气气氛下，将制得的格氏试剂加入到被冷却至 0℃的 7-氯-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(10.2 g; 53.3 mmol)在 THF(60 mL)中的溶液中。历经 45 分钟，通过套管加入镁试剂溶剂，同时保持温度低于 10℃。然后将混合物在 5℃下再搅拌 30 分钟。向这种混合物中加入 10%氯化铵水溶液(wt/wt)(125 mL)并将其在 22℃下搅拌 30 分钟。分离有机层并用乙酸乙酯对水层进行萃取(2 × 25 mL)。将有机层合并并用硫酸钠干燥。对混合物进行过滤并蒸发掉溶剂。用硅胶快速色谱对该粗品物质进行纯化，用己烷/乙酸乙酯(5/1)进行洗脱，得到标题化合物(8 g, 62%)。ES/MS m/z 246 $(M+1)^+$。

制备例 3a

7-(1-丙基-丁基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶

另一种路径

步骤 1: 3-乙酰基-3-氧代-4-丙基-庚酸乙酯

将氯化镁(14.63 g, 153.70 mmol)加入到二氯甲烷(500 mL)中，然后一次性加入乙酰乙酸乙酯(19.55 mL, 20.00 g, 153.79 mmol)并将其在室温下搅拌 1 小时。将混合物在冰水浴中冷却并滴加吡啶(24.86 mL, 24.32 g, 307.39 mmol)。在 0℃下在氮气下向该白色浆液中滴加二-正丙基乙酰氯(25.00 g,

153.70 mmoles)。在完全加入后，除去冷却浴，温热至环境温度并将其搅拌 16 小时。将反应用 1 N HCl(400 mL)淬灭并分离出底层。将有机部分用硫酸镁干燥，过滤，真空浓缩，得到黄色油状物(34 g, 86%)。将该物质不进行进一步纯化地直接用于下一步。

步骤 2: 5-丙基-辛烷-2,4-二酮

将 3-乙酰基-3-氧代-4-丙基-庚酸乙酯(32.4 g, 126.39 mmol)溶解于二甲基亚砜(100 mL)和水(5 mL)中。将该溶液在 150℃下加热 6 至 8 小时或者用 GC/MS 对反应进行追踪。将反应冷却，用庚烷萃取(3 × 100 mL)。将合并的有机部分用水(100 mL)和盐水(100 mL)洗涤。在 50℃下真空浓缩以除去大部分庚烷。得到 23.29g 油状物并将其直接用于下一步。

步骤 3: 7-(1-丙基-丁基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶

将 5-丙基-辛烷-2,4-二酮(15 g, 81.40 mmol)混合到乙酸(15 mL)中并将其在冰浴上进行冷却。分批加入 5-氨基-3-甲基吡唑(7.91 g, 81.40 mmol)并在环境温度下进行搅拌。在 3 小时后，用 GC/MS 检查是否反应完全。GC 表明与真实样品相比存在正确的区域异构体。蒸馏掉过量的乙酸。加入水(50 mL)并用庚烷(50 mL)萃取。用盐水(50 mL)对庚烷部分进行洗涤。将有机层用 $MgSO_4$ 干燥，过滤，真空浓缩，得到粗品油状物(15.8 g, 79%)。^1H NMR ($CDCl_3$): 6.39(s, 1H); 6.31(s, 1H); 3.75(m, 1H); 2.55(s, 3H); 2.45(s, 3H); 1.71(q, 4H); 1.23(m, 4H); 0.85(t, 6H)。

基本如制备例 3 中所述的那样用任何一种操作制备下面的化合物。用 3-溴戊烷来制备格氏试剂。

制备例号	化学名	物理数据
4	7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶	ES/MS m/z 218.1 $(M+1)^+$

制备例 5

7-(1-丙基-丁基)-3-碘-2,5-二甲基-吡唑并[1,5-*a*]嘧啶

将 7-(1-丙基-丁基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(3.08 g, 12.5 mmol)溶

解于无水乙腈(25 mL)中并以 10 分钟的时间间隔加入 6 份(每份 0.70 g) N-碘琥珀酰亚胺(4.2 g, 18.7 mmol)。在室温下搅拌过周末。除去乙腈，用二氯甲烷(100 mL)对剩下的油状物进行稀释。用饱和氯化铵溶液(2 × 50 mL)洗涤。收集有机相，用无水硫酸镁干燥，过滤，减压浓缩，得到深红色的油状物。用快速色谱对该油状物进行纯化，用 100%己烷/0%(20%乙酸乙酯/己烷)至 0%己烷/100%(20%乙酸乙酯/己烷)以 50%的增量逐步进行梯度洗脱，得到橙色油状物(10.97 g, 87%)。^{1}H NMR (400 MHz, $CDCl_3$): 6.42(s, 1H), 3.74-3.70(m, 1H), 2.58(s, 3H), 2.46(s, 3H), 1.74-1.68(m, 4H), 1.28-1.14(m, 4H), 0.84(t, J = 7.0 Hz, 6H)。

基本如制备例 5 所述的那样制备下面的化合物。

制备例号	化学名	物理数据
6	7-(1-乙基-丙基)-3-碘-2,5-二甲基-吡唑并[1,5-*a*]嘧啶	1H NMR(400 MHz, $CDCl_3$): 6.44(s, 1H), 3.59(m, 1H), 2.61(s, 3H), 2.49(s, 3H), 1.86-1.76(m, 4H), 0.85(t, J = 7.5 Hz, 6H)。

制备例 6 的另一种操作:

将乙酸(1 mL)和 N-碘琥珀酰亚胺(6.7 g, 29.9 mmol)一次性加入到 7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(6 g, 27.5 mmol)在乙腈(60 mL)中的溶液中。将混合物在 22℃下搅拌 2 小时。蒸发掉溶剂，将残余物吸收于水(50 mL)和甲基-叔丁基醚(100 mL)中。将有机部分分离出来，用硫酸钠干燥，过滤，蒸发掉溶剂，得到标题化合物(9.2 g, 96%)。ES/MS m/z 344 $(M+1)^+$。

制备例 7

2-三甲基硅烷基-噻唑

将正丁基锂(20.4 mL, 51.0 mmol, 2.5 M 的己烷溶液)与乙醚(50 mL)在一个配有滴液漏斗和温度计的三颈烧瓶中混合到一起。冷却至-78℃，滴加

噻唑(4.25 g, 50.0 mmol)在乙醚(50 mL)中的溶液。在完全加入后，将反应混合物在-78℃下搅拌 30 分钟，然后加入三甲基氯硅烷(5.4 g, 50 .0 mmol)。在-78℃下搅拌 1 小时，然后温热至室温。通过加入饱和碳酸氢钠来将反应淬灭。用乙醚萃取水层。用盐水洗涤合并的有机部分，用硫酸钠干燥。过滤并减压浓缩，得到残余物。通过蒸馏进行纯化，得到 8.33 g (52-56℃/ 15 mm Hg)标题化合物。^{1}H NMR (400 MHz, $CDCl_3$) 8.13(d, 1H, *J*=2.6 Hz), 7.54(d, 1H, *J* = 2.6 Hz), 0.43(s, 9H)。

制备例 8

5-溴-4-甲基噻唑

在 0℃下，向 4-甲基噻唑(15.0 g, 152 mmol)在乙酸(30 mL)中的溶液中加入溴(9.27 mL, 182 mmol)。将反应混合物缓慢温热至室温并搅拌过夜。用二氯甲烷稀释并用 1 N NaOH 和盐水洗涤。将有机层用硫酸钠干燥，过滤，真空浓缩。用硅胶柱色谱对粗产物进行纯化，用己烷/乙酸乙酯(5/1)进行洗脱，得到标题化合物(9.94 g, 37%)。^{1}H NMR (400 MHz, $CDCl_3$): δ 8.69(s, 1H), 2.43(s, 3H)。

制备例 9

噻唑-2-甲酸乙酯

历经 15 分钟，向 2-三甲基硅烷基噻唑(135 g, 858.1 mmol)在甲苯(1350 mL)中的混合物中加入氯甲酸乙酯(98.4 mL, 1.03 mol)在甲苯(1350 mL)中的溶液。将反应在 22℃下搅拌 2 小时。将该溶液加到 25%(wt/wt)碳酸钠水溶液(5 L)上并搅拌 30 分钟。分离有机层，用二氯甲烷(2 × 1 L)反萃取水层。将有机层合并并蒸发掉溶剂，得到标题化合物(134 g, 99%)。ES/MS m/z 158 $(M+1)^+$。

制备例 10

噻唑-2-甲酰胺

将噻唑-2-甲酸乙酯(150 g, 0.9 mol)加入到甲醇(75 mL)和 30%氢氧化铵水溶液(750 mL)的混合物中，将混合物在回流下加热 1 小时。然后冷却至 22℃，在真空下蒸发掉甲醇。将混合物在室温下搅拌 30 分钟，滤出固体。将分离出来的固体真空干燥，得到标题化合物(98 g, 85%)。ES/MS m/z 129 $(M+1)^+$。

制备例 11

1-甲基-5-噻唑-2-基-1H-[1,2,4]三唑

将 1,1-二甲氧基-*N*,*N*-二甲基-甲胺(240 mL)冷却至 10℃并分三份加入噻唑-2-甲酰胺(60 g, 421 mmol)。将混合物在 10℃下搅拌 30 分钟。然后将混合物在 45 分钟内逐渐加热至回流。蒸馏掉所形成的甲醇，然后将反应加热至 100℃达 1.5 小时。将混合物冷却至 60℃并通过真空蒸馏除去过量的 1,1-二甲氧基-N,N-二甲基-甲胺。将残余物冷却至 22℃，加入己烷(200 mL)。将混合物研磨 15 分钟，过滤，将固体干燥至恒重，然后用于下一步。

将上面分离出来的固体(68 g)加入到乙酸(680 mL)中并将混合物冷却至 10℃。以保持温度低于 15℃的速度加入 *N*-甲基-肼(27 mL, 509 mmol)。将混合物在 30 分钟内温热至 20℃，然后逐渐加热至 90℃。在 90℃下搅拌 30 分钟，然后冷却至 22℃。通过真空蒸馏除去乙酸。将残余物加到水上，通过加入 25%氢氧化钠水溶液调至 pH = 8。用甲基-叔丁基醚对水层进行萃取(3 × 600 mL)。将有机层合并，蒸发掉溶剂。用硅胶色谱对所得的残余物进行纯化，用己烷/异丙醇(9/1)进行洗脱，得到标题化合物(49 g, 70%)。ES/MS m/z 167 $(M+1)^+$。

制备例 12

5-(5-溴-噻唑-2-基)-1-甲基-1*H*-[1,2,4]三唑

历经 1 小时分三批向甲基-5-噻唑-2-基-1H-[1,2,4]三唑(6.55 g; 39.4 mmol)和二甲基甲酰胺(32 mL)的混合物中加入 N-溴琥珀酰亚胺(14 g, 78.8 mmol)。将混合物在 22℃下搅拌 18 小时，然后加入到被冷却至 0-5℃的水

(300 mL)中。分离出水层，用甲基-叔丁基醚(2 × 200 mL)萃取。将有机层合并，用 7%碳酸氢钠水溶液(100 mL)洗涤，然后用硫酸钠干燥。过滤并蒸发掉溶剂，得到标题化合物(9.5 g, 93%)。ES/MS m/z (^{79}Br/^{81}Br) 245/247 $(M+1)^+$。

制备例 13

2,5-二甲基-3-[2-(2-甲基-2*H*-[1,2,4]三唑-3-基)-噻唑-5-基]-7-(1-丙基-丁基)-吡唑并[1,5-*a*]嘧啶

将 5-(5-溴-噻唑-2-基)-1-甲基-1H-[1,2,4]三唑(6.5 g, 23.8 mmol)和 2,5-二甲基-7-(1-丙基-丁基)-吡唑并[1,5-*a*]嘧啶(6 g, 24.5 mmol)合并到 N-甲基吡咯烷酮(58 mL)中并将其在氮气下搅拌至完全形成溶液。然后，加入溴化四-N-丁基铵(5.47 g, 16.7 mmol)和醋酸钾(11.8 g, 119 mmol)并将混合物在氮气气氛下加热至 100℃。通过用真空/氮气清洁三个循环对该热混合物进行脱气。然后加入醋酸钯(216 mg, 0.94 mmol)和三(2,4-二-叔丁基-苯基)-磷烷(787 mg, 1.2 mmol)。将混合物在 125℃下在氮气下加热 4 小时。将混合物冷却至 22℃并加入到水(750 mL)中。用甲基-叔丁基醚(3 × 200 mL)对水层进行萃取，将有机部分合并，蒸发。通过用硅胶垫过滤纯化残余物，用己烷/乙酸乙酯(4/1)进行洗脱。将包含产物的级分合并，蒸发掉溶剂，得到标题化合物(7 g, 72%)。ES/MS m/z 410 $(M+1)^+$。

制备例 14

2,5-二甲基-7-(1-丙基-丁基)-3-噻唑-5-基-吡唑并[1,5-*a*]嘧啶

向一个烘箱干燥的烧瓶中加入被溶解于无水 THF(30 mL)中的 2-三甲基硅烷基噻唑(1.765 g, 11.24 mmol)并将其在惰性气氛下冷却至-78℃。缓慢加入正丁基锂(2.5 M 己烷溶液, 4.5 mL, 11.24 mmol)并在-78℃下搅拌 30 分钟。一次性加入无水氯化锌(2.26 g, 16.58 mmol)并在-78℃下搅拌 30 分钟。使反应升至室温，搅拌 30 分钟，加入 7-(1-丙基-丁基)-3-碘-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(1.624 g, 5.18 mmol)和二氯[1,1'-双(二苯基膦基)二茂铁]钯

(II)二氯甲烷(0.423 g, 0.518 mmol)。在油浴(90℃)中在惰性气氛下回流过夜。将反应冷却至室温，用饱和碳酸氢钠淬灭，用乙酸乙酯(150 mL)稀释。将水相分离出来并用乙酸乙酯(75 mL)萃取。将有机相合并，用无水硫酸镁干燥，过滤，减压浓缩。将所得残余物用快速色谱进行纯化，用 100%己烷/0%(30% THF/己烷)至 0%己烷/100%(30% THF/己烷)以 10%的增量逐步进行梯度洗脱，得到白色固体(0.720 g, 42%)。ES/MS m/z 329.0$(M+1)^+$。

基本如制备例 14 中所述的那样来制备下面的化合物。

制备例号	化学名	物理数据
15	7-(1-乙基-丙基)-2,5-二甲基-3-噻唑-5-基-吡唑并[1,5-*a*]嘧啶	1H NMR(400 MHz, $CDCl_3$): d 8.75(s, 1H), 8.75(s, 1H), 8.28(s, 1H), 6.47(s, 1H), 3.62-3.55(m, 1H), 2.65(s, 3H), 2.61(s, 3H), 1.89-1.75(m, 4H), 0.85(t, 6H, *J* = 7.5 Hz), 0.85(t, 6H, *J* = 7.5 Hz)。

制备例 15 的另一种操作:

在-78℃下，在氮气下，将正丁基锂(76.5 mL, 191 mmol, 2.5 M 的己烷溶液)加入到 2-三甲基硅烷基噻唑(30 g, 191 mmol)在 THF(450 mL)中的溶液中，在加入期间保持温度低于-74℃。将该混合物在-78℃下搅拌 30 分钟，然后一次性加入氯化锌干粉(39.9 g, 286 mmol)并将混合物历经 1 小时温热至 22℃。加入 7-(1-乙基-丙基)-3-碘-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(30 g, 87 mmol)和 1,1'-双(二苯基膦基)二茂铁氯化钯(II)(6.5 g, 8 mmol)并将混合物在氮气下加热至回流达 8 小时。将混合物冷却至 22℃并加入 10%氯化铵水溶液(450 mL)。分离有机层并用甲基-叔丁基醚对水层进行洗涤(2 × 100 mL)。将有机部分合并，用硫酸钠干燥，过滤，蒸发掉溶剂，得到标题化合物(20.4 g, 78%)。ES/MS m/z 301$(M+1)^+$。

制备例 16

7-(1-乙基-丙基)-3-(2-碘-噻唑-5-基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶

在-78℃下，在氮气气氛下，将新制备的二-异丙基氨基锂溶液(150 ml; 62.4 mmol, 0.6 M 的 THF 溶液)加入到 7-(1-乙基-丙基)-2,5-二甲基-3-噻唑-5-基-吡唑并[1,5-*a*]嘧啶(17.8 g, 62.4 mmol)在 THF(100 mL)中的混合物中，在加入期间保持温度低于-74℃。将该混合物在-78℃下搅拌 1 小时，然后加入 N-碘琥珀酰亚胺(15 g, 63 mmol)在 THF(100 mL)中的溶液，同时保持温度低于-74℃。将反应逐渐温热至 22℃，然后加入 10%氯化铵水溶液(300 mL)。分离有机层，用甲基-叔丁基醚对水层进行洗涤(2 × 200 mL)。将有机层合并，用硫酸钠干燥，过滤并蒸发掉溶剂。用硅胶色谱对所得的残余物进行纯化，用己烷/丙酮(5/1)进行洗脱，得到标题化合物(15 g, 60%)。ES/MS m/z 427 $(M+1)^+$。

实施例 1

7-(1-乙基-丙基)-2,5-二甲基-3-(4-甲基-噻唑-5-基)-吡唑并[1,5-*a*]嘧啶

将 Rieke®锌(10 g，位于 100 mL THF 中, 13.2 mL, 18.48 mmol)加入到 5-溴-4-甲基噻唑(2.13 g, 18.48 mmol)中并在回流下加热 2 小时。将混合物冷却至室温，通过离心使锌沉降。向位于干燥四氢呋喃(10 mL)中的 7-(1-乙基-丙基)-3-碘-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(900 mg, 2.62 mmol)中用氮气鼓泡，加入有机溴化锌溶液，然后加入[1,1'-双(二苯基膦基)二茂铁]-二氯钯(II)(106 mg, 0.13 mmol)。将反应混合物在回流下搅拌过夜，然后冷却至室温。向反应混合物中加入氯化铵溶液并用二氯甲烷萃取。将有机部分用硫酸钠干燥，过滤，真空除去溶剂。用硅胶柱色谱对粗产物进行纯化，用己烷/乙酸乙酯(3/1)进行洗脱，得到标题化合物(652 mg, 79%)。^{1}H NMR (400 MHz, $CDCl_3$): δ 8.80(s, 1H), 6.48(s, 1H), 3.63(m, 1H), 2.57(s, 3H), 2.43(s, 3H), 2.40(s, 3H), 1.85(m, 4H), 0.90(t, 6H, J = 7.3Hz)。ES/MS m/z 315 $(M+1)^+$。

制备例 17

3-(2,4-二溴-噻唑-5-基)-2,5-二甲基-7-(1-丙基-丁基)-吡唑并[1,5-*a*]嘧啶

将 2,5-二甲基-7-(1-丙基-丁基)-3-噻唑-5-基-吡唑并[1,5-*a*]嘧啶(3.15 g, 9.59 mmol)溶解于乙腈(100 mL)中并一次性加入 N-溴琥珀酰亚胺(4.27 g, 24.0 mmol)。在惰性气氛下搅拌过夜并用 TLC 证实反应进行完全。减压浓缩，用二氯甲烷(150 mL)稀释油状物，用水(75 mL)洗涤。将有机相用无水硫酸镁干燥，过滤，减压浓缩。将所得的油状物用快速色谱进行纯化，用 100%己烷/0%(30% THF/己烷)至 0%己烷/100%(30% THF/己烷)逐步梯度洗脱(0-10-15-20-25-30-35-40-45-50-100%的 30%THF/己烷)，得到黄色晶体(3.70 g, 79%)。ES/MS m/z ($^{79}Br^{81}Br$) 486.7 $(M+1)^+$。

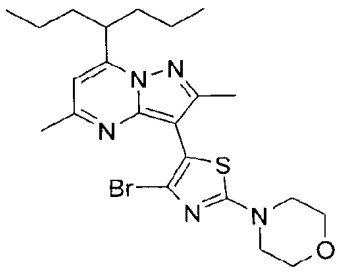
基本如制备 17 中所述的那样制备下面的化合物，不同的是用二氯甲烷作为溶剂。

实施例编号	化学名	物理数据
2	3-(2,4-二溴-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶	MS (APCI) m/z ($^{79}Br^{81}Br$) 459.1 $(M+1)^+$
3*	3-(2-溴-4-甲基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶	ES/MS m/z (^{79}Br) 393 $(M+1)^+$

*使用 1.1 eq NBS，搅拌 3 天。

实施例 4

3-(4-溴-2-吗啉-4-基-噻唑-5-基)-2,5-二甲基-7-(1-丙基-丁基)-吡唑并[1,5-*a*]嘧啶



向一个烘箱干燥的烧瓶中加入 3-(2,4-二溴噻唑-5-基)-2,5-二甲基-7-(1-

丙基-丁基)-吡唑并[1,5-*a*]嘧啶(0.973 g, 2.00 mmol)、无水二噁烷(20 mL)、吗啉(0.872 g, 10.0 mmol)和碳酸铯(1.95 g, 6.00 mmol)。在油浴(105℃)中在惰性气氛下回流过夜。用 LC/MS 证实反应完全。用乙酸乙酯(100 mL)稀释，用水(50 mL)洗涤，用乙酸乙酯(50 mL)反萃取水相。将有机相合并，用硫酸镁干燥，过滤，减压浓缩。将所得残余物用快速色谱进行纯化，用100%己烷/0%(40%二氯甲烷/20%乙酸乙酯/2% 7 N 氨的甲醇溶液/38%己烷)至 0%己烷/100%(40%二氯甲烷/20%乙酸乙酯/2% 7 N 氨的甲醇溶液/38%己烷)以 10%的增量逐步梯度洗脱，得到灰白色固体(0.878 g, 89%)。ES/MS m/z (^{79}Br) 491.7$(M+1)^+$。

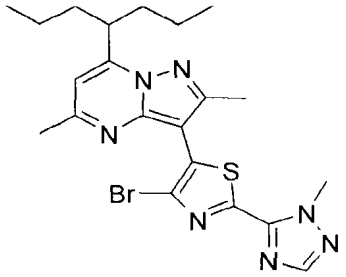
基本如实施例 4 中所述的那样用 2.0 二甲基胺/THF 或吗啉作为胺、用 THF 或二噁烷作为溶剂制备下面的实施例。这些反应是在密封的容器或 Schlenk 管中进行的。

实施例号	化学名	物理数据
5*	{4-溴-5-[2,5-二甲基-7-(1-丙基-丁基)-吡唑并[1,5-*a*]嘧啶-3-基]-噻唑-2-基}-二甲基胺	ES/MS (^{79}Br) 449.8 $(M+1)^+$。
6	3-(4-溴-2-吗啉-4-基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶	MS (APCI) m/z (^{81}Br) 466.5 $(M+1)^+$
7	{4-溴-5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶-3-基]-噻唑-2-基}-二甲基-胺	MS (APCI) m/z (^{81}Br) 424.4 $(M+1)^+$
8	{5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶-3-基]-4-甲基-噻唑-2-基}-二甲基-胺	ES/MS m/z 358 $(M+1)^+$
9	7-(1-乙基-丙基)-2,5-二甲基-3-(4-甲基-2-吗啉-4-基-噻唑-5-基)-吡唑并[1,5-*a*]嘧啶	ES/MS m/z 400 $(M+1)^+$

*使用 6 eq 碳酸铯。

实施例 10

3-[4-溴-2-(2-甲基-2*H*-[1,2,4]三唑-3-基)-噻唑-5-基]-2,5-二甲基-7-(1-丙基-丁基)-吡唑并[1,5-*a*]嘧啶



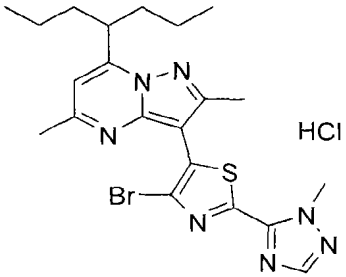
向一个烘箱干燥的烧瓶中加入 1-甲基-1,2,4-三唑(0.498, 6.00 mmol)和无水 THF(20 mL)，在惰性气氛下冷却至-78℃。缓慢加入正丁基锂(2.5M 己烷溶液, 2.4 mL, 6.0 mmol)并搅拌 30 分钟。一次性加入无水氯化锌(1.36 g, 10.0 mmol)并在-78℃下搅拌 30 分钟。使反应温热至室温，搅拌 30 分钟，加入 3-(2,4-二溴噻唑-5-基)-2,5-二甲基-7-(1-丙基-丁基)-吡唑并[1,5-*a*]嘧啶(0.973 g, 2.00 mmol)和二氯[1,1'-双(二苯基膦基)二茂铁]钯(II)二氯甲烷(0.163 g, 0.200 mmol)。将反应在油浴(90℃)中在惰性气氛下回流过夜。将反应冷却至室温，用水淬灭，用乙酸乙酯(100 mL)稀释。分离出含水部分，用二氯甲烷(50 mL)萃取。将有机相合并，用无水硫酸镁干燥，过滤，减压浓缩。将所得残余物用快速色谱进行纯化，用 100%己烷/0%(10%乙腈/40% THF/50%己烷)至 0%己烷/100%(10%乙腈/40% THF/50%己烷)以 10%的增量逐步梯度洗脱，得到白色固体(0.090 g, 9%)。ES/MS m/z (^{79}Br) 487.7 $(M+1)^+$。

另一种制备方法：

向 2,5-二甲基-3-[2-(2-甲基-2H-[1,2,4]三唑-3-基)-噻唑-5-基]-7-(1-丙基-丁基)-吡唑并[1,5-*a*]嘧啶(6 g, 14.65 mmol)在乙腈(60 mL)中的溶液中一次性加入 N-溴琥珀酰亚胺(2.74 g, 15.4 mmol)并在 22℃下搅拌 10 小时。蒸发掉溶剂，将残余物溶解于水(50 mL)和甲基-叔丁基醚(100 mL)的混合物中。分离有机层，用另外的甲基-叔丁基醚对水层进行萃取(2 × 50 mL)。将有机部分合并，蒸发掉溶剂。通过用硅胶垫过滤来对所得物质进行纯化，用己烷/乙酸乙酯(3/1)进行洗脱。将包含产物的级分合并，蒸发掉溶剂。加入庚烷(25 mL)并对固体进行研磨。将固体滤出，真空干燥，得到标题化合物(5.5 g, 77%)。ES/MS m/z (^{79}Br/^{81}Br) 488/490$(M+1)^+$。

实施例 10a

3-[4-溴-2-(2-甲基-2*H*-[1,2,4]三唑-3-基)-噻唑-5-基]-2,5-二甲基-7-(1-丙基-丁基)-吡唑并[1,5-*a*]嘧啶，盐酸盐



将 3-[4-溴-2-(2-甲基-2*H*-[1,2,4]三唑-3-基)-噻唑-5-基]-2,5-二甲基-7-(1-丙基-丁基)-吡唑并[1,5-*a*]嘧啶(750 mg, 1.54 mmol)溶解于丙酮(5 mL)中并加入 1 M HCl 的乙醚溶液(1.84 mL, 1.84 mmol)。将混合物在室温下搅拌 3 小时并真空浓缩。将残余物溶解于乙醚/己烷= 1/1(5 mL)中，使所需的 HCl 盐结晶(526 mg, 65%)。ES/MS m/z (^{81}Br) 490 $(M+1)^+$; ^{1}H-NMR ($CDCl_3$): 8.20(s, 1H), 6.82(s, 1H), 4.21(s, 3H), 3.64(m, 1H), 2.49(m, 3H), 2.44(s, 3H), 1.75(m, 4H), 1.96(m, 4H), 0.81(m, 6H)。

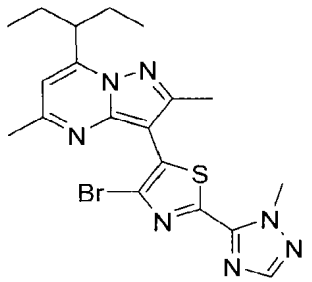
基本如实施例 10 中所述的那样来制备下面的化合物。

实施例编号	化学名	物理数据
11*	7-(1-乙基-丙基)-2,5-二甲基-3-[4-甲基-2-(2-甲基-2H-[1,2,4]三唑-3-基)-噻唑-5-基]-吡唑并[1,5-*a*]嘧啶	ES/MS m/z 396 $(M+1)^+$

* 用 0.5 M 位于 THF 中的氯化锌代替无水氯化锌。在 80℃下加热 3 天。

实施例 12

3-(4-溴-2-(2-甲基-2H-[1,2,4]三唑-3-基)-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶



在-78℃下，在氮气气氛下，将正丁基锂(2.5M 己烷溶液, 0.6 mL, 1.5 mmol)加入到 1-甲基-1,2,4-三唑(124.5 mg, 1.5 mmol)在 THF(3 mL)中的溶液中并搅拌 30 分钟。加入无水氯化锌(409 mg, 3.0 mmol)，继续搅拌 30 分钟，温热至室温并搅拌 2 小时。加入 3-(2,4-二溴-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(229 mg, 0.5 mmol)，然后加入四(三苯基膦)钯(58 mg, 0.05 mmol)并回流过夜。冷却至室温，用乙酸乙酯稀释，用饱和氯化铵洗涤。将有机部分用硫酸钠干燥，过滤，浓缩，获得残余物。用快速色谱对粗品物质进行纯化，用己烷:乙酸乙酯(10:2.5)进行洗脱，得到黄色泡沫形式的标题化合物(77 mg)。MS (APCI) m/z (^{79}Br) 460.4 $(M+1)^+$。

从实施例 21 开始的另一种制备方法:

将乙酸(1 mL)和 N-溴琥珀酰亚胺(4.1 g, 22 mmol)加入到 7-(1-乙基-丙基)-2,5-二甲基-3-[2-(2-甲基-2H-[1,2,4]三唑-3-基)-噻唑-5-基]-吡唑并[1,5-*a*]嘧啶(8 g, 21 mmol)在乙腈(80 mL)中的溶液中。将混合物在 22℃下搅拌 2 小时。然后蒸发掉溶剂，向所得的残余物中加入水(50 mL)和甲基-叔丁基醚(100 mL)。将有机部分分离出来，用硫酸钠干燥，过滤，蒸发掉溶剂。将所得的残余物用异丙醇重结晶，得到标题化合物(8.7 g, 90%)。ES/MS (m/z): (^{79}Br/^{81}Br) 460/462 $(M+1)^+$。

实施例 12a

3-(4-溴-2-(2-甲基-2H-[1,2,4]三唑-3-基)-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶, 对-甲苯磺酸

将 3-(4-溴-2-(2-甲基-2H-[1,2,4]三唑-3-基)-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(50 mg, 0.109 mmol)溶解于丙酮(3 mL)

中。加入 0.25 M 对-甲苯磺酸水溶液(434.4 μL, 0.109 mmol)，将所得混合物蒸发至干。加入乙酸乙酯(12 mL)以使固体部分溶解。加入甲醇(1 mL)以获得澄清溶液。通过缓慢蒸发来对该溶液进行浓缩，直至观察到晶体。通过过滤分离出晶体并在 25℃下真空干燥，得到约 50 mg 标题化合物。

用使用下列 HPLC 条件的离子色谱测定该盐的化学计量：柱：Phenomenex Phenosphere SAX, 4.6 × 150 mm，30℃；流动相：50%乙腈/50% 0.025 M 磷酸钠缓冲液, pH = 4.5；流速= 1.5 mL/min；检测：UV, 205 nm；进样体积= 5 μL；运行时间= 3 分钟。理论量计算值：27.2%甲苯磺酸根；实测值：28.4%甲苯磺酸根(三次 HPLC 运行的均值)。

实施例 13

{4-氯-5-[2,5-二甲基-7-(1-丙基-丁基)-吡唑并[1,5-*a*]嘧啶-3-基]-噻唑-2-基}-二甲基胺

向一个烘箱干燥的烧瓶中加入{4-溴-5-[2,5-二甲基-7-(1-丙基-丁基)-吡唑并[1,5-*a*]嘧啶-3-基]-噻唑-2-基-二甲基胺(0.20, 0.44 mmol)和无水 THF (3.0 mL)并在惰性气氛下冷却至-78℃。缓慢加入正丁基锂(1.6 M 己烷溶液, 0.42 mL, 0.67 mmol)并搅拌 30 分钟。一次性加入 N-氯琥珀酰亚胺(0.120 g, 0.889 mmol)并在-78℃下搅拌 30 分钟。使反应温热至室温，搅拌 5 小时，用 LC/MS 检查反应进程。用乙酸乙酯(100 mL)稀释，用饱和氯化铵(50 mL)洗涤，用二氯甲烷(50 mL)反萃取水相。将有机相合并，用无水硫酸镁干燥，过滤，减压浓缩。将所得残余物用快速色谱进行纯化，用 100%己烷/0%(25% THF/己烷)至 0%己烷/100%(25% THF/己烷)以 10%的增量逐步梯度洗脱，得到白色固体(0.087 g, 48%)。ES/MS m/z (^{35}Cl) 406.0 $(M+1)^+$。

基本如实施例 13 所述的那样用上面制得的适宜溴代噻唑来制备下面的实施例。

实施例号	化学名	物理数据
14	3-(4-氯-2-吗啉-4-基-噻唑-5-基)-2,5-二甲基-7-(1-丙基-丁基)-吡唑并[1,5-*a*]嘧啶	ES/MS m/z (^{35}Cl) 448.0 $(M+1)^+$
15	{4-氯-5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶-3-基]-噻唑-2-基}-二甲基-胺	MS (APCI) m/z (^{35}Cl) 378.0 $(M+1)^+$

制备例 18

2,4-二氯噻唑

将噻唑烷-2,4-二酮(50 g, 0.43 mol)在三氯氧化磷(240 mL)中的混合物冷却至5℃，历经15分钟加入吡啶(34 mL, 0.43 mol)。将混合物加热至125℃达4小时，然后冷却至22℃。通过真空蒸馏除去过量的三氯氧化磷并将残余物加入到被冷却至5℃的水(1 L)中。将混合物用二氯甲烷(3 × 400 mL)萃取。将有机部分合并，蒸发掉溶剂，得到标题化合物(50 g, 76%)。EI/MS m/z: (^{35}Cl^{35}Cl/^{35}Cl^{37}Cl/^{37}Cl^{37}Cl) 153/155/157 $(M+1)^+$。

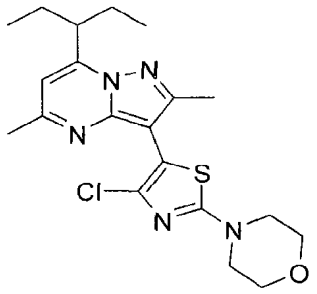
制备例 19

4-氯-2-吗啉代-噻唑

向2,4-二氯噻唑(34 g, 0.22 mol)在乙腈(425 mL)中的混合物中加入碳酸钾(60.9 g, 0.44 mol)，然后历经30分钟滴加吗啉(21.2 mL, 0.225 mol)。将混合物在40℃下回流，然后冷却至22℃。对混合物过滤并蒸发滤液。将残余物用异丙醇(60 mL)在22℃下研磨1小时。将固体滤出并真空干燥至恒重，得到标题化合物(34.5 g, 76%)。ES/MS m/z (^{35}Cl) 205 $(M+1)^+$。

实施例 16

3-(4-氯-2-吗啉-4-基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶



在氮气气氛下，将 3-(4-溴-2-吗啉-4-基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(116 mg, 0.25 mmol)溶解于 THF(1.5 mL)中并冷却至-78℃。加入正丁基锂(0.1 mL. 2.5 M 己烷溶液, 0.25 mmol)并在-78℃下搅拌 30 分钟。加入 N-氯琥珀酰亚胺(33.4 mg, 0.25 mmol)并再搅拌 30 分钟，同时缓慢温热至室温。在搅拌过夜后，通过加入饱和氯化铵溶液将反应淬灭，用乙酸乙酯萃取。用盐水洗涤有机层，用硫酸钠干燥，过滤，浓缩，获得残余物。用快速色谱对该粗品物质进行纯化，用己烷:二氯甲烷:乙酸乙酯(5:5:2)洗脱，得到标题化合物(54 mg)。MS (APCI) m/z (^{35}Cl) 420.6 $(M+1)^+$; ^{1}H NMR (400 MHz, $CDCl_3$): 6.44(s, 1H), 3.79(t, 4H, *J*=4.8 Hz), 3.63-3.56(m, 1H), 3.47(t, 4H, *J*=4.8 Hz), 2.55(s, 3H), 2.45(s, 3H), 1.88-1.75(m, 4H), 0.87(t, 6H, *J*=7.5 Hz)。

从制备例 6 开始的另一种制备方法:

将 7-(1-乙基-丙基)-3-碘-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(9 g, 26.2 mmol)和 4-氯-2-吗啉代-噻唑(7.5 g, 36.7 mmol)合并到之前用氮气进行了脱气的二甲基甲酰胺(90 mL)中。加入碳酸铯(17.8 g, 55 mmol)、碘化亚铜(250 mg, 1.31 mmol)、三苯基膦(550 mg, 2.09 mmol)和醋酸钯(117 mg, 0.52 mmol)。将混合物加热至 125℃达 16 小时，然后冷却至 22℃。加入水(900 mL)并用甲基-叔丁基醚(3 × 200 mL)萃取。将有机部分合并，蒸发掉溶剂。用硅胶色谱进行纯化，用己烷/乙酸乙酯(4/1)进行洗脱，得到标题化合物(6.4 g, 62%)。ES/MS m/z (^{35}Cl) 420 $(M+1)^+$。

实施例 16a

3-(4-氯-2-吗啉-4-基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-

吡唑并[1,5-*a*]嘧啶，盐酸盐

在 50℃下，将 3-(4-氯-2-吗啉-4-基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(1.40 g, 3.33 mmol)溶解于丙酮(10 mL)中并冷却至室温。加入氯化氢(2M 乙醚溶液, 2.0 mL, 4.0 mmol)并在超声波仪中充分搅拌。将溶液浓缩少量，加入最低限度的乙醚以使 HCl 盐结晶。将混合物在冷藏器中冷却过夜。加入另外的氯化氢(2M 乙醚溶液, 2.0 mL, 4.0 mmol)，在冷藏器中冷却。将结晶性物质滤出，干燥，得到标题化合物(1.15 g, 75%)。ES/MS m/z (^{35}Cl) 420 $(M+1)^+$; ^{1}H NMR (CDCl3): 9.18(br, 1H), 6.86(s, 1H), 3.72(m, 4H), 3.49(m, 1H), 3.39(m, 4H), 2.48(s, 3H), 2.38(s, 3H), 1.79(m, 4H), 0.79(m, 6H)。

实施例 17

3-(4-溴-2-丁基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶

在-78℃下，在氮气气氛下，将正丁基锂(2.5M 己烷溶液, 0.2 mL, 0.5 mmol)加入到 3-(2,4-二溴-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(230 mg, 0.5 mmol)在 THF(3 mL)中的溶液中。在 30 分钟后，加入 1-碘丁烷(138 mg, 0.75 mmol)并继续搅拌 1 小时。温热至室温并搅拌 1 小时。通过加入饱和氯化铵溶液来将反应淬灭并用乙酸乙酯萃取。将有机部分用硫酸钠干燥，过滤，浓缩，获得残余物。用快速色谱对该粗品物质进行纯化，用己烷/乙酸乙酯(10/1.5)进行洗脱，得到橙色泡沫形式的标题化合物(78 mg)。ES/MS m/z (^{81}Br) 437.4 $(M+1)^+$。

基本如实施例 17 所述的那样制备下面的实施例。

实施例号	化学名	物理数据
18	3-(4-溴-2-甲氧基甲基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶	MS (APCI) m/z (^{81}Br) 424.6 $(M+1)^+$

实施例 19

7-(1-乙基-丙基)-3-(2-甲氧基甲基-噻唑-5-基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶

在-78℃下，在氮气气氛下，将正丁基锂(2.5M 己烷溶液，0.4 mL，1.0 mmol)加入到 7-(1-乙基-丙基)-2,5-二甲基-3-噻唑-5-基-吡唑并[1,5-a]嘧啶(300 mg, 1.0 mmol)在 THF(3 mL)中的溶液中。搅拌 30 分钟，加入 1-碘甲基甲基醚(205 mg, 1.2 mmol)。继续搅拌 1 小时，然后缓慢温热至室温并搅拌过夜。通过加入饱和氯化铵溶液来将反应淬灭，用乙酸乙酯萃取。将有机层用盐水洗涤，用硫酸钠干燥，过滤，真空浓缩，获得残余物。用快速色谱对该粗品物质进行纯化，用己烷/乙酸乙酯(10/2)进行洗脱，得到黄色泡沫形式的标题化合物(184 mg)。MS (APCI) m/z 345.3 $(M+1)^+$。

实施例 20

3-(4-氯-2-甲氧基甲基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶

在-78 °C 下，将正丁基锂(2.5 M 己烷溶液, 174 mL, 0.43 mmol)加入到进行着搅拌的 7-(1-乙基-丙基)-3-(2-甲氧基甲基-噻唑-5-基)-2,5-二甲基-吡唑并[1,5-a]嘧啶(150 mg, 0.43 mmol)在 THF(3 mL)中的溶液中。搅拌 30 分钟并加入 N-氯琥珀酰亚胺(87 mg, 0.653 mmol)。搅拌 30 分钟，然后将反应缓慢温热至室温，使反应继续进行过夜。通过加入饱和氯化铵溶液来将反应淬灭，用乙酸乙酯萃取，用硫酸钠干燥，过滤，浓缩，获得残余物。用快速色谱进行纯化，用己烷/乙酸乙酯(10/2)进行洗脱，得到标题化合物(7 mg)。MS (APCI) m/z (^{35}Cl) 379.3 $(M+1)^+$。

实施例 21

7-(1-乙基-丙基)-2,5-二甲基-3-[2-(2-甲基-2H-[1,2,4]三唑-3-基)-噻唑-5-基)-吡唑并[1,5-*a*]嘧啶

将 3-(4-溴-2-(2-甲基-2H-[1,2,4]三唑-3-基)-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(175 mg, 0.38 mmol)和氯化亚铜(I)(132

mg, 1.33 mmol)在 DMF(5 mL)中进行混合并加热至 120℃达 24 小时。冷却至室温，用乙酸乙酯稀释并用盐水洗涤。将有机层用硫酸钠干燥，过滤，真空浓缩，得到残余物。用快速色谱进行纯化，用己烷、然后用己烷/EtOAc (10/1.8)进行洗脱，得到黄色-橙色固体(45 mg)。ES/MS m/z 382.0$(M+1)^+$。

另一种制备方法:

在-78℃下，在氮气气氛下，将正丁基锂(2.5 M 己烷溶液, 57.6 mL, 144 mmol)加入到 *N*-甲基三唑(11.95 g, 144 mmol)在 THF(600 mL)中的溶液中，在加入期间保持温度低于-74℃。然后一次性加入氯化锌干粉(26 g, 192 mmol)并将混合物在 1 小时内温热至 22℃。一次性加入 7-(1-乙基-丙基)-3-(2-碘-噻唑-5-基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(12.5 g, 29 mmol)和四(三苯基)膦钯催化剂(1.15 g, 0.01 mol)，将混合物在氮气下加热至回流达 8 小时。将混合物冷却至 22℃并加入水(300 mL)。分离有机层并用甲基-叔丁基醚萃取水层(2 × 200 mL)。将有机部分合并，用硫酸钠干燥，过滤，蒸发掉溶剂。通过硅胶垫来进行纯化，用己烷/乙酸乙酯(4/1)进行洗脱，得到标题化合物(8 g, 72%)。ES/MS m/z 382 $(M+1)^+$。

实施例 22

3-(4-氯-2-(2-甲基-2H-[1,2,4]三唑-3-基)-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶

将 7-(1-乙基-丙基)-2,5-二甲基-3-[2-(2-甲基-2H-[1,2,4]三唑-3-基)-噻唑-5-基)-吡唑并[1,5-*a*]嘧啶(20 mg, 0.052 mmol)和 N-氯琥珀酰亚胺(7.6 mg, 0.0569 mmol)在二氯甲烷(0.5 mL)和乙腈(0.5 mL)中的混合物在室温下在一个小瓶中搅拌过夜。浓缩，获得残余物。用快速色谱进行纯化，用己烷、然后用己烷/乙酸乙酯(10/1.5)洗脱，得到标题化合物(16 mg)。ES/MS m/z (^{35}Cl) 416.0 $(M+1)^+$。

实施例 23

7-(1-乙基-丙基)-3-(4-甲氧基-2-吗啉-4-基-噻唑-5-基)-2,5-二甲基-

吡唑并[1,5-*a*]嘧啶

将 3-(4-溴-2-吗啉-4-基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(162 mg, 0.35 mmol)、甲醇钠(57 mg, 1.05 mmol)和碘化亚铜(I)(67 mg, 0.35 mmol)在甲醇(3 mL)中的混合物在一个密封的 4-mL 小瓶中在 120℃下搅拌 15 小时。冷却至室温，通过过滤除去固体，将滤液真空浓缩。用快速色谱对残余物进行纯化，用己烷/THF(10/2)进行洗脱。用甲醇将该物质重结晶，得到标题化合物(20 mg)。ES/MS m/z 416.0 $(M+1)^+$。

制备例 20

4-溴-5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶-3-基]-噻唑-2-甲醛

在-78℃下，在氮气气氛下，将正丁基锂(1.6 M 己烷溶液, 0.312 mL, 0.50 mmol)加入到 3-(2,4-二溴-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(230 mg, 0.50 mmol)的 THF 溶液(2.5 mL)中并搅拌 30 分钟。加入 N-甲酰基吗啉(58 mg, 0.50 mmol)的 THF(0.5 mL)溶液。搅拌 1 小时，然后将反应在-20℃下储存过夜。将反应温热至室温，用醚稀释，通过加入 4 N HCl (4 mL)将其淬灭。将有机相分离出来并用 4 N HCl (2 × 4 mL)萃取。将含水部分合并，用碳酸氢钠固体处理至 pH = 8 至 9，然后用乙醚萃取。将所有的有机层合并，用盐水洗涤，用硫酸钠干燥，过滤，浓缩，获得残余物。用快速色谱对该粗品物质进行纯化，用己烷/二氯甲烷/乙酸乙酯(5/5/1)洗脱，得到标题化合物(154 mg)。MS (APCI) m/z (^{81}Br) 409.0 $(M+1)^+$。

实施例 24

3-(4-溴-2-吗啉-4-基-甲基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶

将 4-溴-5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶-3-基]-噻唑-2-甲醛(150 mg, 0.368 mmol)、吗啉(35 mg, 0.405 mmol)和三乙酰氧基硼氢化钠(97 mg, 0.46 mmol)合并到二氯甲烷(3 mL)和甲醇(0.5 mL)中。搅拌过夜，再加入一些吗啉(35 mg, 0.405 mmol)和三乙酰氧基硼氢化钠(97 mg,

0.46 mmol)，再搅拌 4 小时。真空除去溶剂，用二氯甲烷稀释，用盐水洗涤。将有机相用硫酸钠干燥，过滤并真空浓缩。将所得的物质用快速色谱进行纯化，用二氯甲烷:2 M 氨的甲醇溶液(10:1)洗脱，得到一种混合物。用反相柱对混合物进行纯化，用水/乙腈(80/20)至水/乙腈(10/90)洗脱，得到标题化合物(20 mg)。ES/MS m/z (^{81}Br) 480.0 $(M+1)^+$。

实施例 25

3-(4-溴-2-吡啶-4-基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-a]嘧啶

在-78℃下，在氮气气氛下，将正丁基锂(1.6 M 己烷溶液, 0.312 mL, 0.5 mmol)加入到 3-(2,4-二溴-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(229 mg, 0.5 mmol)在 THF(4 mL)中的溶液中。搅拌 30 分钟后，加入无水氯化锌(264 mg, 1.5 mmol)，继续搅拌 30 分钟。将反应温热至室温并搅拌 1 小时。加入 4-碘吡啶(103 mg, 0.5 mmol)，然后加入 1,1'-双(二苯基膦基)二茂铁氯化钯(II)(二氯甲烷加合物)(0.40.8 mg, 0.05 mmol)。将反应加热至回流过夜。冷却至室温，用乙酸乙酯稀释，用水和盐水洗涤。将有机层用硫酸钠干燥，过滤，浓缩，获得残余物。用快速色谱对该粗品物质进行纯化，用二氯甲烷:2 N 氨的甲醇溶液(10:0.75)洗脱，得到一种混合物。用反相柱对混合物进行纯化，用水:乙腈= 80:20 至水:乙腈= 10:90 进行洗脱，得到标题化合物(19 mg)。ES/MS m/z (^{79}Br) 456.0 $(M+1)^+$。

制备例 21

4-三氟甲基-噻唑-2-基胺

将硫脲(4.0 g, 52.3 mmol)和 3-溴-1,1,1-三氟丙-2-酮(5.5 mL, 10 g, 52.3 mmol)加入到乙醇(100 mL)中并在 50℃下加热 2 小时。冷却至室温，浓缩至干。将残余物溶解于水中并用 2M NaOH 将 pH 调至>12。用乙醚萃取(4×)。将合并的有机萃取物用硫酸钠干燥，过滤并真空浓缩。用硅胶色谱对所得材料进行纯化(CH_2Cl_2)，得到标题化合物(6.9 g, 79%)。ES/MS m/z

169 $(M+1)^+$。

制备例 22

5-溴-4-三氟甲基-噻唑-2-基胺，氢溴酸盐

将溴(2.0 mL, 6.28 g, 39.3 mmol)滴加到冰浴冷却的 4-三氟甲基-噻唑-2-基胺(6.0 g, 35.7 mmol)在乙醚(60 mL)中的溶液中。在完全加入后搅拌 1 小时，然后温热至室温。通过过滤收集固体并用乙醚洗涤，得到标题化合物(10.5 g, 90%)。ES/MS m/z ($^{79}Br/^{81}Br$) 247/249 $(M+1)^+$。

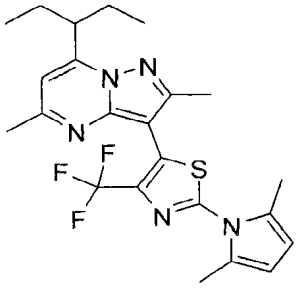
制备例 23

5-溴-2-(2,5-二甲基-吡咯-1-基)-4-三氟甲基-噻唑

将己烷-2,5-二酮(3.5 mL, 3.4 g, 30.2 mmol)加入到 5-溴-4-三氟甲基-噻唑-2-基胺氢溴酸盐(9.0 g, 27.4 mmol)在甲醇(60 mL)中的溶液中。在室温下搅拌过夜。加入磷酸盐缓冲液(50 mL, pH = 7)。通过过滤收集沉淀，用水洗涤。将滤饼溶解于 CH_2Cl_2 中，用硫酸钠干燥。过滤并真空浓缩，得到标题化合物(8.2 g, 92%)。^1H NMR (400 MHz, $CDCl_3$) δ 5.91(s, 2H), 2.27(s, 6H)。

实施例 26

3-[2-(2,5-二甲基-吡咯-1-基)-4-三氟甲基-噻唑-5-基]-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶



将 5-溴-2-(2,5-二甲基-吡咯-1-基)-4-三氟甲基-噻唑(2.2 g, 6.6 mmol)在 THF(25 mL)中的溶液在干冰浴中进行冷却。滴加叔丁基锂(1.7 M 的戊烷溶

液, 8.5 mL, 14.5 mmol)。搅拌 45 分钟，然后滴加氯化锌(0.5 M 的 THF 溶液, 14.6 mL, 7.3 mmol)。搅拌 5 分钟并除去冷却浴。搅拌 30 分钟，然后加入 7-(1-乙基-丙基)-3-碘-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(1.5 g, 4.4 mmol)和双(三-叔丁基膦)钯(0)(450 mg, 0.9 mmol)。回流 24 小时。将反应冷却，将混合物倾倒到乙醚中，用水(2×)洗涤。将合并的水层用乙醚洗涤。将合并的有机部分用硫酸钠干燥，过滤，真空浓缩至干。用硅胶色谱对所得的残余物进行纯化(75-100% CH_2Cl_2 的己烷溶液)，得到标题化合物(1.47 g, 72%)。HR-ToF-MS m/z $C_{23}H_{26}F_3N_5S+H^+$的计算值：462.1939，实测值：462.1915。

实施例 27

5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶-3-基]-4-三氟甲基-噻唑-2-基胺

将羟胺(2 mL, 50%的水溶液)加入到 3-[2-(2,5-二甲基-吡咯-1-基)-4-三氟甲基-噻唑-5-基]-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(1.1 g, 2.3 mmol)在乙酸(10 mL)中的溶液中。将反应加热至 80℃达 6 小时。冷却至室温。倾倒到乙醚中，用 2 M NaOH 洗涤 2 次，然后用水洗涤一次。将有机部分用硫酸钠干燥，过滤，浓缩，真空浓缩至干。用硅胶色谱对所得的残余物进行纯化(40%乙酸乙酯的己烷溶液)，得到标题化合物(0.76 g, 87%)。ES/MS m/z 384 $(M+1)^+$。

制备例 24

3-(2-溴-4-三氟甲基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶

将溴化铜(II)(540 mg, 2.4 mmol)和亚硝酸叔丁酯(0.36 mL, 310 mg, 3.0 mmol)在乙腈(20 mL)中的混合物加热至 60℃。加入固体形式的 5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-a]嘧啶-3-基]-4-三氟甲基-噻唑-2-基胺(755 mg, 2.0 mmol)。将反应加热至 80℃达 2 小时。将反应冷却，倾倒到乙醚中，

用水萃取(3×)。将有机层用硫酸钠干燥，过滤，浓缩，真空浓缩至干。用硅胶色谱对所得的残余物进行纯化(CH_2Cl_2)，得到标题化合物(0.77 g, 87%)。ES/MS m/z ($^{79}Br/^{81}Br$) 447, 449 $(M+1)^+$。

实施例 28

{5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶-3-基]-4-三氟甲基-噻唑-2-基}-二甲基-胺

将一个包含位于甲醇(4 mL)中的 3-(2-溴-4-三氟甲基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(313 mg, 0.7 mmol)和二甲胺(2 M 的 THF 溶液, 4 mL, 8 mmol)的密封管加热至 80℃达 2 小时。将反应冷却并真空浓缩。用硅胶色谱对所得的残余物进行纯化，用 0-30%乙酸乙酯的 CH_2Cl_2 溶液洗涤，得到标题化合物(0.28 g, 97%)。HR-ToF-MS m/z $C_{19}H_{24}F_3N_5S+H^+$的计算值: 412.1770, 实测值: 412.1783。

基本如实施例 28 中所述的那样制备下面的实施例，不同的是将反应回流 8 小时。

实施例号	化学名	物理数据
29	7-(1-乙基-丙基)-2,5-二甲基-3-(2-吗啉-4-基-4-三氟甲基-噻唑-5-基)-吡唑并[1,5-*a*]嘧啶	HR-ToF-MS m/z $C_{21}H_{26}F_3N_5OS+H^+$ 的计算值: 454.1873, 实测值: 454.1888

实施例 30

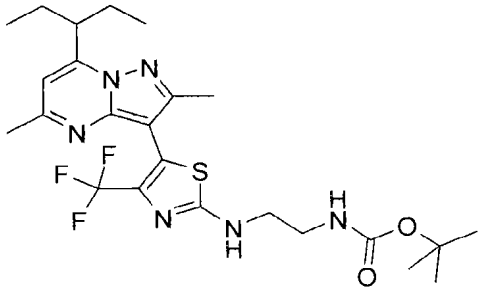
N-{5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶-3-基]-4-三氟甲基-噻唑-2-基}-乙酰胺

将羟胺(50%的水溶液, 5 mL)加入到 3-[2-(2,5-二甲基-吡咯-1-基)-4-三氟甲基-噻唑-5-基]-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(2.4 g, 5.2 mmol)在乙酸(25 mL)中的溶液中。将反应加热至 80℃达 72 小时。将反应冷却至室温，倾倒到乙醚中，用 2 M NaOH(2×)洗涤，然后用水洗涤一次。

将有机部分用硫酸钠干燥，过滤并浓缩至干。用柱色谱对所得残余物进行纯化，用 40%乙酸乙酯的己烷溶液洗脱，得到标题化合物(0.28 g, 13%)。HR-ToF-MS m/z $C_{19}H_{22}F_3N_5OS+H^+$ 的计算值：426.1575，实测值：426.1565。

实施例 31

(2-{5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶-3-基]-4-三氟甲基-噻唑-2-基氨基}-乙基)-氨基甲酸叔丁酯



将(2-氨基-乙基)-氨基甲酸叔丁酯(0.5 mL, 506 mg, 3.2 mmol)加入到3-(2-溴-4-三氟甲基-噻唑-5-基)-7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶(100 mg, 0.22 mmol)和三乙胺(0.3 mL, 222 mg, 2.2 mmol)在甲醇(1 mL)中的溶液中。蒸发掉甲醇并加热至 80℃过夜。将反应冷却至室温并真空浓缩。用柱色谱对所得残余物进行纯化，用 10-50%乙酸乙酯的 CH_2Cl_2 溶液洗脱，得到标题化合物(0.11 g, 97%)。ES/MS m/z 527.2 $(M+1)^+$。

用适宜的胺基本如实施例 31 中所述的那样制备下面的化合物。

制备例或实施例号	化学名	物理数据
制备例 25*	[2-({5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶-3-基]-4-三氟甲基-噻唑-2-基}-丙基-氨基)-乙基]-氨基甲酸叔丁酯	ES/MS m/z 569.3$(M+1)^+$
制备例 26**	(S)-(1-{5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶-3-基]-4-三氟甲基-噻唑-2-基}-吡咯烷-3-基)-氨基甲酸叔丁酯	ES/MS m/z 553.3$(M+1)^+$

实施例 32***	*N*-{5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶-3-基]-4-三氟甲基-噻唑-2-基}-*N*,*N*'-二丙基-乙烷-1,2-二胺，盐酸盐	ES/MS m/z 511.2$(M+1)^+$

* 在 EtOH 中在回流下加热过夜。蒸发掉 EtOH，在 110℃下加热 24 小时。

** 在 EtOH 中在 80℃下加热过夜。

*** 制备该 HCl 盐，用 EtOAc/己烷重结晶。

实施例 33

N^1-{5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶-3-基]-4-三氟甲基-噻唑-2-基}-乙烷-1,2-二胺，盐酸盐

将(2-{5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶-3-基]-4-三氟甲基-噻唑-2-基氨基}-乙基)-氨基甲酸叔丁酯(101 mg,0.192 mmol)加入到 1 M HCl 的甲醇溶液(1 mL)中。将反应在 70℃下加热过夜。将反应冷却并由甲醇/乙酸乙酯真空浓缩。用乙酸乙酯对所得的残余物进行研磨，得到标题化合物(64 mg, 78%)。ES/MS m/z 427.0 $(M+1)^+$。

基本如实施例 33 中所述的那样制备下面的实施例。

实施例号	化学名	物理数据
34	N^1-{5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-*a*]嘧啶-3-基]-4-三氟甲基-噻唑-2-基}-N^1-丙基-乙烷-1,2-二胺，盐酸盐	ES/MS m/z 469.2$(M+1)^+$
35	(*S*)-1-{5-[7-(1-乙基-丙基)-2,5-二甲基-吡唑并[1,5-a]嘧啶-3-基]-4-三氟甲基-噻唑-2-基}-吡咯烷-3-基胺，盐酸盐	ES/MS m/z 453.0$(M+1)^+$

实施例 A

用离体结合进行的体内效力评估

为了评估体内效力，用离体结合对本发明的化合物进行评价。使用 D.

R. Gehlert 等人, *EJP* 509: 145-153(2005)中所提供的操作，通过口服途径将化合物施用于大鼠。然后如 Gehlert 等人所述的那样对 ^{125}I-蛙皮降压肽与小脑的离体结合进行评估。例如，实施例 15 在 10 mg/kg 下提供 65%的抑制。

实施例 B

CRF1 滤膜结合测定法

在产生具有足够受体密度以开展结合测定法的重组细胞系方面，通过使用由 Stanford 许可的 Phoenix 逆转录病毒表达系统克服了以质粒为基础的人 CRF1 表达的限制。用稳定的 HEK-hCRF1 细胞系制备膜，如下建立结合反应(200 µL): 50 µL ^{125}I-蛙皮降压肽(终浓度为 0.2 nM)、50 µL 化合物和 100 µL CRF1 膜(25 µg/反应)。将反应在室温下孵育 2 小时，然后通过用预处理的 FB 微孔玻璃纤维滤板(96 孔)过滤来终止反应。将板用冰冷的测定缓冲液(50 mM tris, 12.5 mM NaCl, 1 mM EDTA, 10 mM $MgCl_2$, 0.05% BSA, pH 7.2)洗涤两次，风干过夜，用 100 µL Microscint 40 在 MicroBeta 计数器中进行计数。在存在 0.5 µM 未标记的蛙皮降压肽的情况下测定非特异性结合(NSB)。通常一式三份地进行测定，用 Graph Pad Prism 对中值数据点作图。

用这种测定法，本发明具体给出的化合物以低于 1 µM 的 Ki(抑制常数)抑制滚动(roller)/粘附细胞中 ^{125}I-蛙皮降压肽(4 nM)的结合。例如，实施例 15 表现出 6.2 nM 的 Ki。

实施例 C

CRF2 滤膜结合测定法

在产生具有足够受体密度以开展结合测定法的重组细胞系方面，通过使用由 Stanford 许可的 Phoenix 逆转录病毒表达系统克服了以质粒为基础的人 CRF2 表达的限制。用稳定的 HEK-hCRF2 细胞系制备膜，如下建立结合反应(200 µL): 50 µL ^{125}I-蛙皮降压肽(终浓度为 0.2 nM)、50 µL 化合物和 100 µL CRF2 膜(25 µg/反应)。将反应在室温下孵育 2 小时，然后通过用预处理的 FB 微孔玻璃纤维滤板(96 孔)过滤来终止反应。将板用冰冷的

测定缓冲液(50 mM tris, 12.5 mM NaCl, 1 mM EDTA, 10 mM $MgCl_2$, 0.05% BSA, pH 7.2)洗涤两次，风干过夜，用 100 μL Microscint 40 在 MicroBeta 计数器中进行计数。在存在 0.5 μM 未标记的蛙皮降压肽的情况下测定非特异性结合(NSB)。或者，用闪烁亲近测定法来对化合物进行评价。如下建立该测定法：50 μL ^{125}I-蛙皮降压肽(终浓度为 0.2 nM)、50 μL 化合物或未标记的蛙皮降压肽(NSB)和 100 μL 含 250 μg 麦胚凝集素(WGA)的 SPA 小珠和 CRF2 膜(1.5 μg/反应)。将板在室温下孵育 4-5 小时，然后在 200 X g 下离心 10 分钟。用 Wallac Trilux 闪烁计数器来对结合的放射性进行评估。通常使用一式三份的测定来对结合进行评估，用 Graph Pad Prism 对中值数据点作图。首先在固定浓度下对化合物进行筛选，如果注意到足够的活性，则随后产生浓度-响应曲线。

在所述 CRF2 结合测定法中对本发明的具体给出的化合物进行了试验，它们对 CRF2 受体表现出弱亲合性。例如，实施例 15 在 50 μM 的浓度下表现出 11%的抑制。这一结果表明本发明的化合物对 CRF1 受体具有选择性(相对于 CRF2 而言)。

实施例 D

生物利用度和药动学性质

分布容积(Vdist)是指药物在机体中的量与药物在血液或血浆中的浓度之比。分布容积指的是在与血液或血浆中的浓度相同的浓度下，包含机体中的全部药物量所需的体液体积：Vdist = 机体中的药物量/血液或血浆中的药物浓度(Goodman and Gillman's)。对于 10 mg 的剂量和 10 mg/L 的血浆浓度而言，分布容积将为 1 升。分布容积反映了药物在血管外组织中的存在程度。大的分布容积反映了与血浆蛋白结合相比化合物倾向于与组织组分结合。在临床情况中，可以用 Vdist 来确定获得稳态浓度的负荷剂量。

为了测定分布容积，给雄性 Sprague Dawley 大鼠(N=3)施用 1 mg/kg 的化合物静脉内单剂量。在给药后 0.08 至 24 小时，在一些时间点收集多个血浆样品。用 LC/MS/MS 对血浆样品进行分析以测定血浆浓度。进行血浆药动学计算以确定包括 Vdist 和血浆清除率(Clp)在内的药动学参数。

本发明的化合物优选具有良好的生物利用度。例如，大部分可商购的CNS和心血管药物表现出<10 L/Kg的人Vdist。与CRF拮抗剂CP154526(Schulz等人, *Proc. Natl. Acad. Sci. (USA)*, 93:10477 (1996))和NBI30775(Chen等人, *Drug Development Research*, 65:216 (2005))相比(当单独进行分析时，CP154526和NBI30775分别表现出114 L/Kg和76 L/Kg的大鼠Vdist)，当单独进行分析时，本发明的实施例15在给予1 mg/kg的静脉内单剂量后仅表现出7.2 L/Kg的大鼠Vdist。

此外，血浆清除率(CLp)是药物由机体除去速率的量度。按照静脉内剂量和一级动力学，可以用下列方程确定血浆清除率：CLp = 剂量/AUC，其中AUC是描述血浆药物浓度与从0至无穷大时间的函数关系的曲线下总面积。当单独进行分析时，在单次静脉内剂量后，参比物CRF拮抗剂CP154526和NBI37582分别表现出83和306 mL/min/kg的大鼠清除率(CLp)，而当单独进行分析时，本发明的实施例15仅表现出23.6 mL/min/kg的大鼠CLp。

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor:	HOWERTON; Alexis et al.	Group Art Unit:	
Serial Number:	19/043,264	Examiner:	Not Yet Assigned
Filing or 371 (c) Date:	2025-01-31	**CONFIRMATION NO:**	**6961**
Title:	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS		

FILED ELECTRONICALLY ON: February 5, 2025

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT UNDER 37 CFR § 1.97

Commissioner for Patents:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP § 609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in § 1.56.

A. ☒ *37 CFR § 1.97 (b).* This Information Disclosure Statement should be considered by the Office because:

☐ (1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under § 1.53 (d);

-- OR --

☐ (2) It is being filed within 3 months of entry of the national stage as set forth in § 1.491 in an international application;

-- OR --

☒ (3) It is being filed before the mailing of a first Office action on the merits;

-- OR --

☐ (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under § 1.114.

B. ☐ *37 CFR § 1.97(c).* Although this Information Disclosure Statement is being filed after the period specified in *37 CFR § 1.97(b),* above, it is filed before the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, this Information Disclosure Statement should be considered because it is accompanied by one of:

☐ a statement as specified in §1.97 (e) provided concurrently herewith;

-- OR --

☐ a fee of [$280.00/112.00/56.00] as set forth in § 1.17 (p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.

C. ☐ *37 CFR § 1.97 (d).* Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, it is being filed before payment of the issue fee and should be considered because it is accompanied by:

i. a statement as specified in § 1.97 (e);

-- AND --

ii. a fee of [$280.00/112.00/56.00] as set forth in §1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.

D. ☐ *37 CFR §1.97 (e).* Statement.

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (c);

-- AND/OR --

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (d);

-- AND/OR --

☐ A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under 37 C.F.R. § 1.97(e) (1) as provided for under MPEP 609.04(b) V.

E. ☐ *Statement Under 37 C.F.R. §1.704(d).* Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office or is a communication that was issued by a

patent office in a counterpart foreign or international application or by the Office that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay. The Patent Term Adjustment Statement Under 37 CFR 1.704(d) on Form PTO/SB/133 is filed herewith.

F. ☒ *37 CFR §1.98 (a) (2).* The content of the Information Disclosure Statement is as follows:

☐ Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.

-- OR --

☒ Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 are not enclosed.

-- AND/OR --

☒ Copies of Foreign Patent Documents and/or Non Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).

-- AND/OR --

☒ Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98 (a) (2) (iii).

G. ☒ *37 CFR §1.98(a)(3).* The Information Disclosure Statement includes non-English patents and/or references.

☐ Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.

☐ Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.

-- OR --

☐ A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____

☒ Pursuant to 37 CFR §1.98(a) (3) (ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.

H. ☐ *37 CFR §1.98(d).* Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:

☐ Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.

Application in which the information was submitted: _____

Information Disclosure Statement(s) filed on: _____

AND

☐ The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

I. ☒ *Fee Authorization*. The Commissioner is hereby authorized to charge the above-referenced fees of $200.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 23-2415 (50535-710.306).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI

Dated: February 5, 2025

By: /Victoria Cheng/
Victoria Cheng, Reg. No. 79641

650 Page Mill Road
Palo Alto, CA 94304-1050
(650) 493-9300
Customer No. 21971

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	1	of	9	Attorney Docket Number	50535-710.306

U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	001	US-4328245	05-04-1982	YU; Cheng-Der et al.	
	002	US-4409239	10-11-1983	YU; Cheng-Der	
	003	US-4410545	10-18-1983	YU; Cheng-Der et al.	
	004	US-4861760	08-29-1989	MAZUEL; Claude et al.	
	005	US-4911920	03-27-1990	JANI; Rajni et al.	
	006	US-5212162	05-18-1993	MISSEL; Paul J. T. et al.	
	007	US-5403841	04-04-1995	LANG; John C. et al.	
	008	US-10849908	12-01-2020	HOWERTON; Alexis et al.	
	009	US-11007201	05-18-2021	HOWERTON; Alexis et al.	
	010	US-11304950	04-19-2022	HOWERTON et al.	
	011	US-11311549	04-26-2022	HOWERTON; Alexis et al.	
	012	US-11344557	05-31-2022	HOWERTON; Alexis et al.	
	013	US-11351177	06-07-2022	HOWERTON; Alexis et al.	
	014	US-12115166	10-15-2024	HOWERTON; Alexis et al.	
	015	US-20100196389	08-05-2010	EVANS-FREKE; Stephen	
	016	US-20100222339	09-02-2010	CHEN; Zhaogen et al.	
	017	US-20170020877	01-26-2017	GRIGORIADIS; Dimitri E.	
	018	US-20220143037	05-12-2022	HOWERTON; Alexis et al.	
	019	US-20220211711	07-07-2022	HOWERTON; Alexis et al.	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	2	of	9	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	001	CN-101516887-A	08-26-2009	LILLY CO ELI [US]	Abstract:	☒
	002	CN-106102740-A	11-09-2016	NEUROCRINE BIOSCIENCES INC.	Abstract:	☒
	003	JP-2010504344-A	02-12-2010	ELI LILLY AND CO.	Machine Translation:	☒
	004	JP-2017503030-A	01-26-2017	NEUROCRINE BIOSCIENCES, INC.	Machine Translation:	☒
	005	JP-H0971528-A	03-18-1997	HAYASHIBARA BIOCHEM LAB [JP]	Abstract:	☒
	006	WO-0040529-A1	07-13-2000	ANGIOGENE PHARM LTD [GB], et al.		☐
	007	WO-0041669-A2	07-20-2000	ANGIOGENE PHARM LTD [GB], et al.		☐
	008	WO-0192224-A1	12-06-2001	ASTRAZENECA AB [SE], et al.		☐
	009	WO-0204434-A1	01-17-2002	ANGIOGENE PHARM LTD [GB], et al.		☐
	010	WO-0208213-A1	01-31-2002	ANGIOGENE PHARM LTD [GB], et al.		☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	3	of	9	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	011	WO-2007109853-A1	10-04-2007	VELACOR THERAPEUTICS PTY LTD [AU], et al.		☐
	012	WO-2008036579-A1	03-27-2008	LILLY CO ELI [US], et al.		☐
	013	WO-2015112642-A1	07-30-2015	NEUROCRINE BIOSCIENCES INC [US]		☐
	014	WO-2019036472-A1	02-21-2019	SPRUCE BIOSCIENCES INC [US]		☐
	015	WO-2019036503-A1	02-21-2019	SPRUCE BIOSCIENCES INC [US]		☐
	016	WO-2019210266-A1	10-31-2019	SPRUCE BIOSCIENCES INC [US]		☐
	017	WO-9722596-A1	06-26-1997	ZENECA LTD [GB], et al.		☐
	018	WO-9730035-A1	08-21-1997	ZENECA LTD [GB], et al.		☐
	019	WO-9732856-A1	09-12-1997	ZENECA LTD [GB], et al.		☐
	020	WO-9813354-A1	04-02-1998	ZENECA LTD [GB], et al.		☐
	021	WO-9902166-A1	01-21-1999	ANGIOGENE PHARM LTD [GB], et al.		☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	4	of	9	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	001	AL-MUHAMMED, J. et al. In-vivo studies on dexamethasone sodium phosphate liposomes. Journal of microencapsulation 13(3):293-306 (1996).	☐
	002	BERGE, Stephen M. et al. Pharmaceutical Salts. Journal of Pharmaceutical Sciences 66(1):1-19 (1977).	☐
	003	CHEN, Hua-dong. et al. Ovarian adrenal rest tumors undetected by imaging studies and identified at surgery in three females with congenital adrenal hyperplasia unresponsive to increased hormone therapy dosage. Endocrine pathology 28(2):146-151 (2017).	☐
	004	CHONN, Archadio et al. Recent Advances in Liposomal Drug-delivery Systems. Current Opinion in Biotechnology 6(6):698-708 (1995).	☐
	005	Co-pending U.S. Application No. 18/941,832, inventors HOWERTON; Alexis et al., filed on November 08, 2024.	☐
	006	Co-pending U.S. Application No. 19/043,275, inventors HOWERTON; Alexis et al., filed on January 31, 2025.	☐
	007	Co-pending U.S. Application No. 19/043,303, inventors HOWERTON; Alexis et al., filed on January 31, 2025.	☐
	008	EYLES et al. Oral delivery and fate of poly(lactic acid) microsphere-encapsulated interferon in rats. J Pharm Pharmacol. 49(7):669-74 (1997).	☐
	009	GAO, Zhi-Hui. et al. Controlled Release of a Contraceptive Steroid From Biodegradable and Injectable Gel Formulations: in Vitro Evaluation. Pharmaceutical research 12(6):857-863 (1995).	☐
	010	JIN et al. Testicular adrenal rest tumors in a patient with untreated congenital adrenal hyperplasia. Korean J Pediatr 2011;54(3):137-140.	☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	5	of	9	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	011	KAPRARA, et al. The corticotropin releasing factor system in cancer: expression and pathophysiological implications. Cellular and molecular life sciences 67(8):1293-1306 (2010).				☐
	012	MENDES-DOS-SANTOS et al. Prevalence of Testicular Adrenal Rest Tumor and Factors Associated with Its Development in Congenital Adrenal Hyperplasia. Hormone Res. In Paed. 90:161-168 (2018).				☐
	013	MUNSON, Peter et al. Ligand: a Versatile Computerized Approach for Characterization of Ligand-binding Systems. Analytical Biochemistry 107(1):220-239 (1980).				☐
	014	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Decision Granting Institution of Post-Grant Review of U.S. Patent No. 11,007,201 dated December 1, 2023 (pp. 1-38) (PGR2022-00025).				☐
	015	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Final Written Decision of Post-Grant Review of U.S. Patent No. 10,849,908 dated November 26, 2024 (pp. 1-62) (PGR2021-00088).				☐
	016	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Final Written Decision of Post-Grant Review of U.S. Patent No. 11,007,201 dated November 27, 2024 (pp. 1-60) (PGR2022-00025).				☐
	017	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Petition for Post Grant Review of U.S. Patent No. 10,849,908 dated May 28, 2021 (pp. 1-90) (PGR2021-00088).				☐
	018	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Petition for Post Grant Review of U.S. Patent No. 11,007,201 dated February 18, 2022 (pp 1-92) (PGR2022-00025).				☐
	019	OSTRO, Mark J. et al. Use of Liposomes as Injectable-drug Delivery Systems. American Journal of Hospital Pharmacy 46(8):1576-1587 (1989).				☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	6	of	9	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	020	PCT/US2019/029486 International Invitation to Pay Additional Fees dated June 4, 2019.				☐
	021	PCT/US2019/029486 International Search Report and Written Opinion dated August 12, 2019.				☐
	022	RAO. Recent developments of collagen-based materials for medical applications and drug delivery systems. J Biomater Sci Polym Ed. 7(7):623-45 (1995).				☐
	023	REICHMAN et al. Fertility in Patients with Congenital Adrenal Hyperplasia. Fertility and Sterility, Vol. 101, No. 2, 2014. 301-309.				☐
	024	REISCH et al. Testicular adrenal rest tumors develop independently of long-term disease control: a longitudinal analysis of 50 adult men with congenital adrenal hyperplasia due to classic 21-hydroxylase deficiency. J. Clin. Endocrinol. Metab. 98(11):E1820-E1826 (2013).				☐
	025	SMEETS et. al. Molecular characterization of testicular adrenal rest tumors in congenital adrenal hyperplasia: lesions with both adrenocortical and Leydig cell features. JCEM 100(3):E524-E530 (2015).				☐
	026	TIOSANO et al. Ovarian adrenal rest tumor in a congenital adrenal hyperplasia patient with adrenocorticotropin hypersecretion following adrenalectomy. Hormone Res. in Paediatrics 74:223-228 (2010).				☐
	027	U.S. Serial No. 17/081,694 Office Action dated February 26, 2021.				☐
	028	U.S. Serial No. 17/081,694 Office Action dated June 11, 2021.				☐
	029	U.S. Serial No. 17/667,285 Office Action dated December 29, 2023.				☐
	030	U.S. Serial No. 17/667,285 Office Action dated June 10, 2024.				☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	7	of	9	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	031	ZAAROUR et al. Bilateral Ovary Adrenal Rest Tumor in a Congenital Adrenal Hyperplasia Following Adrenalectomy. Endocrinol. Pract. 20(4):e69-e74 (2014).				☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	8	of	9	Attorney Docket Number	50535-710.306

TIMING STATEMENT

Please see 37 CFR 1.97(e) to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached timing statement under 37 CFR 1.97(e).

☒ A timing statement under 37 CFR 1.97(e) is not submitted herewith.

TIMING FEE

☐ The fee set forth in 37 CFR 1.17(p) has been submitted herewith.

COPIES

☐ An identification of an earlier application pursuant to 37 CFR 1.98(d)(1) is attached.

SIZE FEE ASSERTION

Please see 37CFR 1.17(v) and attached instructions for completing this form to make the appropriate selection of an assertion under 37 CFR 1.98. For the information disclosure statement (IDS) submitted herewith, the applicant or patent owner certifies the following with respect to the cumulative number of applicant-provided or patent-owner provided items of information submitted to date including those in the accompanying IDS (select only one):

☐ No IDS size fee is required under 37 CFR 1.17 (v) at this time.

☒ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(1).

☐ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(2).

☐ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(3).

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 11.18. Please see CFR 1.4(d) for the form of the signature.

Signature:	**/Victoria Cheng/**	Date (YYYY-MM-DD)	**2025-02-05**
Name/Print	Victoria Cheng	Registration Number	79641

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	9	of	9	Attorney Docket Number	50535-710.306

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor:	HOWERTON; Alexis et al.	Group Art Unit:	
Serial Number:	19/043,264	Examiner:	Not Yet Assigned
Filing or 371 (c) Date:	2025-01-31	**CONFIRMATION NO:**	**6961**
Title:	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS		

FILED ELECTRONICALLY ON: February 5, 2025

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT UNDER 37 CFR § 1.97

Commissioner for Patents:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP § 609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in § 1.56.

A. ☒ *37 CFR § 1.97 (b).* This Information Disclosure Statement should be considered by the Office because:

☐ (1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under § 1.53 (d);

-- OR --

☐ (2) It is being filed within 3 months of entry of the national stage as set forth in § 1.491 in an international application;

-- OR --

☒ (3) It is being filed before the mailing of a first Office action on the merits;

-- OR --

☐ (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under § 1.114.

B. ☐ *37 CFR § 1.97(c).* Although this Information Disclosure Statement is being filed after the period specified in *37 CFR § 1.97(b),* above, it is filed before the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, this Information Disclosure Statement should be considered because it is accompanied by one of:

☐ a statement as specified in §1.97 (e) provided concurrently herewith;

-- OR --

☐ a fee of [$280.00/112.00/56.00] as set forth in § 1.17 (p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.

C. ☐ *37 CFR § 1.97 (d).* Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, it is being filed before payment of the issue fee and should be considered because it is accompanied by:

i. a statement as specified in § 1.97 (e);

-- AND --

ii. a fee of [$280.00/112.00/56.00] as set forth in §1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.

D. ☐ *37 CFR §1.97 (e).* Statement.

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (c);

-- AND/OR --

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (d);

-- AND/OR --

☐ A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under 37 C.F.R. § 1.97(e) (1) as provided for under MPEP 609.04(b) V.

E. ☐ *Statement Under 37 C.F.R. §1.704(d).* Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office or is a communication that was issued by a

patent office in a counterpart foreign or international application or by the Office that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay. The Patent Term Adjustment Statement Under 37 CFR 1.704(d) on Form PTO/SB/133 is filed herewith.

F. ☒ *37 CFR §1.98 (a) (2).* The content of the Information Disclosure Statement is as follows:

☐ Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.

-- OR --

☒ Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 are not enclosed.

-- AND/OR --

☒ Copies of Foreign Patent Documents and/or Non Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).

-- AND/OR --

☒ Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98 (a) (2) (iii).

G. ☒ *37 CFR §1.98(a)(3).* The Information Disclosure Statement includes non-English patents and/or references.

☐ Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.

☐ Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.

-- OR --

☐ A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____

☒ Pursuant to 37 CFR §1.98(a) (3) (ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.

H. ☐ *37 CFR §1.98(d).* Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:

☐ Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.

Application in which the information was submitted: _____

Information Disclosure Statement(s) filed on: _____

AND

☐ The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

I. ☒ *Fee Authorization*. The Commissioner is hereby authorized to charge the above-referenced fees of $200.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 23-2415 (50535-710.306).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI

Dated: February 5, 2025

By: /Victoria Cheng/
Victoria Cheng, Reg. No. 79641

650 Page Mill Road
Palo Alto, CA 94304-1050
(650) 493-9300
Customer No. 21971

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	1	of	9	Attorney Docket Number	50535-710.306

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	001	US-4328245	05-04-1982	YU; Cheng-Der et al.	
	002	US-4409239	10-11-1983	YU; Cheng-Der	
	003	US-4410545	10-18-1983	YU; Cheng-Der et al.	
	004	US-4861760	08-29-1989	MAZUEL; Claude et al.	
	005	US-4911920	03-27-1990	JANI; Rajni et al.	
	006	US-5212162	05-18-1993	MISSEL; Paul J. T. et al.	
	007	US-5403841	04-04-1995	LANG; John C. et al.	
	008	US-10849908	12-01-2020	HOWERTON; Alexis et al.	
	009	US-11007201	05-18-2021	HOWERTON; Alexis et al.	
	010	US-11304950	04-19-2022	HOWERTON et al.	
	011	US-11311549	04-26-2022	HOWERTON; Alexis et al.	
	012	US-11344557	05-31-2022	HOWERTON; Alexis et al.	
	013	US-11351177	06-07-2022	HOWERTON; Alexis et al.	
	014	US-12115166	10-15-2024	HOWERTON; Alexis et al.	
	015	US-20100196389	08-05-2010	EVANS-FREKE; Stephen	
	016	US-20100222339	09-02-2010	CHEN; Zhaogen et al.	
	017	US-20170020877	01-26-2017	GRIGORIADIS; Dimitri E.	
	018	US-20220143037	05-12-2022	HOWERTON; Alexis et al.	
	019	US-20220211711	07-07-2022	HOWERTON; Alexis et al.	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	2	of	9	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[2]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	001	CN-101516887-A	08-26-2009	LILLY CO ELI [US]	Abstract:	☒
	002	CN-106102740-A	11-09-2016	NEUROCRINE BIOSCIENCES INC.	Abstract:	☒
	003	JP-2010504344-A	02-12-2010	ELI LILLY AND CO.	Machine Translation:	☒
	004	JP-2017503030-A	01-26-2017	NEUROCRINE BIOSCIENCES, INC.	Machine Translation:	☒
	005	JP-H0971528-A	03-18-1997	HAYASHIBARA BIOCHEM LAB [JP]	Abstract:	☒
	006	WO-0040529-A1	07-13-2000	ANGIOGENE PHARM LTD [GB], et al.		☐
	007	WO-0041669-A2	07-20-2000	ANGIOGENE PHARM LTD [GB], et al.		☐
	008	WO-0192224-A1	12-06-2001	ASTRAZENECA AB [SE], et al.		☐
	009	WO-0204434-A1	01-17-2002	ANGIOGENE PHARM LTD [GB], et al.		☐
	010	WO-0208213-A1	01-31-2002	ANGIOGENE PHARM LTD [GB], et al.		☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	3	of	9	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	011	WO-2007109853-A1	10-04-2007	VELACOR THERAPEUTICS PTY LTD [AU], et al.		☐
	012	WO-2008036579-A1	03-27-2008	LILLY CO ELI [US], et al.		☐
	013	WO-2015112642-A1	07-30-2015	NEUROCRINE BIOSCIENCES INC [US]		☐
	014	WO-2019036472-A1	02-21-2019	SPRUCE BIOSCIENCES INC [US]		☐
	015	WO-2019036503-A1	02-21-2019	SPRUCE BIOSCIENCES INC [US]		☐
	016	WO-2019210266-A1	10-31-2019	SPRUCE BIOSCIENCES INC [US]		☐
	017	WO-9722596-A1	06-26-1997	ZENECA LTD [GB], et al.		☐
	018	WO-9730035-A1	08-21-1997	ZENECA LTD [GB], et al.		☐
	019	WO-9732856-A1	09-12-1997	ZENECA LTD [GB], et al.		☐
	020	WO-9813354-A1	04-02-1998	ZENECA LTD [GB], et al.		☐
	021	WO-9902166-A1	01-21-1999	ANGIOGENE PHARM LTD [GB], et al.		☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	4	of	9	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	001	AL-MUHAMMED, J. et al. In-vivo studies on dexamethasone sodium phosphate liposomes. Journal of microencapsulation 13(3):293-306 (1996).	☐
	002	BERGE, Stephen M. et al. Pharmaceutical Salts. Journal of Pharmaceutical Sciences 66(1):1-19 (1977).	☐
	003	CHEN, Hua-dong. et al. Ovarian adrenal rest tumors undetected by imaging studies and identified at surgery in three females with congenital adrenal hyperplasia unresponsive to increased hormone therapy dosage. Endocrine pathology 28(2):146-151 (2017).	☐
	004	CHONN, Archadio et al. Recent Advances in Liposomal Drug-delivery Systems. Current Opinion in Biotechnology 6(6):698-708 (1995).	☐
	005	Co-pending U.S. Application No. 18/941,832, inventors HOWERTON; Alexis et al., filed on November 08, 2024.	☐
	006	Co-pending U.S. Application No. 19/043,275, inventors HOWERTON; Alexis et al., filed on January 31, 2025.	☐
	007	Co-pending U.S. Application No. 19/043,303, inventors HOWERTON; Alexis et al., filed on January 31, 2025.	☐
	008	EYLES et al. Oral delivery and fate of poly(lactic acid) microsphere-encapsulated interferon in rats. J Pharm Pharmacol. 49(7):669-74 (1997).	☐
	009	GAO, Zhi-Hui. et al. Controlled Release of a Contraceptive Steroid From Biodegradable and Injectable Gel Formulations: in Vitro Evaluation. Pharmaceutical research 12(6):857-863 (1995).	☐
	010	JIN et al. Testicular adrenal rest tumors in a patient with untreated congenital adrenal hyperplasia. Korean J Pediatr 2011;54(3):137-140.	☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	5	of	9	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	011	KAPRARA, et al. The corticotropin releasing factor system in cancer: expression and pathophysiological implications. Cellular and molecular life sciences 67(8):1293-1306 (2010).				☐
	012	MENDES-DOS-SANTOS et al. Prevalence of Testicular Adrenal Rest Tumor and Factors Associated with Its Development in Congenital Adrenal Hyperplasia. Hormone Res. In Paed. 90:161-168 (2018).				☐
	013	MUNSON, Peter et al. Ligand: a Versatile Computerized Approach for Characterization of Ligand-binding Systems. Analytical Biochemistry 107(1):220-239 (1980).				☐
	014	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Decision Granting Institution of Post-Grant Review of U.S. Patent No. 11,007,201 dated December 1, 2023 (pp. 1-38) (PGR2022-00025).				☐
	015	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Final Written Decision of Post-Grant Review of U.S. Patent No. 10,849,908 dated November 26, 2024 (pp. 1-62) (PGR2021-00088).				☐
	016	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Final Written Decision of Post-Grant Review of U.S. Patent No. 11,007,201 dated November 27, 2024 (pp. 1-60) (PGR2022-00025).				☐
	017	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Petition for Post Grant Review of U.S. Patent No. 10,849,908 dated May 28, 2021 (pp. 1-90) (PGR2021-00088).				☐
	018	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Petition for Post Grant Review of U.S. Patent No. 11,007,201 dated February 18, 2022 (pp 1-92) (PGR2022-00025).				☐
	019	OSTRO, Mark J. et al. Use of Liposomes as Injectable-drug Delivery Systems. American Journal of Hospital Pharmacy 46(8):1576-1587 (1989).				☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	6	of	9	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	020	PCT/US2019/029486 International Invitation to Pay Additional Fees dated June 4, 2019.				☐
	021	PCT/US2019/029486 International Search Report and Written Opinion dated August 12, 2019.				☐
	022	RAO. Recent developments of collagen-based materials for medical applications and drug delivery systems. J Biomater Sci Polym Ed. 7(7):623-45 (1995).				☐
	023	REICHMAN et al. Fertility in Patients with Congenital Adrenal Hyperplasia. Fertility and Sterility, Vol. 101, No. 2, 2014. 301-309.				☐
	024	REISCH et al. Testicular adrenal rest tumors develop independently of long-term disease control: a longitudinal analysis of 50 adult men with congenital adrenal hyperplasia due to classic 21-hydroxylase deficiency. J. Clin. Endocrinol. Metab. 98(11):E1820-E1826 (2013).				☐
	025	SMEETS et. al. Molecular characterization of testicular adrenal rest tumors in congenital adrenal hyperplasia: lesions with both adrenocortical and Leydig cell features. JCEM 100(3):E524-E530 (2015).				☐
	026	TIOSANO et al. Ovarian adrenal rest tumor in a congenital adrenal hyperplasia patient with adrenocorticotropin hypersecretion following adrenalectomy. Hormone Res. in Paediatrics 74:223-228 (2010).				☐
	027	U.S. Serial No. 17/081,694 Office Action dated February 26, 2021.				☐
	028	U.S. Serial No. 17/081,694 Office Action dated June 11, 2021.				☐
	029	U.S. Serial No. 17/667,285 Office Action dated December 29, 2023.				☐
	030	U.S. Serial No. 17/667,285 Office Action dated June 10, 2024.				☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	7	of	9	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	031	ZAAROUR et al. Bilateral Ovary Adrenal Rest Tumor in a Congenital Adrenal Hyperplasia Following Adrenalectomy. Endocrinol. Pract. 20(4):e69-e74 (2014).				☐

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	8	of	9	Attorney Docket Number	50535-710.306

TIMING STATEMENT

Please see 37 CFR 1.97(e) to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached timing statement under 37 CFR 1.97(e).

☒ A timing statement under 37 CFR 1.97(e) is not submitted herewith.

TIMING FEE

☐ The fee set forth in 37 CFR 1.17(p) has been submitted herewith.

COPIES

☐ An identification of an earlier application pursuant to 37 CFR 1.98(d)(1) is attached.

SIZE FEE ASSERTION

Please see 37CFR 1.17(v) and attached instructions for completing this form to make the appropriate selection of an assertion under 37 CFR 1.98. For the information disclosure statement (IDS) submitted herewith, the applicant or patent owner certifies the following with respect to the cumulative number of applicant-provided or patent-owner provided items of information submitted to date including those in the accompanying IDS (select only one):

☐ No IDS size fee is required under 37 CFR 1.17 (v) at this time.

☒ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(1).

☐ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(2).

☐ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(3).

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 11.18. Please see CFR 1.4(d) for the form of the signature.

Signature:	**/Victoria Cheng/**	Date (YYYY-MM-DD)	**2025-02-05**
Name/Print	Victoria Cheng	Registration Number	79641

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	9	of	9	Attorney Docket Number	50535-710.306

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
19/043,264	**02/05/2025 07:07:18 PM Z ET**	**50535-710.306**

Title of Invention

METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	6961	FILED BY	Jennifer Vail
PATENT CENTER #	69068511	FILING DATE	-
CUSTOMER #	21971	FIRST NAMED INVENTOR	Alexis HOWERTON
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Victoria Cheng

Documents

TOTAL DOCUMENTS: 2

DOCUMENT		PAGES	DESCRIPTION	SIZE (KB)
Spruce 50535-710_306_IDS.pdf		14	-	459 KB
Spruce 50535-710_306_IDS-TRAN.LET.pdf	(1-4)	4	Transmittal Letter	156 KB
Spruce 50535-710_306_IDS-IDS.pdf	(5-14)	10	Information Disclosure Statement (IDS) Form (SB08)	439 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
Spruce 50535-	D70861711D56587AD0381D85402B6223D4BBB8C3D0283E92E

710_306_IDS.pdf	57E64668AB65E7AAF16D73AEB1BFA09BD550981DAAF96B63EC15A139429F4FA72C50E33DBCBA63A
Spruce 50535-710_306_IDS-TRAN.LET.pdf	94C131008D3A9D083BB5E18633BA43AB7CFDE082323461B3FAD2F4B374FF8A9E66A664D5BEFF6CC1C5121FCE517C59184F98D92FBB1596AA9F955A3B92A36BA9
Spruce 50535-710_306_IDS-IDS.pdf	DE2509C72BF317C9E43E14A3BEBD554B01282BAAA6245B7084EFBACA54FC78E300BB8D6D7C6B20A38B19EEA8DFBF2A17EECC0B58CBA57D68583D7D1A66FA8F50

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
19/043,264	01/31/2025	Alexis HOWERTON	50535-710.306

CONFIRMATION NO. 6961

21971
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

INFORMAL NOTICE

OC00000084754788

Date Mailed: 02/24/2025

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):

  Alexis HOWERTON
  Hal GERBER

  An inventor's oath or declaration in compliance with 37 CFR 1.63 or 1.64 executed by or with respect to each inventor must be submitted no later than the date on which the issue fee is paid in response to a notice requiring such fee. See 37 CFR 1.53(f).

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/nhnguyen/

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
19/043,264	01/31/2025		2800	50535-710.306	21	1

CONFIRMATION NO. 6961

FILING RECEIPT

21971
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

OC000000084759785

Date Mailed: 02/24/2025

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

Alexis HOWERTON, San Francisco, CA;
Hal GERBER, San Francisco, CA;

Applicant(s)

Spruce Biosciences, Inc., South San Francisco, CA;

Power of Attorney: None

Domestic Applications for which benefit is claimed

This application is a CON of 18/941,832 11/08/2024
which is a CON of 17/667,285 02/08/2022 ABN
which is a CON of 17/081,694 10/27/2020 PAT 11,304,950
which is a CON of PCT/US2019/029486 04/26/2019
which claims benefit of 62/822,815 03/23/2019
and claims benefit of 62/663,951 04/27/2018

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) **- None.**
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 02/21/2025

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 19/043,264**

Projected Publication Date: 06/05/2025

Non-Publication Request: No

Early Publication Request: No

Title

METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific

countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop

technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 19/043,264

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	350
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	770
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	880
TOTAL CLAIMS (37 CFR 1.16(i))	21 minus 20 =	* 1			OR	x 200 =	200
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*				x =	0
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	2200

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)	OR	RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

Welcome to the U.S. Patent and Trademark Office (USPTO)

*Congratulations on taking the first step to protect your invention. You can now advertise or mark any products covered by this application for your invention as **"Patent Pending"** for as long as this application is pending!*

We are here to help you on the rest of your journey.

New to the process? The Patent Basics page will take you from "What is a patent?" to assistance with the application process.

- **www.uspto.gov/PatentBasics**

Consider cost savings. You might qualify for small entity or micro entity status, which means a discount on most USPTO patent fees.

- **www.uspto.gov/EntityStatus**

Become a registered electronic filer (eFiler). You'll gain access to the full capabilities of Patent Center. As a registered eFiler, you can electronically view your submission, track the progress of your application, and respond to USPTO correspondence.

- **www.uspto.gov/NewPatentFilers**

Learn how to identify which of your creative ideas might be intellectual property assets and how to protect them by using the USPTO Intellectual Property (IP) Identifier.

- **ipidentifier.uspto.gov**

We also provide Pro Se Assistance, support specifically geared for independent inventors without an attorney or agent. Follow the link below, call us at 866-767-3848, or email proseassistancecenter@uspto.gov.

- **www.uspto.gov/ProSePatents**

Need help near you? We have regional offices across the country, as well as Patent and Trademark Resource Centers in libraries in nearly every state, with specialists standing by to help you at no cost.

- **www.uspto.gov/locations**
- **www.uspto.gov/ptrc**

Contact us:

- General information: USPTO Contact Center
  - **www.uspto.gov/help**
- Patent Center:
  Patent Electronic Business Center
  - **www.uspto.gov/EBC**
- Pre or post-examination: Application Assistance Unit
  - **www.uspto.gov/PatentAssistance**

Want to learn more?

- Access our free services all in one spot. Find all of our contact centers, nationwide services, events, education, and training.
  - **www.uspto.gov/FreeServices**
- Connect with us on Instagram, LinkedIn, Twitter, Facebook, and YouTube.
- Hear about resources for startups, inventors, and entrepreneurs, as well as our initiatives engaging women, the military community, and others. These programs offer helpful tips and tricks on identifying and managing your IP, finding options for funding, and expanding your network to support your business.
  - **www.uspto.gov/startups**
  - **www.uspto.gov/inventors**
  - **www.uspto.gov/initiatives**
- We hope you connect with us by subscribing to receive important notifications by email.
  - **www.uspto.gov/subscribe**

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
19/043,264	01/31/2025	Alexis HOWERTON	50535-710.306	6961

21971 7590 03/25/2025

WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

EXAMINER	
PIHONAK, SARAH	

ART UNIT	PAPER NUMBER
1627	

NOTIFICATION DATE	DELIVERY MODE
03/25/2025	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentdocket@wsgr.com

Decision Granting Request for Prioritized Examination (Track I)	**Application No.** 19/043,264	**Applicant(s)** HOWERTON et al.	
	Examiner IRVIN DINGLE	**Art Unit** OPET	**AIA (FITF) Status** Yes

1. THE REQUEST FILED 31 January 2025 IS **GRANTED** .

The above-identified application has met the requirements for prioritized examination

A. ☑ for an original nonprovisional application (Track I).
B. ☐ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination**. The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

A. filing a **petition for extension of time** to extend the time period for filing a reply;

B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims** , or a multiple dependent claim;

C. filing a **request for continued examination** ;

D. filing a notice of appeal;

E. filing a request for suspension of action;

F. mailing of a notice of allowance;

G. mailing of a final Office action;

H. completion of examination as defined in 37 CFR 41.102; or

I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to IRVIN DINGLE at (571)272-3210. In his/her absence, calls may be directed to Petition Help Desk at (571) 272-3282.

/IRVIN DINGLE/ Lead Paralegal Specialist, OPET	

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
19/043,264	01/31/2025	Alexis HOWERTON	50535-710.306	6961

21971 7590 04/25/2025

WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

EXAMINER	
PIHONAK, SARAH	

ART UNIT	PAPER NUMBER
1627	

NOTIFICATION DATE	DELIVERY MODE
04/25/2025	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentdocket@wsgr.com

Office Action Summary	Application No. 19/043,264	Applicant(s) HOWERTON et al.	
	Examiner SARAH PIHONAK	Art Unit 1627	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 1/31/2025.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 1-21 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 1-21 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 1/31/25 is/are: a)☑ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Priority

This application, filed 01/31/2025 is a Continuation of 18941832, filed 11/08/2024.

18941832 is a Continuation of 17667285, filed 02/08/2022.

17667285 is a Continuation of 17081694, filed 10/27/2020.

17081694 is a Continuation of PCT/US2019/029486, filed 04/26/2019.

PCT/US2019/029486 Claims Priority from Provisional Application 62822815, filed 03/23/2019; and Provisional Application 62663951, filed 04/27/2018. The provisional applications don't provide support to treating menstrual irregularities; therefore, the effective filing and priority date of the claims is 4/26/2019.

Status of Claims

1. Claims 1-21 are currently pending. A track one status has been granted.

2. Claims 1-21 were examined and are rejected.

Claim Rejections-35 USC § 112

3. The following is a quotation of 35 U.S.C. 112(b):

(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 4 is rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor (or for applications subject to pre-AIA 35 U.S.C. 112, the applicant), regards as the invention.

Claim 4 depends directly from claim 1 and recites "wherein the metabolic parameter is insulin sensitivity". However, claim 1 doesn't mention or refer to a metabolic parameter, therefore, there is insufficient antecedent basis for the limitation of claim 4, and the claim is indefinite.

Claim Rejections-35 USC § 102

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

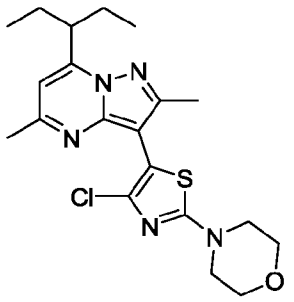
> A person shall be entitled to a patent unless –
>
> (a)(1) the claimed invention was patented, described in a printed publication, or in public use, on sale, or otherwise available to the public before the effective filing date of the claimed invention.
>
> (a)(2) the claimed invention was described in a patent issued under section 151, or in an application for patent published or deemed published under section 122(b), in which the patent or application, as the case may be, names another inventor and was effectively filed before the effective filing date of the claimed invention.

Claim(s) 1-2 and 5-21 is/are rejected under 35 U.S.C. 102(a)(1) as being anticipated by Howerton et. al., WO 2019036503 A1, publ. 2/21/2019, international filing date 8/14/2018, cited in an IDS.

Claim(s) 1-2 and 5-21 is/are rejected under 35 U.S.C. 102(a)(2) as being anticipated by Howerton et. al., WO 2019036503 A1, publ. 2/21/2019, international filing date 8/14/2018.

The applied reference has a common inventor with the instant application. Based upon the earlier effectively filed date of the reference, it constitutes prior art under 35 U.S.C. 102(a)(2). This rejection under 35 U.S.C. 102(a)(2) might be overcome by: (1) a showing under 37 CFR 1.130(a) that the subject matter disclosed in the reference was obtained directly or indirectly from the inventor or a joint inventor of this application and is thus not prior art in accordance with 35 U.S.C. 102(b)(2)(A); (2) a showing under 37 CFR 1.130(b) of a prior public disclosure under 35 U.S.C. 102(b)(2)(B) if the same invention is not being claimed; or (3) a statement pursuant to 35 U.S.C. 102(b)(2)(C) establishing that, not later than the effective filing date of the claimed invention, the subject matter disclosed in the reference and the claimed invention were either owned by the same person or subject to an obligation of assignment to the same person or subject to a joint research agreement.

Howerton discloses 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 of treating congenital adrenal hyperplasia (CAH) and menstrual irregularity comprising administering the compound in a pharmaceutical composition (para [0003-0004], [0041]):



.

Howerton discloses treatment of an adolescent from about 12 years of age (para [0039], [0170]), as well as treatment of an adult of about 20 years of age (para [0039]). Howerton discloses a dose of the above shown compound between about 5-500 mg. (para [0027]), which includes the range recited in instant claim 8. Howerton discloses administration of the compound in a pharmaceutical composition once or twice per day (para [0030]), and administration of the composition to a subject in the fed state (para [0012]). Howerton discloses oral administration in dosage forms inclusive of tablets and capsules (para [0076]). Howerton discloses administration of the compound reduces 17-OHP (para [0047-0048]) and ACTH, with 50% of subjects experiencing a greater than 25% reduction in 17-OHP (para [0165-0166]). Howerton as such anticipates the claims.

Claim Rejections-35 USC § 103

5. The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries for establishing a background for determining obviousness under 35 U.S.C. 103 are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

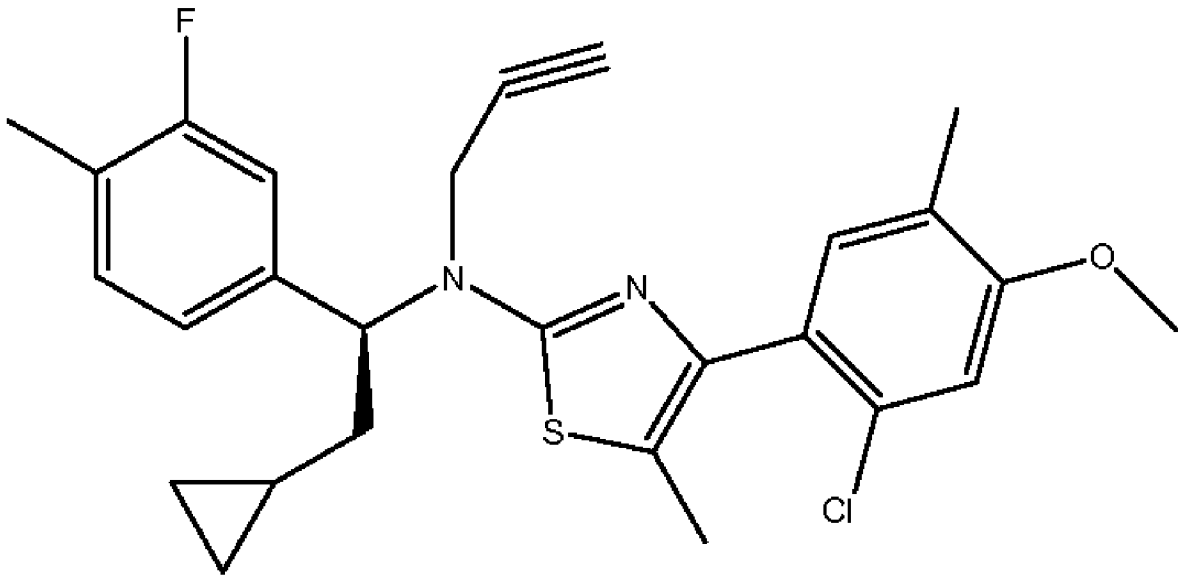
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims the examiner presumes that the subject matter of the various claims was commonly owned as of the effective filing date of the claimed invention(s) absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and effective filing dates of each claim that was not commonly owned as of the effective filing date of the later invention in order for the examiner to consider the applicability of 35 U.S.C. 102(b)(2)(C) for any potential 35 U.S.C. 102(a)(2) prior art against the later invention.

Claim(s) 1, 3-14, and 17-21 is/are rejected under 35 U.S.C. 103 as being unpatentable over Grigoriadis, WO 2015112642 A1, publ. 7/30/2015, cited in an IDS.

Grigoriadis teaches corticotropin releasing factor-1 (CRF1) receptor antagonists for the treatment of congenital adrenal hyperplasia (CAH) and inhibition of ACTH release in these patients (title & abstract; p. 1, lines 3-5). Grigoriadis teaches CAH as a group of autosomal recessive genetic disorders that cause little to no cortisol biosynthesis and often exhibit 21-hydroxylase deficiency; ACTH production is excessive (p. 1, line 6-p. 2, line 3). CAH affects both males and females (p. 7, lines 22-28). Grigoriadis teaches treatment of CAH typically involves glucocorticoids to establish sufficient cortisol production and for reducing elevated ACTH levels, and suppression of ACTH prevents menstrual disturbances in women (p. 7, line 29-p. 8, line 11). An issue with glucocorticoid therapy is that the doses required to achieve sufficient suppression of excess androgens are usually well above the normal physiologic dose, and over time glucocorticoid therapy can lead to increased cardiovascular risk factors, glucose intolerance,

and decreased bone mineral density (p. 9, lines 1-12). Grigoriadis exemplifies the following compound as a CRF1 receptor antagonist (p. 14, see SSR-125543):



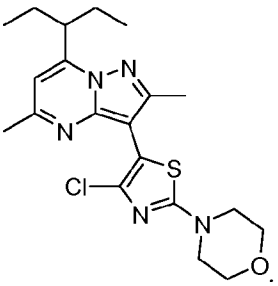
. Grigoriadis teaches treatment of human and non-human animals; a therapeutically effective dose is taught to range from about 50-1000 mg. (p. 24, line 3-p. 25, line 7). Grigoriadis teaches various routes of administration, including oral (p. 25, lines 15-21). Grigoriadis teaches administration of a CRF1 receptor antagonist as described results in a clinically significant reduction of both ACTH and 17-OHP levels relative to placebo, including a reduction of at least 25% (p. 28, lines 10-19). Grigoriadis teaches pharmaceutical compositions comprising CRF1 receptor antagonists in the form of tablets or capsules (p. 28, line 20-p. 29, line 16). Grigoriadis further teaches the dose also depends on the patient's condition, stage of disease, severity of symptoms; although bedtime administration is exemplified, the timing of administration can also be adjusted depending on various factors (p. 25, line 22-p. 26, line 20).

It would have been prima facie obvious to one of ordinary skill in the art, before the effective filing date of the claims to have treated menstrual irregularity in a subject with CAH comprising administering the CRF1 receptor antagonist shown in instant claims 1 and 3, in consideration of Grigoriadis. Grigoriadis teaches CRF1 receptor antagonists for the treatment of CAH, including in females, and exemplifies the compound recited in instant claim 3. Grigoriadis further teaches subjects with CAH have elevated ACTH levels and suppression of ACTH elevation can prevent menstrual disturbances. As Grigoriadis further teaches administering a CRF1 receptor antagonist can also suppress elevated ACTH levels, it would have been prima facie obvious that this treatment would have similarly treated or prevented menstrual disturbances, i.e., menstrual irregularities in a female with CAH and elevated ACTH levels. One of ordinary skill in the art would have arrived at the claimed method of treatment and have had a reasonable expectation of success. Since menstruating females include adolescents and subjects aged 18 years and older, it would have been prima facie obvious to have treated menstrual irregularities in a female with CAH who is an adolescent, as recited by instant claim 6, or who is at least 18 years of ager, as recited by instant claim 7.

As Grigoriadis teaches the dose and timing of administration of the CRF1 receptor antagonist can be adjusted based on various factors, it would have been prima facie obvious to have administered the CRF1 receptor antagonist once per day as recited by instant claim 13, or twice per day as recited by instant claim 14.

6. Claim(s) 1-2, 5-14, and 17-21 is/are rejected under 35 U.S.C. 103 as being unpatentable over Chen et. al., WO 2008036579 A1, publ. 3/27/2008, cited in an IDS in view of Grigoriadis, WO 2015112642 A1, publ. 7/30/2015.

Chen teaches thiazole pyrazolopyrimidine derivatives as CRF1 antagonists (title & abstract; p. 1, lines 3-7). Chen further teaches CRF1 antagonists to have activity for treating numerous conditions, including congenital adrenal hyperplasia (p. 3, lines 11-19; p. 3, line 28-p. 4, line 6). Chen exemplifies the following compound as a CRF1 antagonist (Ex. 16, p. 37, line 19-p. 38, line 18):



Oral administration of the CRF1 antagonist in a pharmaceutical composition is taught (p. 7, lines 24-28).

Chen doesn't explicitly teach or suggest treating menstrual irregularity.

Grigoriadis teaches corticotropin releasing factor-1 (CRF1) receptor antagonists for the treatment of congenital adrenal hyperplasia (CAH) (title & abstract; p. 1, lines 3-5). Grigoriadis teaches CAH as a group of autosomal recessive genetic disorders that cause little to no cortisol biosynthesis and often exhibit 21-hydroxylase deficiency; ACTH production is excessive (p. 1, line 6-p. 2, line 3). CAH affects both males and females (p. 7, lines 22-28). Grigoriadis teaches treatment of CAH typically involves glucocorticoids to establish sufficient cortisol production and for reducing elevated ACTH levels, and suppression of ACTH prevents menstrual

disturbances in women (p. 7, line 29-p. 8, line 11). An issue with glucocorticoid therapy is that the doses required to achieve sufficient suppression of excess androgens are usually well above the normal physiologic dose, and over time glucocorticoid therapy can lead to increased cardiovascular risk factors, glucose intolerance, and decreased bone mineral density (p. 9, lines 1-12). Grigoriadis teaches treatment of human and non-human animals; a therapeutically effective dose is taught to range from about 50-1000 mg. (p. 24, line 3-p. 25, line 7). Grigoriadis teaches various routes of administration, including oral (p. 25, lines 15-21). Grigoriadis teaches administration of a CRF1 receptor antagonist as described results in a clinically significant reduction of both ACTH and 17-OHP levels relative to placebo, including a reduction of at least 25% (p. 28, lines 10-19). Grigoriadis teaches pharmaceutical compositions comprising CRF1 receptor antagonists in the form of tablets or capsules (p. 28, line 20-p. 29, line 16). Grigoriadis further teaches the dose also depends on the patient's condition, stage of disease, severity of symptoms; although bedtime administration is exemplified, the timing of administration can also be adjusted depending on various factors (p. 25, line 22-p. 26, line 20).

It would have been prima facie obvious to one of ordinary skill in the art, before the effective filing date of the claims to have arrived at the instantly claimed method of treating menstrual irregularity in a subject with CAH comprising administering a CRF1 receptor antagonist of instant claims 1-2, in consideration of Chen and Grigoriadis. Chen teaches the compound of instant claim 2 as a CRF1 receptor antagonist for treating CAH, while Grigoriadis also teaches treatment of CAH by administering a CRF1 receptor antagonist. Grigoriadis further teaches subjects with CAH have elevated ACTH levels and suppression of ACTH elevation can prevent menstrual disturbances. As Grigoriadis further teaches administering a CRF1 receptor

antagonist can also suppress elevated ACTH levels, it would have been prima facie obvious that this treatment would have similarly treated or prevented menstrual disturbances, i.e., menstrual irregularities in a female with CAH and elevated ACTH levels. One of ordinary skill in the art would have arrived at the claimed method of treatment and have had a reasonable expectation of success. Since menstruating females include female adolescents and female subjects aged 18 years and older, it would have been prima facie obvious to have treated menstrual irregularities in a female with CAH who is an adolescent, as recited by instant claim 6, or who is at least 18 years or older, as recited by instant claim 7.

The dose of CRF1 receptor antagonist from about 50-1000 mg. as taught by Grigoriadis overlaps with the amounts recited by instant claims 8-12. Therefore, it would have been prima facie obvious to one of ordinary skill in the art to have administered the CRF1 receptor antagonist taught by Chen within the same dosage range, for treating CAH in a subject having menstrual irregularities. As Grigoriadis teaches the dose and timing of administration of the CRF1 receptor antagonist can be adjusted based on various factors, it would have been prima facie obvious to have administered the CRF1 receptor antagonist once per day as recited by instant claim 13, or twice per day as recited by instant claim 14.

Claim Rejections-Nonstatutory Double Patenting

7. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is

appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP § 2146 *et seq.* for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The filing of a terminal disclaimer by itself is not a complete reply to a nonstatutory double patenting (NSDP) rejection. A complete reply requires that the terminal disclaimer be accompanied by a reply requesting reconsideration of the prior Office action. Even where the NSDP rejection is provisional the reply must be complete. See MPEP § 804, subsection I.B.1. For a reply to a non-final Office action, see 37 CFR 1.111(a). For a reply to final Office action, see 37

CFR 1.113(c). A request for reconsideration while not provided for in 37 CFR 1.113(c) may be filed after final for consideration. See MPEP §§ 706.07(e) and 714.13.

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The actual filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/apply/applying-online/eterminal-disclaimer.

Claims 1-3, 5, 8-14, and 17-21 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4, 7-8, 11-14, 17-18, and 21-22 of U.S. Patent No. 10849908 B2 in view of Grigoriadis, WO 2015112642 A1, publ. 7/30/2015. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims encompass administering a CRF1 antagonist to a subject having CAH. In particular, the patented claims recite treating a human having CAH by administering a therapeutically effective amount of a CRF1 antagonist, resulting in a reduction of the ACTH level by at least 10% from baseline. The instant claims similarly recite administering small molecule CRF1 antagonists to treat a subject having CAH (see instant claims 1 and 5); although the patented claims don't recite treating menstrual irregularity as required by the instant claims, this patient population would have been prima facie obvious in view of Grigoriadis. Grigoriadis teaches corticotropin releasing factor-1 (CRF1) receptor antagonists for the treatment of congenital adrenal hyperplasia (CAH) (title & abstract; p. 1, lines 3-5). Grigoriadis teaches CAH

as a group of autosomal recessive genetic disorders that cause little to no cortisol biosynthesis and often 21-hydroxylase deficiency; ACTH production is excessive (p. 1, line 6-p. 2, line 3). CAH affects both males and females (p. 7, lines 22-28). Grigoriadis teaches treatment of CAH typically involves glucocorticoids to establish sufficient cortisol production and for reducing elevated ACTH levels, and suppression of ACTH prevents menstrual disturbances in women (p. 7, line 29-p. 8, line 11). An issue with glucocorticoid therapy is that the doses required to achieve sufficient suppression of excess androgens are usually well above the normal physiologic dose, and over time glucocorticoid therapy can lead to increased cardiovascular risk factors, glucose intolerance, and decreased bone mineral density (p. 9, lines 1-12). Grigoriadis teaches treatment of human and non-human animals; a therapeutically effective dose is taught to range from about 50-1000 mg. (p. 24, line 3-p. 25, line 7). Grigoriadis teaches various routes of administration, including oral (p. 25, lines 15-21). Grigoriadis teaches administration of a CRF1 receptor antagonist as described results in a clinically significant reduction of both ACTH and 17-OHP levels relative to placebo, including a reduction of at least 25% (p. 28, lines 10-19). Grigoriadis teaches pharmaceutical compositions comprising CRF1 receptor antagonists in the form of tablets or capsules (p. 28, line 20-p. 29, line 16). Grigoriadis further teaches the dose also depends on the patient's condition, stage of disease, severity of symptoms; although bedtime administration is exemplified, the timing of administration can also be adjusted depending on various factors (p. 25, line 22-p. 26, line 20). Therefore, as the patented claims recite the method provides a reduction in ACTH levels, and elevated ACTH levels are associated with menstrual irregularities as taught by Grigoriadis, it would have been prima facie obvious to have modified the patented method by treating menstrual irregularities in a subject with CAH,

and have had a reasonable expectation of success. Additionally, the amount of CRF1 antagonist administered is overlapping (from 50-1600 mg/day in patented claim 2 compared to 10-400 mg/day as recited by instant claim 8); wherein the CRF1 antagonist is formulated as a capsule or tablet (see instant claim 18 & patented claim 8). As Grigoriadis teaches the dose and timing of administration of the CRF1 receptor antagonist can be adjusted based on various factors, it would have been prima facie obvious to have administered the CRF1 receptor antagonist once per day as recited by instant claim 13, or twice per day as recited by instant claim 14. For these reasons the instant and patented claims are obvious variants of each other and are not patentably distinct.

8. Claims 1-3, 5, 8-14, and 17-21 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4, 7-8, and 11-16 of U.S. Patent No. 11007201 B2 in view of Grigoriadis, WO 2015112642 A1, publ. 7/30/2015. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims encompass administering a CRF1 antagonist to a subject having CAH. In particular, the patented claims recite treating a human having CAH by administering a therapeutically effective amount of a CRF1 antagonist, resulting in a reduction of the androstenedione (A4) level. The instant claims similarly recite administering small molecule CRF1 antagonists to treat a subject having CAH (see instant claims 1 and 5); although the patented claims don't recite treating menstrual irregularity as required by the instant claims, this patient population would have been prima facie obvious in view of Grigoriadis. Grigoriadis teaches corticotropin releasing factor-1 (CRF1) receptor antagonists for the treatment of congenital adrenal hyperplasia (CAH) (title & abstract;

p. 1, lines 3-5). Grigoriadis teaches CAH as a group of autosomal recessive genetic disorders that cause little to no cortisol biosynthesis and often 21-hydroxylase deficiency; ACTH production is excessive (p. 1, line 6-p. 2, line 3). CAH affects both males and females (p. 7, lines 22-28). Grigoriadis teaches treatment of CAH typically involves glucocorticoids to establish sufficient cortisol production and for reducing elevated ACTH levels, and suppression of ACTH prevents menstrual disturbances in women (p. 7, line 29-p. 8, line 11). An issue with glucocorticoid therapy is that the doses required to achieve sufficient suppression of excess androgens are usually well above the normal physiologic dose, and over time glucocorticoid therapy can lead to increased cardiovascular risk factors, glucose intolerance, and decreased bone mineral density (p. 9, lines 1-12). Grigoriadis teaches treatment of human and non-human animals; a therapeutically effective dose is taught to range from about 50-1000 mg. (p. 24, line 3-p. 25, line 7). Grigoriadis teaches various routes of administration, including oral (p. 25, lines 15-21). Grigoriadis teaches administration of a CRF1 receptor antagonist as described results in a clinically significant reduction of both ACTH and 17-OHP levels relative to placebo, including a reduction of at least 25% (p. 28, lines 10-19). Grigoriadis teaches pharmaceutical compositions comprising CRF1 receptor antagonists in the form of tablets or capsules (p. 28, line 20-p. 29, line 16). Grigoriadis further teaches the dose also depends on the patient's condition, stage of disease, severity of symptoms; although bedtime administration is exemplified, the timing of administration can also be adjusted depending on various factors (p. 25, line 22-p. 26, line 20). Therefore, as the patented claims recite the method of treating CAH by administering a CRF1 antagonist, and elevated ACTH levels are associated with menstrual irregularities as taught by Grigoriadis, it would have been prima facie obvious to have modified the patented method by

treating menstrual irregularities in a subject with CAH, and have had a reasonable expectation of success. Additionally, the amount of CRF1 antagonist administered is overlapping (from 50-1600 mg/day in patented claim 2 compared to 10-400 mg/day as recited by instant claim 8); wherein the CRF1 antagonist is formulated as a capsule or tablet (see instant claim 18 & patented claim 8). As Grigoriadis teaches the dose and timing of administration of the CRF1 receptor antagonist can be adjusted based on various factors, it would have been prima facie obvious to have administered the CRF1 receptor antagonist once per day as recited by instant claim 13, or twice per day as recited by instant claim 14. For these reasons the instant and patented claims are obvious variants of each other and are not patentably distinct.

9. Claims 1-2, 5, 8-14, and 17-21 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4, 7-8, and 11-16 of U.S. Patent No. 11311549 B2 in view of Grigoriadis, WO 2015112642 A1, publ. 7/30/2015. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims encompass administering a CRF1 antagonist to a subject having CAH. In particular, the patented claims recite treating a human having CAH by administering a therapeutically effective amount of a CRF1 antagonist, resulting in a reduction of the androstenedione (A4) level. The instant claims similarly recite administering small molecule CRF1 antagonists to treat a subject having CAH (see instant claims 1 and 5); although the patented claims don't recite treating menstrual irregularity as required by the instant claims, this patient population would have been prima facie obvious in view of Grigoriadis. Grigoriadis teaches corticotropin releasing factor-1 (CRF1) receptor antagonists for the treatment of congenital adrenal hyperplasia (CAH) (title & abstract;

p. 1, lines 3-5). Grigoriadis teaches CAH as a group of autosomal recessive genetic disorders that cause little to no cortisol biosynthesis and often 21-hydroxylase deficiency; ACTH production is excessive (p. 1, line 6-p. 2, line 3). CAH affects both males and females (p. 7, lines 22-28). Grigoriadis teaches treatment of CAH typically involves glucocorticoids to establish sufficient cortisol production and for reducing elevated ACTH levels, and suppression of ACTH prevents menstrual disturbances in women (p. 7, line 29-p. 8, line 11). An issue with glucocorticoid therapy is that the doses required to achieve sufficient suppression of excess androgens are usually well above the normal physiologic dose, and over time glucocorticoid therapy can lead to increased cardiovascular risk factors, glucose intolerance, and decreased bone mineral density (p. 9, lines 1-12). Grigoriadis teaches treatment of human and non-human animals; a therapeutically effective dose is taught to range from about 50-1000 mg. (p. 24, line 3-p. 25, line 7). Grigoriadis teaches various routes of administration, including oral (p. 25, lines 15-21). Grigoriadis teaches administration of a CRF1 receptor antagonist as described results in a clinically significant reduction of both ACTH and 17-OHP levels relative to placebo, including a reduction of at least 25% (p. 28, lines 10-19). Grigoriadis teaches pharmaceutical compositions comprising CRF1 receptor antagonists in the form of tablets or capsules (p. 28, line 20-p. 29, line 16). Grigoriadis further teaches the dose also depends on the patient's condition, stage of disease, severity of symptoms; although bedtime administration is exemplified, the timing of administration can also be adjusted depending on various factors (p. 25, line 22-p. 26, line 20). Therefore, as the patented claims recite the method of treating CAH by administering a CRF1 antagonist, and elevated ACTH levels are associated with menstrual irregularities as taught by Grigoriadis, it would have been prima facie obvious to have modified the patented method by

treating menstrual irregularities in a subject with CAH, and have had a reasonable expectation of success. Additionally, the amount of CRF1 antagonist administered is overlapping (from 50-1600 mg/day in patented claim 2 compared to 10-400 mg/day as recited by instant claim 8); wherein the CRF1 antagonist is formulated as a capsule or tablet (see instant claim 18 & patented claim 8). As Grigoriadis teaches the dose and timing of administration of the CRF1 receptor antagonist can be adjusted based on various factors, it would have been prima facie obvious to have administered the CRF1 receptor antagonist once per day as recited by instant claim 13, or twice per day as recited by instant claim 14. For these reasons the instant and patented claims are obvious variants of each other and are not patentably distinct.

10. Claims 1-3, 5, 8-14, and 17-21 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4, 7-8, 11-16, and 19-24 of U.S. Patent No. 11344557 B2 in view of Grigoriadis, WO 2015112642 A1, publ. 7/30/2015. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims encompass administering a CRF1 antagonist to a subject having CAH. In particular, the patented claims recite treating a human having CAH by administering a therapeutically effective amount of a CRF1 antagonist, resulting in a reduction of the ACTH level by at least 10% from baseline. The instant claims similarly recite administering small molecule CRF1 antagonists to treat a subject having CAH (see instant claims 1 and 5); although the patented claims don't recite treating menstrual irregularity as required by the instant claims, this patient population would have been prima facie obvious in view of Grigoriadis. Grigoriadis teaches corticotropin releasing factor-1 (CRF1) receptor antagonists for the treatment of congenital adrenal

hyperplasia (CAH) (title & abstract; p. 1, lines 3-5). Grigoriadis teaches CAH as a group of autosomal recessive genetic disorders that cause little to no cortisol biosynthesis and often 21-hydroxylase deficiency; ACTH production is excessive (p. 1, line 6-p. 2, line 3). CAH affects both males and females (p. 7, lines 22-28). Grigoriadis teaches treatment of CAH typically involves glucocorticoids to establish sufficient cortisol production and for reducing elevated ACTH levels, and suppression of ACTH prevents menstrual disturbances in women (p. 7, line 29-p. 8, line 11). An issue with glucocorticoid therapy is that the doses required to achieve sufficient suppression of excess androgens are usually well above the normal physiologic dose, and over time glucocorticoid therapy can lead to increased cardiovascular risk factors, glucose intolerance, and decreased bone mineral density (p. 9, lines 1-12). Grigoriadis teaches treatment of human and non-human animals; a therapeutically effective dose is taught to range from about 50-1000 mg. (p. 24, line 3-p. 25, line 7). Grigoriadis teaches various routes of administration, including oral (p. 25, lines 15-21). Grigoriadis teaches administration of a CRF1 receptor antagonist as described results in a clinically significant reduction of both ACTH and 17-OHP levels relative to placebo, including a reduction of at least 25% (p. 28, lines 10-19). Grigoriadis teaches pharmaceutical compositions comprising CRF1 receptor antagonists in the form of tablets or capsules (p. 28, line 20-p. 29, line 16). Grigoriadis further teaches the dose also depends on the patient's condition, stage of disease, severity of symptoms; although bedtime administration is exemplified, the timing of administration can also be adjusted depending on various factors (p. 25, line 22-p. 26, line 20). Therefore, as the patented claims recite the method provides a reduction in ACTH levels, and elevated ACTH levels are associated with menstrual irregularities as taught by Grigoriadis, it would have been prima facie obvious to have modified the patented

method by treating menstrual irregularities in a subject with CAH, and have had a reasonable expectation of success. Additionally, the amount of CRF1 antagonist administered is overlapping (from 50-1600 mg/day in patented claim 2 compared to 10-400 mg/day as recited by instant claim 8); wherein the CRF1 antagonist is formulated as a capsule or tablet (see instant claim 18 & patented claim 8). Both sets of claims further recite a reduction of 17-OHP levels in the treated subject (see patented claims 1, 19-24; and instant claims 19-21). As Grigoriadis teaches the dose and timing of administration of the CRF1 receptor antagonist can be adjusted based on various factors, it would have been prima facie obvious to have administered the CRF1 receptor antagonist once per day as recited by instant claim 13, or twice per day as recited by instant claim 14. For these reasons the instant and patented claims are obvious variants of each other and are not patentably distinct.

11. Claims 1-2, 5, 8-14, and 17-21 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4, 7-8, and 14-19 of U.S. Patent No. 11351177 B2 in view of Grigoriadis, WO 2015112642 A1, publ. 7/30/2015. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims encompass administering a CRF1 antagonist to a subject having CAH. In particular, the patented claims recite treating a human having CAH by administering a therapeutically effective amount of a CRF1 antagonist, resulting in a reduction of the ACTH level by at least 10% from baseline. The instant claims similarly recite administering small molecule CRF1 antagonists to treat a subject having CAH (see instant claims 1 and 5); although the patented claims don't recite treating menstrual irregularity as required by the instant claims, this patient population would

have been prima facie obvious in view of Grigoriadis. Grigoriadis teaches corticotropin releasing factor-1 (CRF1) receptor antagonists for the treatment of congenital adrenal hyperplasia (CAH) (title & abstract; p. 1, lines 3-5). Grigoriadis teaches CAH as a group of autosomal recessive genetic disorders that cause little to no cortisol biosynthesis and often 21-hydroxylase deficiency; ACTH production is excessive (p. 1, line 6-p. 2, line 3). CAH affects both males and females (p. 7, lines 22-28). Grigoriadis teaches treatment of CAH typically involves glucocorticoids to establish sufficient cortisol production and for reducing elevated ACTH levels, and suppression of ACTH prevents menstrual disturbances in women (p. 7, line 29-p. 8, line 11). An issue with glucocorticoid therapy is that the doses required to achieve sufficient suppression of excess androgens are usually well above the normal physiologic dose, and over time glucocorticoid therapy can lead to increased cardiovascular risk factors, glucose intolerance, and decreased bone mineral density (p. 9, lines 1-12). Grigoriadis teaches treatment of human and non-human animals; a therapeutically effective dose is taught to range from about 50-1000 mg. (p. 24, line 3-p. 25, line 7). Grigoriadis teaches various routes of administration, including oral (p. 25, lines 15-21). Grigoriadis teaches administration of a CRF1 receptor antagonist as described results in a clinically significant reduction of both ACTH and 17-OHP levels relative to placebo, including a reduction of at least 25% (p. 28, lines 10-19). Grigoriadis teaches pharmaceutical compositions comprising CRF1 receptor antagonists in the form of tablets or capsules (p. 28, line 20-p. 29, line 16). Grigoriadis further teaches the dose also depends on the patient's condition, stage of disease, severity of symptoms; although bedtime administration is exemplified, the timing of administration can also be adjusted depending on various factors (p. 25, line 22-p. 26, line 20). Therefore, as the patented claims recite the method provides a

reduction in ACTH levels, and elevated ACTH levels are associated with menstrual irregularities as taught by Grigoriadis, it would have been prima facie obvious to have modified the patented method by treating menstrual irregularities in a subject with CAH, and have had a reasonable expectation of success. Additionally, the amount of CRF1 antagonist administered is overlapping (from 50-1600 mg/day in patented claim 2 compared to 10-400 mg/day as recited by instant claim 8); wherein the CRF1 antagonist is formulated as a capsule or tablet (see instant claim 18 & patented claim 8). As Grigoriadis teaches the dose and timing of administration of the CRF1 receptor antagonist can be adjusted based on various factors, it would have been prima facie obvious to have administered the CRF1 receptor antagonist once per day as recited by instant claim 13, or twice per day as recited by instant claim 14. For these reasons the instant and patented claims are obvious variants of each other and are not patentably distinct.

12. Claims 1-3, 5, 8-14, and 17-21 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-15 and 19 of U.S. Patent No. 11304950 B2 in view of Stikkelbroeck et. al., Fertility & Sterility, vol. 80(4), pp. 1030-1036, publ. 2003; and Grigoriadis, WO 2015112642 A1, publ. 7/30/2015. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims encompass administering a CRF1 antagonist to a subject having CAH (see patented claims 1-2). The instant claims similarly recite administering small molecule CRF1 antagonists to treat a subject having CAH (see instant claims 1 and 5). The instant and patented claims differ from each other as the patented claims are drawn to treating ovarian adrenal rest tumors in a

subject with CAH, and not menstrual irregularities. However, Stikkelbroeck teaches females with CAH can have irregular menstrual cycles due to adrenal androgen excess (p. 1030, 1st para). Stikkelbroeck further teaches females with CAH can develop adrenal rest tumors in the ovaries which replace normal ovarian tissue, causing impaired menstrual cycling (p. 1034, right col., last para-p. 1035, left col., top para). Therefore, it would have been prima facie obvious to have applied the instantly claimed method to treat menstrual irregularities in a subject with CAH and ovarian adrenal rest tumors, and have had a reasonable expectation of success.

Furthermore, Grigoriadis teaches corticotropin releasing factor-1 (CRF1) receptor antagonists for the treatment of congenital adrenal hyperplasia (CAH) (title & abstract; p. 1, lines 3-5). Grigoriadis teaches CAH as a group of autosomal recessive genetic disorders that cause little to no cortisol biosynthesis and often 21-hydroxylase deficiency; ACTH production is excessive (p. 1, line 6-p. 2, line 3). CAH affects both males and females (p. 7, lines 22-28). Grigoriadis teaches treatment of CAH typically involves glucocorticoids to establish sufficient cortisol production and for reducing elevated ACTH levels, and suppression of ACTH prevents menstrual disturbances in women (p. 7, line 29-p. 8, line 11). An issue with glucocorticoid therapy is that the doses required to achieve sufficient suppression of excess androgens are usually well above the normal physiologic dose, and over time glucocorticoid therapy can lead to increased cardiovascular risk factors, glucose intolerance, and decreased bone mineral density (p. 9, lines 1-12). Grigoriadis teaches treatment of human and non-human animals; a therapeutically effective dose is taught to range from about 50-1000 mg. (p. 24, line 3-p. 25, line 7). Grigoriadis teaches various routes of administration, including oral (p. 25, lines 15-21). Grigoriadis teaches administration of a CRF1 receptor antagonist as described results in a

clinically significant reduction of both ACTH and 17-OHP levels relative to placebo, including a reduction of at least 25% (p. 28, lines 10-19). Grigoriadis teaches pharmaceutical compositions comprising CRF1 receptor antagonists in the form of tablets or capsules (p. 28, line 20-p. 29, line 16). Grigoriadis further teaches the dose also depends on the patient's condition, stage of disease, severity of symptoms; although bedtime administration is exemplified, the timing of administration can also be adjusted depending on various factors (p. 25, line 22-p. 26, line 20). Therefore, as the patented claims recite the method provides a reduction in ACTH levels, and elevated ACTH levels are associated with menstrual irregularities as taught by Grigoriadis, it would have been prima facie obvious to have modified the patented method by treating menstrual irregularities in a subject with CAH and ovarian adrenal rest tumors, and have had a reasonable expectation of success. Additionally, the amount of CRF1 antagonist administered is the same (from 10-400 mg/day in patented claim 13 compared to 10-400 mg/day as recited by instant claim 8); wherein the CRF1 antagonist is formulated as a capsule or tablet (see instant claim 18 & patented claim 15). Both sets of claims explicitly recite the CRF1 antagonist of instant claims 1-2 (see patented claim 11). As Grigoriadis teaches the dose and timing of administration of the CRF1 receptor antagonist can be adjusted based on various factors, it would have been prima facie obvious to have administered the CRF1 receptor antagonist once per day as recited by instant claim 13, or twice per day as recited by instant claim 14. For these reasons the instant and patented claims are obvious variants of each other and are not patentably distinct.

13. Claims 1-3, and 5-21 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 72-91 of copending application 18941832 in view of Stikkelbroeck et. al., Fertility & Sterility, vol. 80(4), pp. 1030-1036, publ. 2003. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims encompass administering a CRF1 antagonist to a subject having CAH (see copending claims 72 and 76). The instant claims similarly recite administering small molecule CRF1 antagonists to treat a subject having CAH (see instant claims 1 and 5). The instant and copending claims differ from each other as the copending claims are drawn to treating a benign tumor, including ovarian adrenal rest tumors in a subject with CAH (see claims 72 and 75), and not menstrual irregularities. However, Stikkelbroeck teaches females with CAH can have irregular menstrual cycles due to adrenal androgen excess (p. 1030, 1st para). Stikkelbroeck further teaches females with CAH can develop adrenal rest tumors in the ovaries which replace normal ovarian tissue, causing impaired menstrual cycling (p. 1034, right col., last para-p. 1035, left col., top para). Therefore, it would have been prima facie obvious to have applied the instantly claimed method to treat menstrual irregularities in a subject with CAH and ovarian adrenal rest tumors, and have had a reasonable expectation of success.

Additionally, the amount of CRF1 antagonist administered is overlapping (from 5-800 mg/day in copending claim 78 compared to 10-400 mg/day as recited by instant claim 8); wherein the CRF1 antagonist is formulated as a capsule or tablet (see instant claim 18 & copending claim 91); wherein the treated subject is in a fed state (copending claim 84 and instant claims 14-15); and wherein the CRF1 antagonist is administered twice per day (copending claim 83 & instant claim 14). Both sets of claims explicitly recite the same CRF1

antagonists (see copending claim 72 & instant claim 1); and reduction of the same biomarkers selected from 17-OHP, ACTH, A4, LH, FSH, SHBG, renin, or aldosterone (see instant claim 19 & copending claim 86). As CAH affects females, including women and adolescent females (see Stikkelbroeck, p. 1030, right col., last para-p. 1031, left col., top para), it would have been prima facie obvious to have applied the copending method to treat an adolescent female as re cited by instant claim 6, and a female at least 18 years or older as recited by instant claim 7. For these reasons the instant and copending claims are obvious variants of each other and are not patentably distinct.

14. Claims 1-3, and 5-21 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-5 and 9-21 of copending application 19043275 in view of Stikkelbroeck et. al., Fertility & Sterility, vol. 80(4), pp. 1030-1036, publ. 2003. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims encompass administering a CRF1 antagonist to a subject having CAH (see copending claims 1 & 5). The instant claims similarly recite administering small molecule CRF1 antagonists to treat a subject having CAH (see instant claims 1 and 5). The instant and copending claims differ from each other as the copending claims are drawn to treating a benign tumor, including ovarian adrenal rest tumors in a subject with CAH (see copending claim 4), and not menstrual irregularities. However, Stikkelbroeck teaches females with CAH can have irregular menstrual cycles due to adrenal androgen excess (p. 1030, 1st para). Stikkelbroeck further teaches females with CAH can develop adrenal rest tumors in the ovaries which replace normal ovarian tissue, causing impaired menstrual cycling (p. 1034, right col., last para-p. 1035,

left col., top para). Therefore, it would have been prima facie obvious to have applied the instantly claimed method to treat menstrual irregularities in a subject with CAH and ovarian adrenal rest tumors, and have had a reasonable expectation of success.

Additionally, the amount of CRF1 antagonist administered is the same (10-400 mg/day as recited by instant claim 8 and copending claim 11); wherein the CRF1 antagonist is formulated as a capsule or tablet (see instant claim 18 & copending claim 21); wherein the treated subject is in a fed state (copending claims 18-19 and instant claims 14-15); and wherein the CRF1 antagonist is administered once per day (copending claim 16 & instant claim 13); and the CRF1 antagonist is administered twice per day (copending claim 17 & instant claim 14). Both sets of claims explicitly recite the same CRF1 antagonists (see copending claim 1 & instant claim 1); treatment of an adolescent (see copending claim 9 & instant claim 6); and treatment of a subject at least 18 years of age (copending claim 10 & instant claim 7). For these reasons the instant and copending claims are obvious variants of each other and are not patentably distinct.

15. Claims 1-3, 5-13, and 17-21 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-19 of copending application 19043303 in view of Stikkelbroeck et. al., Fertility & Sterility, vol. 80(4), pp. 1030-1036, publ. 2003. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims encompass administering a CRF1 antagonist to a subject having CAH (see copending claims 1 & 5). The instant claims similarly recite administering small molecule CRF1 antagonists to treat a subject having CAH (see instant claims 1 and 5). The instant and

copending claims differ from each other as the copending claims are drawn to treating ovarian adrenal rest tumors in a subject with CAH (see copending claims 1 & 5), and not menstrual irregularities. However, Stikkelbroeck teaches females with CAH can have irregular menstrual cycles due to adrenal androgen excess (p. 1030, 1st para). Stikkelbroeck further teaches females with CAH can develop adrenal rest tumors in the ovaries which replace normal ovarian tissue, causing impaired menstrual cycling (p. 1034, right col., last para-p. 1035, left col., top para). Therefore, it would have been prima facie obvious to have applied the instantly claimed method to treat menstrual irregularities in a subject with CAH and ovarian adrenal rest tumors, and have had a reasonable expectation of success.

Additionally, the amount of CRF1 antagonist administered is the same (10-400 mg/day as recited by instant claim 8 and copending claim 8); wherein the CRF1 antagonist is formulated as a capsule or tablet (see instant claim 18 & copending claim 16); wherein the CRF1 antagonist is administered once per day (copending claim 13 & instant claim 13); and the CRF1 antagonist is administered twice per day (copending claim 14 & instant claim 14). Both sets of claims explicitly recite the same CRF1 antagonists (see copending claim 1 & instant claim 1); treatment of an adolescent (see copending claim 9 & instant claim 6); and treatment of a subject at least 18 years of age (copending claim 10 & instant claim 7). As CAH affects females, including women and adolescent females (see Stikkelbroeck, p. 1030, right col., last para-p. 1031, left col., top para), it would have been prima facie obvious to have applied the copending method to treat an adolescent female as recited by instant claim 6, and a female at least 18 years or older as recited by instant claim 7. For these reasons the instant and copending claims are obvious variants of each other and are not patentably distinct.

Information Disclosure Statements

16. The IDS filed on 2/5/25 have been considered.

Conclusion

17. Claims 1-21 are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SARAH PIHONAK whose telephone number is (571)270-7710. The examiner can normally be reached Monday-Friday 9:00-5:30 EST.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Kortney Klinkel can be reached on 571-270-5239. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: https://patentcenter.uspto.gov. Visit https://www.uspto.gov/patents/apply/patent-center for more information about Patent Center and https://www.uspto.gov/patents/docx for

information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

SARAH . PIHONAK
Primary Examiner
Art Unit 1627

/SARAH PIHONAK/
Primary Examiner, Art Unit 1627

Notice of References Cited	Application/Control No. 19/043,264	Applicant(s)/Patent Under Reexamination HOWERTON et al.	
	Examiner SARAH PIHONAK	Art Unit 1627	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date YYYY-MM-DD	Name	CPC Classification	US Classification
	A					
	B					
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date YYYY-MM-DD	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title, Date, Publisher, Edition or Volume, Pertinent Pages
	U	Stikkelbroeck et. al., Fertility & Sterility, vol. 80(4), pp. 1030-1036, publ. 2003 (Year: 2003)
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in YYYY-MM-DD format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	19/043,264	HOWERTON et al.
	Examiner	Art Unit
	SARAH PIHONAK	1627

CPC - Searched*		
Symbol	Date	Examiner
a61k31/519; a61p5/06,08	04/21/2025	s.p.

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
a61k31/519; a61p5/06,08	04/21/2025	s.p.
inventor & applicant search in PE2E, patent data portal	04/21/2025	s.p.
invention & claims search in stn, PE2E	04/21/2025	s.p.

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

/SARAH PIHONAK/ Primary Examiner, Art Unit 1627	

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

BIB DATA SHEET

CONFIRMATION NO. 6961

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
19/043,264	01/31/2025 RULE	514	1627	50535-710.306

APPLICANTS
Spruce Biosciences, Inc., South San Francisco, CA;

INVENTORS
Alexis HOWERTON, San Francisco, CA;
Hal GERBER, San Francisco, CA;

**** CONTINUING DATA ***************************
This application is a CON of 18/941,832 11/08/2024
which is a CON of 17/667,285 02/08/2022 ABN
which is a CON of 17/081,694 10/27/2020 PAT 11304950
which is a CON of PCT/US2019/029486 04/26/2019
which claims benefit of 62/822,815 03/23/2019
and claims benefit of 62/663,951 04/27/2018

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****
02/21/2025

Foreign Priority claimed	35 USC 119(a-d) conditions met	Verified and Acknowledged	Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
☐ Yes ☑ No	☐ Yes ☑ No	/SARAH PIHONAK/ Examiner's Signature	☐ SP Initials	CA	14	21	1

ADDRESS
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050
UNITED STATES

TITLE
METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

FILING FEE RECEIVED		
0.00	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 9

Complete if Known	
Application Number	18/941,832
Filing Date	11-08-2024
First Named Inventor	HOWERTON; Alexis
Art Unit	
Examiner Name	Not yet assigned
Attorney Docket Number	50535-710.303

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	001	US-4328245	05-04-1982	YU; Cheng-Der et al.	
	002	US-4409239	10-11-1983	YU; Cheng-Der	
	003	US-4410545	10-18-1983	YU; Cheng-Der et al.	
	004	US-4861760	08-29-1989	MAZUEL; Claude et al.	
	005	US-4911920	03-27-1990	JANI; Rajni et al.	
	006	US-5212162	05-18-1993	MISSEL; Paul J. T. et al.	
	007	US-5403841	04-04-1995	LANG; John C. et al.	
	008	US-10849908	12-01-2020	HOWERTON; Alexis et al.	
	009	US-11007201	05-18-2021	HOWERTON; Alexis et al.	
	010	US-11304950	04-19-2022	HOWERTON et al.	
	011	US-11311549	04-26-2022	HOWERTON; Alexis et al.	
	012	US-11344557	05-31-2022	HOWERTON; Alexis et al.	
	013	US-11351177	06-07-2022	HOWERTON; Alexis et al.	
	014	US-12115166	10-15-2024	HOWERTON; Alexis et al.	
	015	US-20100196389	08-05-2010	EVANS-FREKE; Stephen	
	016	US-20100222339	09-02-2010	CHEN; Zhaogen et al.	
	017	US-20170020877	01-26-2017	GRIGORIADIS; Dimitri E.	
	018	US-20220143037	05-12-2022	HOWERTON; Alexis et al.	
	019	US-20220211711	07-07-2022	HOWERTON; Alexis et al.	

Examiner Signature	/SARAH PIHONAK/	Date Considered	04/21/2025

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	18/941,832
				Filing Date	11-08-2024
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not yet assigned
Sheet	2	of	9	Attorney Docket Number	50535-710.303

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[2]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	001	CN-101516887-A	08-26-2009	LILLY CO ELI [US]	Abstract:	☒
	002	CN-106102740-A	11-09-2016	NEUROCRINE BIOSCIENCES INC.	Abstract:	☒
	003	JP-2010504344-A	02-12-2010	ELI LILLY AND CO.	Machine Translation:	☒
	004	JP-2017503030-A	01-26-2017	NEUROCRINE BIOSCIENCES, INC.	Machine Translation:	☒
	005	JP-H0971528-A	03-18-1997	HAYASHIBARA BIOCHEM LAB [JP]	Abstract:	☒
	006	WO-0040529-A1	07-13-2000	ANGIOGENE PHARM LTD [GB], et al.		☐
	007	WO-0041669-A2	07-20-2000	ANGIOGENE PHARM LTD [GB], et al.		☐
	008	WO-0192224-A1	12-06-2001	ASTRAZENECA AB [SE], et al.		☐
	009	WO-0204434-A1	01-17-2002	ANGIOGENE PHARM LTD [GB], et al.		☐
	010	WO-0208213-A1	01-31-2002	ANGIOGENE PHARM LTD [GB], et al.		☐

Examiner Signature	/SARAH PIHONAK/	Date Considered	04/21/2025

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	18/941,832
				Filing Date	11-08-2024
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not yet assigned
Sheet	3	of	9	Attorney Docket Number	50535-710.303

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	011	WO-2007109853-A1	10-04-2007	VELACOR THERAPEUTICS PTY LTD [AU], et al.		☐
	012	WO-2008036579-A1	03-27-2008	LILLY CO ELI [US], et al.		☐
	013	WO-2015112642-A1	07-30-2015	NEUROCRINE BIOSCIENCES INC [US]		☐
	014	WO-2019036472-A1	02-21-2019	SPRUCE BIOSCIENCES INC [US]		☐
	015	WO-2019036503-A1	02-21-2019	SPRUCE BIOSCIENCES INC [US]		☐
	016	WO-2019210266-A1	10-31-2019	SPRUCE BIOSCIENCES INC [US]		☐
	017	WO-9722596-A1	06-26-1997	ZENECA LTD [GB], et al.		☐
	018	WO-9730035-A1	08-21-1997	ZENECA LTD [GB], et al.		☐
	019	WO-9732856-A1	09-12-1997	ZENECA LTD [GB], et al.		☐
	020	WO-9813354-A1	04-02-1998	ZENECA LTD [GB], et al.		☐
	021	WO-9902166-A1	01-21-1999	ANGIOGENE PHARM LTD [GB], et al.		☐

Examiner Signature	/SARAH PIHONAK/	Date Considered	04/21/2025

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 4 of 9

Complete if Known	
Application Number	18/941,832
Filing Date	11-08-2024
First Named Inventor	HOWERTON; Alexis
Art Unit	
Examiner Name	Not yet assigned
Attorney Docket Number	50535-710.303

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	001	AL-MUHAMMED, J. et al. In-vivo studies on dexamethasone sodium phosphate liposomes. Journal of microencapsulation 13(3):293-306 (1996).	☐
	002	BERGE, Stephen M. et al. Pharmaceutical Salts. Journal of Pharmaceutical Sciences 66(1):1-19 (1977).	☐
	003	CHEN, Hua-dong. et al. Ovarian adrenal rest tumors undetected by imaging studies and identified at surgery in three females with congenital adrenal hyperplasia unresponsive to increased hormone therapy dosage. Endocrine pathology 28(2):146-151 (2017).	☐
	004	CHONN, Archadio et al. Recent Advances in Liposomal Drug-delivery Systems. Current Opinion in Biotechnology 6(6):698-708 (1995).	☐
	005	Co-pending U.S. Application No. 19/043,264, inventors HOWERTON; Alexis et al., filed on January 31, 2025.	☐
	006	Co-pending U.S. Application No. 19/043,275, inventors HOWERTON; Alexis et al., filed on January 31, 2025.	☐
	007	Co-pending U.S. Application No. 19/043,303, inventors HOWERTON; Alexis et al., filed on January 31, 2025.	☐
	008	EYLES et al. Oral delivery and fate of poly(lactic acid) microsphere-encapsulated interferon in rats. J Pharm Pharmacol. 49(7):669-74 (1997).	☐
	009	GAO, Zhi-Hui. et al. Controlled Release of a Contraceptive Steroid From Biodegradable and Injectable Gel Formulations: in Vitro Evaluation. Pharmaceutical research 12(6):857-863 (1995).	☐
	010	JIN et al. Testicular adrenal rest tumors in a patient with untreated congenital adrenal hyperplasia. Korean J Pediatr 2011;54(3):137-140.	☐

Examiner Signature	/SARAH PIHONAK/	Date Considered	04/21/2025

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	18/941,832
				Filing Date	11-08-2024
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not yet assigned
Sheet	5	of	9	Attorney Docket Number	50535-710.303

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	011	KAPRARA, et al. The corticotropin releasing factor system in cancer: expression and pathophysiological implications. Cellular and molecular life sciences 67(8):1293-1306 (2010).	☐
	012	MENDES-DOS-SANTOS et al. Prevalence of Testicular Adrenal Rest Tumor and Factors Associated with Its Development in Congenital Adrenal Hyperplasia. Hormone Res. In Paed. 90:161-168 (2018).	☐
	013	MUNSON, Peter et al. Ligand: a Versatile Computerized Approach for Characterization of Ligand-binding Systems. Analytical Biochemistry 107(1):220-239 (1980).	☐
	014	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Decision Granting Institution of Post-Grant Review of U.S. Patent No. 11,007,201 dated December 1, 2023 (pp. 1-38) (PGR2022-00025).	☐
	015	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Final Written Decision of Post-Grant Review of U.S. Patent No. 10,849,908 dated November 26, 2024 (pp. 1-62) (PGR2021-00088).	☐
	016	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Final Written Decision of Post-Grant Review of U.S. Patent No. 11,007,201 dated November 27, 2024 (pp. 1-60) (PGR2022-00025).	☐
	017	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Petition for Post Grant Review of U.S. Patent No. 10,849,908 dated May 28, 2021 (pp. 1-90) (PGR2021-00088).	☐
	018	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Petition for Post Grant Review of U.S. Patent No. 11,007,201 dated February 18, 2022 (pp 1-92) (PGR2022-00025).	☐

Examiner Signature	/SARAH PIHONAK/	Date Considered	04/21/2025

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	18/941,832
				Filing Date	11-08-2024
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not yet assigned
Sheet	6	of	9	Attorney Docket Number	50535-710.303

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	019	OSTRO, Mark J. et al. Use of Liposomes as Injectable-drug Delivery Systems. American Journal of Hospital Pharmacy 46(8):1576-1587 (1989).	☐
	020	PCT/US2019/029486 International Invitation to Pay Additional Fees dated June 4, 2019.	☐
	021	PCT/US2019/029486 International Search Report and Written Opinion dated August 12, 2019.	☐
	022	RAO. Recent developments of collagen-based materials for medical applications and drug delivery systems. J Biomater Sci Polym Ed. 7(7):623-45 (1995).	☐
	023	REICHMAN et al. Fertility in Patients with Congenital Adrenal Hyperplasia. Fertility and Sterility, Vol. 101, No. 2, 2014. 301-309.	☐
	024	REISCH et al. Testicular adrenal rest tumors develop independently of long-term disease control: a longitudinal analysis of 50 adult men with congenital adrenal hyperplasia due to classic 21-hydroxylase deficiency. J. Clin. Endocrinol. Metab. 98(11):E1820-E1826 (2013).	☐
	025	SMEETS et. al. Molecular characterization of testicular adrenal rest tumors in congenital adrenal hyperplasia: lesions with both adrenocortical and Leydig cell features. JCEM 100(3):E524-E530 (2015).	☐
	026	TIOSANO et al. Ovarian adrenal rest tumor in a congenital adrenal hyperplasia patient with adrenocorticotropin hypersecretion following adrenalectomy. Hormone Res. in Paediatrics 74:223-228 (2010).	☐
	027	U.S. Serial No. 17/081,694 Office Action dated February 26, 2021.	☐
	028	U.S. Serial No. 17/081,694 Office Action dated June 11, 2021.	☐
	029	U.S. Serial No. 17/667,285 Office Action dated December 29, 2023.	☐

Examiner Signature	/SARAH PIHONAK/	Date Considered	04/21/2025

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	18/941,832
				Filing Date	11-08-2024
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not yet assigned
Sheet	7	of	9	Attorney Docket Number	50535-710.303

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	030	U.S. Serial No. 17/667,285 Office Action dated June 10, 2024.	☐
	031	ZAAROUR et al. Bilateral Ovary Adrenal Rest Tumor in a Congenital Adrenal Hyperplasia Following Adrenalectomy. Endocrinol. Pract. 20(4):e69-e74 (2014).	☐

Examiner Signature	/SARAH PIHONAK/	Date Considered	04/21/2025

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	18/941,832
				Filing Date	11-08-2024
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not yet assigned
Sheet	8	of	9	Attorney Docket Number	50535-710.303

TIMING STATEMENT

Please see 37 CFR 1.97(e) to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached timing statement under 37 CFR 1.97(e).

☒ A timing statement under 37 CFR 1.97(e) is not submitted herewith.

TIMING FEE

☐ The fee set forth in 37 CFR 1.17(p) has been submitted herewith.

COPIES

☐ An identification of an earlier application pursuant to 37 CFR 1.98(d)(1) is attached.

SIZE FEE ASSERTION

Please see 37CFR 1.17(v) and attached instructions for completing this form to make the appropriate selection of an assertion under 37 CFR 1.98. For the information disclosure statement (IDS) submitted herewith, the applicant or patent owner certifies the following with respect to the cumulative number of applicant-provided or patent-owner provided items of information submitted to date including those in the accompanying IDS (select only one):

☐ No IDS size fee is required under 37 CFR 1.17 (v) at this time.

☒ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(1).

☐ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(2).

☐ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(3).

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 11.18. Please see CFR 1.4(d) for the form of the signature.

Signature:	**/Victoria Cheng/**	Date (YYYY-MM-DD)	**2025-02-05**
Name/Print	Victoria Cheng	Registration Number	79641

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	18/941,832
				Filing Date	11-08-2024
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not yet assigned
Sheet	9	of	9	Attorney Docket Number	50535-710.303

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	1	of	9	Attorney Docket Number	50535-710.306

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	001	US-4328245	05-04-1982	YU; Cheng-Der et al.	
	002	US-4409239	10-11-1983	YU; Cheng-Der	
	003	US-4410545	10-18-1983	YU; Cheng-Der et al.	
	004	US-4861760	08-29-1989	MAZUEL; Claude et al.	
	005	US-4911920	03-27-1990	JANI; Rajni et al.	
	006	US-5212162	05-18-1993	MISSEL; Paul J. T. et al.	
	007	US-5403841	04-04-1995	LANG; John C. et al.	
	008	US-10849908	12-01-2020	HOWERTON; Alexis et al.	
	009	US-11007201	05-18-2021	HOWERTON; Alexis et al.	
	010	US-11304950	04-19-2022	HOWERTON et al.	
	011	US-11311549	04-26-2022	HOWERTON; Alexis et al.	
	012	US-11344557	05-31-2022	HOWERTON; Alexis et al.	
	013	US-11351177	06-07-2022	HOWERTON; Alexis et al.	
	014	US-12115166	10-15-2024	HOWERTON; Alexis et al.	
	015	US-20100196389	08-05-2010	EVANS-FREKE; Stephen	
	016	US-20100222339	09-02-2010	CHEN; Zhaogen et al.	
	017	US-20170020877	01-26-2017	GRIGORIADIS; Dimitri E.	
	018	US-20220143037	05-12-2022	HOWERTON; Alexis et al.	
	019	US-20220211711	07-07-2022	HOWERTON; Alexis et al.	

Examiner Signature	/SARAH PIHONAK/	Date Considered	04/21/2025

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 9

Complete if Known	
Application Number	19/043,264
Filing Date	01-31-2025
First Named Inventor	HOWERTON; Alexis
Art Unit	
Examiner Name	Not Yet Assigned
Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[2]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	001	CN-101516887-A	08-26-2009	LILLY CO ELI [US]	Abstract:	☒
	002	CN-106102740-A	11-09-2016	NEUROCRINE BIOSCIENCES INC.	Abstract:	☒
	003	JP-2010504344-A	02-12-2010	ELI LILLY AND CO.	Machine Translation:	☒
	004	JP-2017503030-A	01-26-2017	NEUROCRINE BIOSCIENCES, INC.	Machine Translation:	☒
	005	JP-H0971528-A	03-18-1997	HAYASHIBARA BIOCHEM LAB [JP]	Abstract:	☒
	006	WO-0040529-A1	07-13-2000	ANGIOGENE PHARM LTD [GB], et al.		☐
	007	WO-0041669-A2	07-20-2000	ANGIOGENE PHARM LTD [GB], et al.		☐
	008	WO-0192224-A1	12-06-2001	ASTRAZENECA AB [SE], et al.		☐
	009	WO-0204434-A1	01-17-2002	ANGIOGENE PHARM LTD [GB], et al.		☐
	010	WO-0208213-A1	01-31-2002	ANGIOGENE PHARM LTD [GB], et al.		☐

Examiner Signature	/SARAH PIHONAK/	Date Considered	04/21/2025

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	3	of	9	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	011	WO-2007109853-A1	10-04-2007	VELACOR THERAPEUTICS PTY LTD [AU], et al.		☐
	012	WO-2008036579-A1	03-27-2008	LILLY CO ELI [US], et al.		☐
	013	WO-2015112642-A1	07-30-2015	NEUROCRINE BIOSCIENCES INC [US]		☐
	014	WO-2019036472-A1	02-21-2019	SPRUCE BIOSCIENCES INC [US]		☐
	015	WO-2019036503-A1	02-21-2019	SPRUCE BIOSCIENCES INC [US]		☐
	016	WO-2019210266-A1	10-31-2019	SPRUCE BIOSCIENCES INC [US]		☐
	017	WO-9722596-A1	06-26-1997	ZENECA LTD [GB], et al.		☐
	018	WO-9730035-A1	08-21-1997	ZENECA LTD [GB], et al.		☐
	019	WO-9732856-A1	09-12-1997	ZENECA LTD [GB], et al.		☐
	020	WO-9813354-A1	04-02-1998	ZENECA LTD [GB], et al.		☐
	021	WO-9902166-A1	01-21-1999	ANGIOGENE PHARM LTD [GB], et al.		☐

Examiner Signature	/SARAH PIHONAK/	Date Considered	04/21/2025

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	4	of	9	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	001	AL-MUHAMMED, J. et al. In-vivo studies on dexamethasone sodium phosphate liposomes. Journal of microencapsulation 13(3):293-306 (1996).	☐
	002	BERGE, Stephen M. et al. Pharmaceutical Salts. Journal of Pharmaceutical Sciences 66(1):1-19 (1977).	☐
	003	CHEN, Hua-dong. et al. Ovarian adrenal rest tumors undetected by imaging studies and identified at surgery in three females with congenital adrenal hyperplasia unresponsive to increased hormone therapy dosage. Endocrine pathology 28(2):146-151 (2017).	☐
	004	CHONN, Archadio et al. Recent Advances in Liposomal Drug-delivery Systems. Current Opinion in Biotechnology 6(6):698-708 (1995).	☐
	005	Co-pending U.S. Application No. 18/941,832, inventors HOWERTON; Alexis et al., filed on November 08, 2024.	☐
	006	Co-pending U.S. Application No. 19/043,275, inventors HOWERTON; Alexis et al., filed on January 31, 2025.	☐
	007	Co-pending U.S. Application No. 19/043,303, inventors HOWERTON; Alexis et al., filed on January 31, 2025.	☐
	008	EYLES et al. Oral delivery and fate of poly(lactic acid) microsphere-encapsulated interferon in rats. J Pharm Pharmacol. 49(7):669-74 (1997).	☐
	009	GAO, Zhi-Hui. et al. Controlled Release of a Contraceptive Steroid From Biodegradable and Injectable Gel Formulations: in Vitro Evaluation. Pharmaceutical research 12(6):857-863 (1995).	☐
	010	JIN et al. Testicular adrenal rest tumors in a patient with untreated congenital adrenal hyperplasia. Korean J Pediatr 2011;54(3):137-140.	☐

Examiner Signature	/SARAH PIHONAK/	Date Considered	04/21/2025

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO				*Complete if Known*	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT *(Use as many sheets as necessary)*				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	5	of	9	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document — Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	011	KAPRARA, et al. The corticotropin releasing factor system in cancer: expression and pathophysiological implications. Cellular and molecular life sciences 67(8):1293-1306 (2010).				☐
	012	MENDES-DOS-SANTOS et al. Prevalence of Testicular Adrenal Rest Tumor and Factors Associated with Its Development in Congenital Adrenal Hyperplasia. Hormone Res. In Paed. 90:161-168 (2018).				☐
	013	MUNSON, Peter et al. Ligand: a Versatile Computerized Approach for Characterization of Ligand-binding Systems. Analytical Biochemistry 107(1):220-239 (1980).				☐
	014	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Decision Granting Institution of Post-Grant Review of U.S. Patent No. 11,007,201 dated December 1, 2023 (pp. 1-38) (PGR2022-00025).				☐
	015	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Final Written Decision of Post-Grant Review of U.S. Patent No. 10,849,908 dated November 26, 2024 (pp. 1-62) (PGR2021-00088).				☐
	016	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Final Written Decision of Post-Grant Review of U.S. Patent No. 11,007,201 dated November 27, 2024 (pp. 1-60) (PGR2022-00025).				☐
	017	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Petition for Post Grant Review of U.S. Patent No. 10,849,908 dated May 28, 2021 (pp. 1-90) (PGR2021-00088).				☐
	018	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Petition for Post Grant Review of U.S. Patent No. 11,007,201 dated February 18, 2022 (pp 1-92) (PGR2022-00025).				☐
	019	OSTRO, Mark J. et al. Use of Liposomes as Injectable-drug Delivery Systems. American Journal of Hospital Pharmacy 46(8):1576-1587 (1989).				☐

Examiner Signature	/SARAH PIHONAK/	Date Considered	04/21/2025

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	6	of	9	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	020	PCT/US2019/029486 International Invitation to Pay Additional Fees dated June 4, 2019.				☐
	021	PCT/US2019/029486 International Search Report and Written Opinion dated August 12, 2019.				☐
	022	RAO. Recent developments of collagen-based materials for medical applications and drug delivery systems. J Biomater Sci Polym Ed. 7(7):623-45 (1995).				☐
	023	REICHMAN et al. Fertility in Patients with Congenital Adrenal Hyperplasia. Fertility and Sterility, Vol. 101, No. 2, 2014. 301-309.				☐
	024	REISCH et al. Testicular adrenal rest tumors develop independently of long-term disease control: a longitudinal analysis of 50 adult men with congenital adrenal hyperplasia due to classic 21-hydroxylase deficiency. J. Clin. Endocrinol. Metab. 98(11):E1820-E1826 (2013).				☐
	025	SMEETS et. al. Molecular characterization of testicular adrenal rest tumors in congenital adrenal hyperplasia: lesions with both adrenocortical and Leydig cell features. JCEM 100(3):E524-E530 (2015).				☐
	026	TIOSANO et al. Ovarian adrenal rest tumor in a congenital adrenal hyperplasia patient with adrenocorticotropin hypersecretion following adrenalectomy. Hormone Res. in Paediatrics 74:223-228 (2010).				☐
	027	U.S. Serial No. 17/081,694 Office Action dated February 26, 2021.				☐
	028	U.S. Serial No. 17/081,694 Office Action dated June 11, 2021.				☐
	029	U.S. Serial No. 17/667,285 Office Action dated December 29, 2023.				☐
	030	U.S. Serial No. 17/667,285 Office Action dated June 10, 2024.				☐

Examiner Signature	/SARAH PIHONAK/	Date Considered	04/21/2025

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	7	of	9	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	031	ZAAROUR et al. Bilateral Ovary Adrenal Rest Tumor in a Congenital Adrenal Hyperplasia Following Adrenalectomy. Endocrinol. Pract. 20(4):e69-e74 (2014).				☐

Examiner Signature	/SARAH PIHONAK/	Date Considered	04/21/2025

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. Applicant's unique citation designation number (optional). See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	8	of	9	Attorney Docket Number	50535-710.306

TIMING STATEMENT

Please see 37 CFR 1.97(e) to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached timing statement under 37 CFR 1.97(e).

☒ A timing statement under 37 CFR 1.97(e) is not submitted herewith.

TIMING FEE

☐ The fee set forth in 37 CFR 1.17(p) has been submitted herewith.

COPIES

☐ An identification of an earlier application pursuant to 37 CFR 1.98(d)(1) is attached.

SIZE FEE ASSERTION

Please see 37CFR 1.17(v) and attached instructions for completing this form to make the appropriate selection of an assertion under 37 CFR 1.98. For the information disclosure statement (IDS) submitted herewith, the applicant or patent owner certifies the following with respect to the cumulative number of applicant-provided or patent-owner provided items of information submitted to date including those in the accompanying IDS (select only one):

☐ No IDS size fee is required under 37 CFR 1.17 (v) at this time.

☒ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(1).

☐ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(2).

☐ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(3).

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 11.18. Please see CFR 1.4(d) for the form of the signature.

Signature:	**/Victoria Cheng/**	Date (YYYY-MM-DD)	**2025-02-05**
Name/Print	Victoria Cheng	Registration Number	79641

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	
				Examiner Name	Not Yet Assigned
Sheet	9	of	9	Attorney Docket Number	50535-710.306

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/

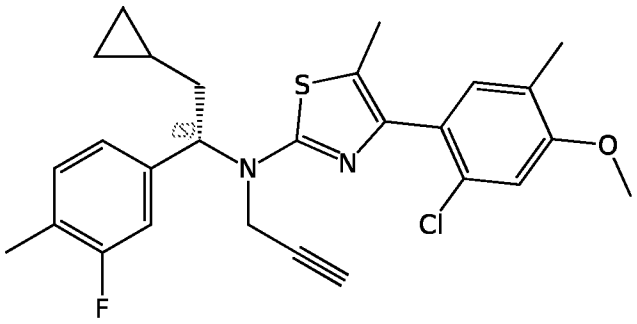
Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.P/



Absolute stereochemistry shown
Rotation (-)

```
OS.CITING REF COUNT:      34    THERE ARE 34 CAPLUS RECORDS THAT CITE THIS
                                RECORD (34 CITINGS)
REFERENCE COUNT:          55    THERE ARE 55 CITED REFERENCES AVAILABLE FOR THIS
                                RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT


=> d his

     (FILE 'HOME' ENTERED AT 10:42:00 ON 21 APR 2025)

     FILE 'CAPLUS' ENTERED AT 10:42:21 ON 21 APR 2025
                E "MENSTRUAL DISORDERS"/CT
                SET EXPAND CONTINUOUS
                E E3+ALL/CT
L1         33351 S E35-E48,E52-E53

     FILE 'REGISTRY' ENTERED AT 10:43:11 ON 21 APR 2025
L2                STRUCTURE UPLOADED
L3              1 S L2 SSS SAM
L4              5 S L2 SSS FULL
L5                STRUCTURE UPLOADED
L6              1 S L5 SSS SAM
L7              8 S L5 SSS FULL

     FILE 'CAPLUS' ENTERED AT 10:50:57 ON 21 APR 2025
L8             67 S L4 OR L7
L9              7 S L1 AND L8
L10             3 S L8 AND (MENSTRUAL OR MENSTRUATION? OR DYSMENORRHEA? OR AMENOR
L11             7 S L9 OR L10
L12             1 S L11 AND (PRY<=2019 OR PY<=2019 OR AY<=2019)

     FILE 'CAPLUS' ENTERED AT 12:17:57 ON 21 APR 2025
                E "CORTICOTROPIN RELEASING FACTOR RECEPTOR ANTAGONISTS"/CT
                E E57+ALL/CT
L13            75 S E72-E78
L14             2 S L1 AND L13
L15             1 S L13 AND (MENSTRUATION OR MENSTRUAL? OR DYSMENORRHEA OR AMENOR
L16             0 S L15 NOT L14
                E E63+ALL/CT
L17          2184 S E81
L18            17 S L17 AND L1
L19             6 S L18 AND (PRY<=2019 OR PY<=2019 OR AY<=2019)
L20            12 S L17 AND (MENSTRUATION OR MENSTRUAL? OR DYSMENORRHEA OR AMENOR
L21             8 S L20 AND (PRY<=2019 OR PY<=2019 OR AY<=2019)
L22             2 S L21 NOT L19

     FILE 'CAPLUS' ENTERED AT 13:01:31 ON 21 APR 2025
L23            37 S L8 AND L17
L24            30 S L23 AND (PRY<=2019 OR PY<=2019 OR AY<=2019)

=>
```

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L1	652	(corticotropin adj8 factor adj8 antagon*) AND (menstruation OR menstrual)	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2025/04/18 05:36 PM
L2	200	((corticotropin adj8 factor adj8 antagon*) OR (CRF1 adj8 antagon*)).ab,ti,clm. AND L1	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2025/04/18 05:37 PM
L3	276	(corticotropin adj8 factor adj8 antagon*) AND (dysmenorrhea)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2025/04/18 05:42 PM
L4	42	((corticotropin adj8 factor adj8 antagon*) OR (CRF1 adj8	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY,	OR	ON	ON	2025/04/18 05:42 PM

		antagon*)).ab,ti,clm. AND L3	CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L5	192	(((@pd OR @ptad OR @prad OR @ad OR @rlad)< ("20190426")) AND (L2 OR L4)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2025/04/18 05:53 PM
L6	31	((("Spruce") near3 ("Biosciences") near3 ("Inc"))).AS,AANM.	(US-PGPUB; USPAT)	OR	ON	ON	2025/04/18 06:11 PM
L7	21	((("HOWERTON") near3 ("Alexis"))).INV.	(US-PGPUB; USPAT; USOCR)	OR	ON	ON	2025/04/18 06:11 PM
L8	21	((("GERBER") near3 ("Hal"))).INV.	(US-PGPUB; USPAT; USOCR)	OR	ON	ON	2025/04/18 06:11 PM
L9	23	(L2 OR L4) AND (L6 OR L7 OR L8)	(US-PGPUB; USPAT; USOCR)	OR	ON	ON	2025/04/18 06:12 PM
L10	6002	(a61k31/519 OR a61p5/06,08).cpc. AND (menstrual OR menstruation OR dysmenorrhea OR dysmennorhea OR dysmennorrhea)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2025/04/18 06:14 PM
L11	140	(corticotropin adj8 factor	(US-PGPUB; USPAT;	OR	ON	ON	2025/04/18

		adj8 antagon*) AND L10	USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				06:14 PM
L12	96	((@pd OR @ptad OR @prad OR @ad OR @rlad)< ("20190426")) AND L11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2025/04/18 06:15 PM
L13	19	("10849908" OR "11007201" OR "11304950" OR "11311549" OR "11344557" OR "11351177" OR "12115166" OR "20100196389" OR "20100222339" OR "20170020877" OR "20220143037" OR "20220211711" OR "4328245" OR "4409239" OR "4410545" OR "4861760" OR "4911920" OR "5212162" OR "5403841").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2025/04/21 09:32 AM
L14	940	(a61k31/519 OR a61p5/06,08).cpc. AND (corticotropin adj8 releas* adj8 factor adj8 antagon*)	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS,	OR	ON	ON	2025/04/21 10:26 AM

			IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L15	877	(((@pd OR @ptad OR @prad OR @ad OR @rlad)< ("20190426")) AND L14	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2025/04/21 10:26 AM
L16	286	L15 AND (corticotropin adj8 releas* adj8 factor adj8 antagon*).ab,ti,clm.	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2025/04/21 10:27 AM
L17	484	(corticotropin adj8 factor adj8 antagon*) AND (amenorrhea)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2025/04/21 12:48 PM

L18	489	(corticotropin adj8 factor adj8 antagon*) AND (amenorrhea OR menorrhagia)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2025/04/21 12:48 PM
L19	423	(((@pd OR @ptad OR @prad OR @ad OR @rlad)< ("20190426")) AND L18	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2025/04/21 12:49 PM
L20	31	"1200419".pn.	(FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2025/04/21 01:07 PM
L21	1	"6586456".pn.	(USPAT)	OR	ON	ON	2025/04/21 01:08 PM
L22	272	(congenital adj6 adrenal adj6 hyperplasia) WITH (menorrhagia OR dysmenorrhea OR menstrual OR menstruation)	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV,	OR	ON	ON	2025/04/21 03:18 PM

			MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L23	216	(((@pd OR @ptad OR @prad OR @ad OR @rlad)< ("20190426")) AND L22	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2025/04/21 03:18 PM
L24	7	"2015112642".pn.	(FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2025/04/21 04:20 PM
L25	3	L24 AND (time OR frequency)	(FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2025/04/21 04:21 PM
L26	2	L24 AND (one OR once OR twice)	(FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP,	OR	ON	ON	2025/04/21 04:21 PM

			ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)				
L27	1	L24 AND (day OR daily)	(FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2025/04/21 04:21 PM
L28	1	"20100222339".pn.	(US-PGPUB)	OR	ON	ON	2025/04/21 04:48 PM
L29	1	L28 AND adrenal	(US-PGPUB)	OR	ON	ON	2025/04/21 04:48 PM
L30	0	L28 AND adrenal AND (tablet* OR capsule*)	(US-PGPUB)	OR	ON	ON	2025/04/21 04:48 PM
L31	1	"12098152".pn.	(USPAT)	OR	ON	ON	2025/04/21 05:46 PM

PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
19/043,264	01/31/2025	Alexis HOWERTON	50535-710.306

CONFIRMATION NO. 6961

21971
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

PUBLICATION NOTICE

OC10000008973276 9

Date Mailed: 06/05/2025

Title:METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS

Publication No.US-2025-0177403-A1
Publication Date:06/05/2025

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be viewed using the USPTO's publicly available Searchable Databases via the Patent Public Search tool at www.uspto.gov. The direct link to access the Patent Public Search tool is currently https://ppubs.uspto.gov/pubwebapp/static/pages/ppubsbasic.html.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Public Records Division. The Public Records Division can be reached by telephone at (571) 272-3150 or (800) 972-6382, by facsimile at (571) 273-3250, by mail addressed to the United States Patent and Trademark Office, Public Records Division, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through Patent Center, the USPTO's electronic patent application filing and management system. The direct link to access this status information is currently https://patentcenter.uspto.gov/. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of Patent Center.

Further assistance in electronically accessing the publication, or about Patent Center, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor:	HOWERTON; Alexis et al.	Group Art Unit:	1627
Serial Number:	19/043,264	Examiner:	PIHONAK, SARAH
Filing or 371 (c) Date:	2025-01-31	**CONFIRMATION NO:**	**6961**
Title:	METHODS FOR TREATING TESTICULAR AND OVARIAN ADRENAL REST TUMORS		

FILED ELECTRONICALLY ON: July 24, 2025

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT UNDER 37 CFR § 1.97

Commissioner for Patents:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP § 609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in § 1.56.

A. ☐ *37 CFR § 1.97 (b).* This Information Disclosure Statement should be considered by the Office because:

☐ (1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under § 1.53 (d);

-- OR --

☐ (2) It is being filed within 3 months of entry of the national stage as set forth in § 1.491 in an international application;

-- OR --

☐ (3) It is being filed before the mailing of a first Office action on the merits;

-- OR --

☐ (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under § 1.114.

B. ☒ *37 CFR § 1.97(c).* Although this Information Disclosure Statement is being filed after the period specified in *37 CFR § 1.97(b),* above, it is filed before the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, this Information Disclosure Statement should be considered because it is accompanied by one of:

☐ a statement as specified in §1.97 (e) provided concurrently herewith;

-- OR --

☒ a fee of [$280.00/112.00/56.00] as set forth in § 1.17 (p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.

C. ☐ *37 CFR § 1.97 (d).* Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, it is being filed before payment of the issue fee and should be considered because it is accompanied by:

i. a statement as specified in § 1.97 (e);

-- AND --

ii. a fee of [$280.00/112.00/56.00] as set forth in §1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.

D. ☐ *37 CFR §1.97 (e).* Statement.

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (c);

-- AND/OR --

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (d);

-- AND/OR --

☐ A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under 37 C.F.R. § 1.97(e) (1) as provided for under MPEP 609.04(b) V.

E. ☐ *Statement Under 37 C.F.R. §1.704(d).* Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office or is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay. The Patent Term Adjustment Statement Under 37 CFR 1.704(d) on Form PTO/SB/133 is filed herewith.

F. ☒ *37 CFR §1.98 (a) (2).* The content of the Information Disclosure Statement is as follows:

☐ Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.

-- OR --

☒ Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 are not enclosed.

-- AND/OR --

☒ Copies of Foreign Patent Documents and/or Non-Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).

-- AND/OR --

☐ Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98 (a) (2) (iii).

G. ☒ *37 CFR §1.98(a)(3).* The Information Disclosure Statement includes non-English patents and/or references.

☐ Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.

☐ Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.

-- OR --

☐ A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____

☒ Pursuant to 37 CFR §1.98(a) (3) (ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.

H. ☐ *37 CFR §1.98(d).* Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:

☐ Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.

Application in which the information was submitted: _____

Information Disclosure Statement(s) filed on: _____

AND

☐ The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

I. ☒ *Fee Authorization*. The Commissioner is hereby authorized to charge the above-referenced fees of $280.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 23-2415 (Docket No. 50535-710.306).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI

Dated: July 24, 2025

By: /Victoria Cheng/
Victoria Cheng, Reg. No. 79641

650 Page Mill Road
Palo Alto, CA 94304-1050
(650) 493-9300
Customer No. 21971

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	1	of	16	Attorney Docket Number	50535-710.306

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	001	US-8030304	10-04-2011	CHEN et al.	
	002	US-8563718	10-22-2013	RIZZO; John Robert et al.	
	003	US-11708372	07-25-2023	REDDY; Dasharatha et al.	
	004	US-11858932	01-02-2024	BARNES; Christopher et al.	
	005	US-12098152	09-24-2024	BARNES; Christopher et al.	
	006	US-20020013357	01-31-2002	NADKARNI; Sreekant et al.	
	007	US-20030008885	01-09-2003	HE; Liqi et al.	
	008	US-20050209250	09-22-2005	ROMANO; Steve	
	009	US-20060078623	04-13-2006	DHOOT; Nikhil et al.	
	010	US-20090076266	03-19-2009	DAUGULIS; Olafs et al.	
	011	US-20100022560	01-28-2010	CHEN; Zhaogen et al.	
	012	US-20100155595	06-24-2010	GHOSHAL; Amit [Us] et al.	
	013	US-20110136865	06-09-2011	BUNTINX; Erik	
	014	US-20110160168	06-30-2011	DHINGRA; Om	
	015	US-20110166345	07-07-2011	RIZZO; John Robert et al.	
	016	US-20130045979	02-21-2013	SANFILIPPO; Louis	
	017	US-20170333126	11-23-2017	SOBOTKA; Paul	

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered and whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.[4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

PTO/SB/08 (01-25)
Approved for use through 11/30/2027. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	2	of	16	Attorney Docket Number	50535-710.306

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	018	US-20180110554	04-26-2018	ZARINS; Denise Marie et al.	
	019	US-20190262349	08-29-2019	HOWERTON; Alexis et al.	
	020	US-20200255436	08-13-2020	HOWERTON; Alexis et al.	
	021	US-20210015827	01-21-2021	HOWERTON; Alexis et al.	
	022	US-20210038604	02-11-2021	HOWERTON; Alexis et al.	
	023	US-20210137935	05-13-2021	HOWERTON; Alexis et al.	
	024	US-20210322430	10-21-2021	HOWERTON; Alexis et al.	
	025	US-20210361664	11-25-2021	HOWERTON; Alexis et al.	
	026	US-20220133742	05-05-2022	GHOSH; Sangita et al.	
	027	US-20220133742	05-05-2022	GHOSH; Sangita et al.	
	028	US-20220143037	05-12-2022	HOWERTON; Alexis et al.	
	029	US-20230159533	05-25-2023	REDDY; Dasharatha et al.	
	030	US-20230321112	10-12-2023	HOWERTON; Alexis et al.	
	031	US-20230414627	12-28-2023	HOWERTON; Alexis et al.	
	032	US-20240043432	02-08-2024	REDDY; Dasharatha et al.	
	033	US-20240261300	08-08-2024	GHOSH; Sangita et al.	

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered and whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.[4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	3	of	16	Attorney Docket Number	50535-710.306

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	034	US-20240293421	09-05-2024	REDDY; Dasharatha et al.	
	035	US-20250064812	02-27-2025	HOWERTON; Alexis et al.	
	036	US-20250177404	06-05-2025	HOWERTON; Alexis et al.	
	037	US-20250195527	06-19-2025	HOWERTON; Alexis et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	001	CN-101516835-A	08-26-2009	OSURF OHIO STATE UNIVERSITY RE [US]	Abstract:	☒
	002	EP-2094709-B1	09-15-2010	LILLY CO ELI [US]		☐
	003	EP-3984523-A1	04-20-2022	NEUROCRINE BIOSCIENCES INC [US], et al.		☐
	004	JP-2000302693-A	10-31-2000	PFIZER PROD INC.	Abstract:	☒
	005	KR-19990067391-A	08-16-1999	JANSSEN PHARMACEUTICA NV [BE], et al.	Machine Translation:	☒

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered and whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.[4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	4	of	16	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	006	WO-0059908-A2	10-12-2000	DU PONT PHARM CO [US]		☐
	007	WO-0123388-A2	04-05-2001	NEUROGEN CORP [US], et al.		☐
	008	WO-02072202-A1	09-19-2002	BRISTOL MYERS SQUIBB PHARMA CO [US]		☐
	009	WO-2005020910-A2	03-10-2005	PHARMACIA CORP [US], et al.		☐
	010	WO-2005063755-A1	07-14-2005	SB PHARMCO INC [US], et al.		☐
	011	WO-2005079868-A2	09-01-2005	SB PHARMCO INC [US], et al.		☐
	012	WO-2006102194-A1	09-28-2006	LILLY CO ELI [US], et al.		☐
	013	WO-2010039678-A1	04-08-2010	LILLY CO ELI [US], et al.		☐
	014	WO-2013160317-A2	10-31-2013	HOLSBOERMASCHMEYER NEUROCHEMIE GMBH [DE]		☐
	015	WO-2020115555-A2	06-11-2020	NEUROCRINE BIOSCIENCES INC [US], et al.		☐

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered and whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.[4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

PTO/SB/08 (01-25)
Approved for use through 11/30/2027. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	5	of	16	Attorney Docket Number	50535-710.306

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	016	WO-2021016208-A1	01-28-2021	SPRUCE BIOSCIENCES INC [US]		☐
	017	WO-2022036123-A1	02-17-2022	SPRUCE BIOSCIENCES INC [US]		☐
	018	WO-2023091684-A1	05-25-2023	SPRUCE BIOSCIENCES INC [US]		☐
	019	WO-9413676-A1	06-23-1994	PFIZER [US], et al.		☐
	020	WO-9729109-A1	08-14-1997	JANSSEN PHARMACEUTICA NV [BE], et al.		☐
	021	WO-9803510-A1	01-29-1998	DU PONT MERCK PHARMA [US]		☐
	022	WO-9808847-A1	03-05-1998	PFIZER [US], et al.		☐

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered and whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.[4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	6	of	16	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	001	ABCAM CP 154526 Hydrochloride, Non-Peptide CRF1 Receptor Antagonist AB141429. [Internet] ABCAM. Retrieved from URL: https://www.abcam.com/products/biochemicals/cp-154526-hydrochloride-non-peptide-crf1-receptor-antagonist-ab141429.html. 2 pages.	☐
	002	BORNSTEIN et al. Diagnosis and Treatment of Primary Adrenal Insufficiency: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 101(2):364-389 (2016).	☐
	003	CHEN, C., Recent Advances in Small Molecule Antagonists of the Corticotropin-Releasing Factor Type-1 Receptor-Focus on Pharmacology and Pharmacokinetics. Current Medicinal Chemistry 13:1261-1282 (2006).	☐
	004	CHEN et al. Design of 2,5-Dimethyl-3-(6-dimethyl-4-methylpyridin-3-yl)-7-dipropyl aminopryazolo[1,5-a]pyrimidine (NBI 30775/R121919) and Structure-Activity Relationships of a Series of Potent and Orally Active Corticotropin- Releasing Factor Receptor Antagonists. J Med Chem 47(19):4787-4798 (2004).	☐
	005	DAVE. Overview of pharmaceutical excipients used in tablets and capsules. https://www.drugtopics.com/view/overview-pharmaceutical-excipients-used-tablets-and-capsules (10/24/2008) 52 pages.	☐
	006	DO, Hien-Quang et al. A General Method for Copper-Catalyzed Arylation of Arene C-H Bonds. J Am Chem Soc. 130(45):15185-15192 (2008).	☐
	007	DO, Hien-Quang et al. Copper-Catalyzed Arylation and Alkenylation of Polyfluoroarene C-H Bonds. Journal of American Chemical Society 130(4):1128-1129 (2008).	☐
	008	DO, Hien-Quang et. al. Copper-catalyzed arylation of C-H bonds; Abstracts of Papers, 236th ACS National Meeting, New Orleans, LA, Apr. 6-10, 2008, Accession No. 2008:390596.	☐

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered and whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.[4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	7	of	16	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	009	DO, Hien-Quang et al. Copper-Catalyzed Arylation of Heterocycle C-H Bonds. J Am Chem Soc. 129(41):12404-12405 (2007).	☐
	010	EL-MAOUCHE, Diala et al. Congenital adrenal hyperplasia. Lancet 390:2194-2210 (2017).	☐
	011	EP3784233 Notice Of Opposition dated March 3, 2025.	☐
	012	ESCOBAR-MORREALE, HF., Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. Nat Rev Endocrinol. 14(5):270-284 (2018).	☐
	013	FAHMY, Hesham et al. Structure and Function of Small Non-Peptide CRF Antagonists and their Potential Clinical Use. Curr Mol Pharmacol 10(4):270-281 (2017).	☐
	014	FUQUA et al. Duration of suppression of adrenal steroids after glucocorticoid administration. Int J Pediatr Endocrinol 2010:712549 (2010).	☐
	015	GEHLERT, Donald R. et al. 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-b]pyridazine: a novel brain-penetrant, orally available corticotropin-releasing factor receptor 1 antagonist with efficacy in animal models of alcoholism. The Journal of neuroscience 27(10):2718-2726 (2007).	☐
	016	GILLIGAN P. et al., The Discovery of 4-(3-Pentylamino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)-pyrazolo-[1,5-alpha]-pyrimidine: A corticotrophin-Releasing Factor (hCRF1)Antagonist. Bioorganic & Medicinal Chemistry 8(1):181-189 (2000).	☐
	017	HE, Liqi et al. 4-(1,3-Dimethoxyprop-2-ylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)pyrazolo[1,5-a]-1,3,5-triazine: A Potent, Orally Bioavailable CRF1 Receptor Antagonist. J Med Chem 43:449-456 (2000).	☐

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered and whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.[4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	8	of	16	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	018	HODGETTS et al. Discovery of N-(1-ethylpropyl)-[3-methoxy-5-(2-methoxy-4-trifluoromethoxyphenyl)-6-methyl-pyrazin-2-yl]amine 59 (NGD 98-2): an orally active corticotropin releasing factor-1 (CRF-1) receptor antagonist. J Med Chem 54:4187-4206 (2011).	☐
	019	KHADILKAR et al., Can polycystic ovarian syndrome be cured? Unfolding the concept of secondary polycystic ovarian syndrome. J Obstet Gynaecol India 69(4):297-302 (2019).	☐
	020	LEE et al. Attenuated Forms of Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency. J Clin Endocrinol Metab. 55(5):866-871 (1982).	☐
	021	MAJO et al. Facile Palladium-Catalyzed Synthesis of 3-Arylpyrazolo-[1,5-a]pyrimidines. Adv. Synth. Catal. 345(5):620-624 (2003).	☐
	022	Malvern Instruments Worldwide. A Basic Guide to Particle Characterization. Inform - White Paper (pp 1-26) (2012).	☐
	023	MARKOPOULOS, Marios C. Hyperandrogenism in women with polycystic ovary syndrome persists after menopause. Journal of Clinical Endocrinology and Metabolism 96(3):623-631 (2011).	☐
	024	MILLION, Mulugeta et al., A Novel Water-Soluble Selective CRF1 Receptor Antagonist, NBI 35965, Blunts Stress-Induced Visceral Hyperalgesia and Colonic Motor Function in Rats. Brain Research, 985(1):32-42 (2003).	☐
	025	MORABBI, Mohammad-Javad et al., Pexacerfont as a CRF1 Antagonist for the Treatment of Withdrawal Symptoms in Men With Heroin/Methamphetamine Dependence: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. International Clinical Psychopharmacology 1-9 (2017).	☐

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered and whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.[4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	9	of	16	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	026	NELLA et al. A Phase 2 Study of Continuous Subcutaneous Hydrocortisone Infusion in Adults With Congenital Adrenal Hyperplasia. J Clin Endocrinol Metab. 101(12):4690-4698 (2016).	☐
	027	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Decision Granting Institution of Post-Grant Review of U.S. Patent No. 10,849,908 dated December 1, 2023 (pp 1-57) (PGR2021-00088).	☐
	028	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Petition for Post Grant Review of U.S. Patent No. 12,115,166 dated February 10, 2025 (pp 1-59).	☐
	029	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Petitioner's Reply to Patent Owner's Response in U.S. Patent No. 10,849,908 dated June 20, 2024 (pp. 1-38) (PGR2021-00088).	☐
	030	Neurocrine Biosciences, Inc. (Petitioner) v. Spruce Biosciences, Inc. (Patent Owner). Petitioner's Reply to Patent Owner's Response in U.S. Patent No. 11,007,201 dated June 20, 2024 (pp. 1-36) (PGR2022-00025).	☐
	031	O'REILLY, Michael W. et al. 11-oxygenated C19 steroids are the predominant androgens in polycystic ovary syndrome. J Clin Endocrinol Metab. 102(3):840-848 (2017).	☐
	032	PCT/US2007/078605 International Search Report and Written Opinion dated January 31, 2008.	☐
	033	PCT/US2007/078605 Written Opinion dated March 20, 2009.	☐
	034	PCT/US2009/058722 International Search Report and Written opinion dated January 25, 2010.	☐
	035	PCT/US2009/058722 International Search Report dated April 8, 2010.	☐

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered and whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.[4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	10	of	16	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	036	PCT/US2009/058722 Written Opinion dated April 2, 2011.	☐
	037	PCT/US2018/046707 International Search Report and Written Opinion dated October 24, 2018.	☐
	038	PCT/US2018/046760 International Search Report and Written Opinion dated October 24, 2018.	☐
	039	PCT/US2020/042820 International Search Report and Written Opinion dated November 25, 2020.	☐
	040	ROSENFIELD et al., The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocrine Reviews 37(5):467-520 (2016).	☐
	041	SARAFOGLOU et al. SUN-LB064 A Phase 2, Dose-Escalation, Safety and Efficacy Study of Tildacerfont (SPR001) for the Treatment of Patients with Classic Congenital Adrenal Hyperplasia. Journal of the Endocrine Society 3(Supplement_1):SUN-LB064 (2017).	☐
	042	SARAFOGLOU, Kyriakie et al. Tildacerfont in Adults with Classic Congenital Adrenal Hyperplasia: Results from Two Phase 2 Studies. The Journal of Clinical Endocrinology and Metabolism 106(11):e4666-e4679 (2021).	☐
	043	SPEISER, Phyllis W. et al., Congenital Adrenal Hyperplasia Due to Steroid 21-Hydroxylase Deficiency: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 95(9):4133-4160 (2010).	☐
	044	SPRUCE BIOSCIENCES, INC. Study of SPR001 in Adults with Classic Congenital Adrenal Hyperplasia. 2017. Pages 1-7.	☐

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered and whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.[4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	11	of	16	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	045	SPRUCE BIOSCIENCES, INC. Study to Evaluate the Safety and Efficacy of SPR001 in Subjects with Classic Congenital Adrenal Hyperplasia. 2018. Pages 1-7.	☐
	046	THAKRAL, Naveen K. et al. Salt Disproportionation in the Solid State: Role of Solubility and Counterion Volatility. Molecular pharmaceutics 13(12):4141-4151 (2016).	☐
	047	TRAPP et al. Congenital adrenal hyperplasia: an update in children. Curr Opin Endocrinol Diabetes Obes. 18(3):166-70 (2011).	☐
	048	TURCU, Adina F. et al. Single-dose study of a corticotropin-releasing factor receptor-1 antagonist in women with 21-hydroxylase deficiency. The Journal of Clinical Endocrinology and Metabolism 101(3):1174-1180 (2016).	☐
	049	U.S. Serial No. 17/586,228 Office Action dated June 10, 2022.	☐
	050	U.S. Serial No. 13/063,226 Office Action dated January 24, 2013.	☐
	051	U.S. Serial No. 16/388,620 Office Action dated July 15, 2019.	☐
	052	U.S. Serial No. 16/388,620 Office Action dated November 25, 2019.	☐
	053	U.S. Serial No. 16/639,540 Office Action dated July 11, 2022.	☐
	054	U.S. Serial No. 16/639,540 Office Action dated March 28, 2024.	☐
	055	U.S. Serial No. 16/639,540 Office Action dated May 25, 2023.	☐

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered and whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.[4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	12	of	16	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	056	U.S. Serial No. 16/639,540 Office Action dated November 16, 2021.	☐
	057	U.S. Serial No. 16/639,540 Office Action dated September 28, 2023.	☐
	058	U.S. Serial No. 16/639,541 Office Action dated April 4, 2022.	☐
	059	U.S. Serial No. 17/063,592 Office Action dated December 7, 2021.	☐
	060	U.S. Serial No. 17/078,054 Office Action dated December 14, 2020.	☐
	061	U.S. Serial No. 17/359,411 Office Action dated January 6, 2022.	☐
	062	U.S. Serial No. 17/359,414 Office Action dated November 16, 2021.	☐
	063	U.S. Serial No. 17/586,228 Office Action dated March 30, 2022.	☐
	064	U.S. Serial No. 18/078,649 Office Action dated March 21,2024.	☐
	065	U.S. Serial No. 18/078,649 Office Action dated October 9, 2024.	☐
	066	U.S. Serial No. 18/078,649 Office Action dated September 8, 2023.	☐
	067	U.S. Serial No. 18/307,718 Office Action dated October 12, 2023.	☐
	068	U.S. Serial No. 19/043,275 Office Action dated May 30, 2025.	☐

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered and whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.[4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	13	of	16	Attorney Docket Number	50535-710.306

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	069	VARMA. Excipients used in the Formulation of Tablets. https://www.rroij.com/openaccess/excipients-used-in-the-formulationof-tablets-.php?aid=78260 Revised date: 26/07/2016.	☐
	070	WILLIAMS, John P. Corticotropin-releasing factor 1 receptor antagonists: a patent review. Expert Opin Ther Pat 23(8):1057-68 (2013).	☐
	071	ZHANG et al. D-Level Essay in Statistics, 2009, How to Analyze Change from Baseline. Available at http://www.statistics.du.se/essays/D09 Zhang%20Ling%20&%20Han%20Kun.pdf (6/10/2009).	☐
	072	ZORRILLA, Eric P. et al., Progress in Corticotropin-Releasing Factor-1 Antagonist Development. Drug Discov Today 15(9-10):371-383 (2010).	☐
	073	ZORRILLA, Eric P. et al. The therapeutic potential of CRF1 antagonists for anxiety. Expert opinion on investigational drugs 13(7):799-828 (2004).	☐

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered and whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.[4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

PTO/SB/08 (01-25)
Approved for use through 11/30/2027. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	14	of	16	Attorney Docket Number	50535-710.306

TIMING STATEMENT

Please see 37 CFR 1.97(e) to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached timing statement under 37 CFR 1.97(e).

☒ A timing statement under 37 CFR 1.97(e) is not submitted herewith.

TIMING FEE

☐ The fee set forth in 37 CFR 1.17(p) has been submitted herewith.

COPIES

☐ An identification of an earlier application pursuant to 37 CFR 1.98(d)(1) is attached.

SIZE FEE ASSERTION

Please see 37 CFR 1.17(v) and attached instructions for completing this form to make the appropriate selection of an assertion under 37 CFR 1.98. For the information disclosure statement (IDS) submitted herewith, the applicant or patent owner certifies the following with respect to the cumulative number of applicant-provided or patent-owner provided items of information submitted to date including those in the accompanying IDS (select only one):

☐ No IDS size fee is required under 37 CFR 1.17 (v) at this time.

☐ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(1).

☒ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(2).

☐ The IDS is accompanied by the IDS size fee under 37 CFR 1.17(v)(3).

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 11.18. Please see CFR 1.4(d) for the form of the signature.

Signature:	/Victoria Cheng/	Date (YYYY-MM-DD)	2025-07-24
Name/Print	Victoria Cheng	Registration Number	79641

PTO/SB/08 (01-25)
Approved for use through 11/30/2027. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** (Use as many sheets as necessary)				Complete if Known	
				Application Number	19/043,264
				Filing Date	01-31-2025
				First Named Inventor	HOWERTON; Alexis
				Art Unit	1627
				Examiner Name	PIHONAK, SARAH
Sheet	15	of	16	Attorney Docket Number	50535-710.306

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 April 2001 (05.04.2001)

PCT

(10) International Publication Number
WO 01/23388 A2

(51) International Patent Classification[7]: C07D 487/04, A61K 31/519, 31/53, A61P 9/00, 25/00, G01N 33/566 // (C07D 487/04, 239:00, 231:00) (C07D 487/04, 251:00, 231:00)

(21) International Application Number: PCT/US00/26885

(22) International Filing Date:
29 September 2000 (29.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/156,868 30 September 1999 (30.09.1999) US

(71) Applicants *(for all designated States except US)*: **NEUROGEN CORPORATION** [US/US]; 35 Northeast Industrial Road, Branford, CT 06405 (US). **PFIZER INC.** [US/US]; 235 East 42nd Street, New York, NY 10017 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **DARROW, James, W.** [US/US]; 4 Dinatale Drive, Wallingford, CT 06492 (US). **DE LOMBAERT, Stephane** [US/US]; 37 Concord Drive, Madison, CT 06443 (US). **BLUM, Charles** [US/US]; 785 West Pond Meadow Rd., Westbrook, CT 06498 (US). **TRAN, Jennifer** [US/US]; 56 Barker Hill drive, Guilford, CT 06437 (US). **GIANGIORDANO, Mark** [US/US]; Building 2-14, 45 Jefferson Road, Branford, CT 06405 (US). **GRIFFITH, David, Andrew** [US/US]; 10 Barley Hill Road, Old Saybrook, CT 06475 (US). **CARPINO, Philip, Albert** [US/US]; 50 Meridian St., Groton, CT 06340 (US).

(74) Agents: **RICHARDS, John**; Ladas & Parry, 26 West 61st Sreet, New York, NY 10023 et al. (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, 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), 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.*

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 01/23388 A2

(54) Title: AMINO SUBSTITUTED PYRAZOLO[1,5,-a]-1,5-PYRIMIDINES AND PYRAZOLO[1,5-a]-1,3,5-TRIAZINES

(57) Abstract: Disclosed are compounds of the formula: (I), where R^1, R^2, R^3, R^4, R^5, R^6, and X are defined herein. These compounds are selective modulators of NPY1 receptors. These compounds are useful in the treatment of a number of CNS disorders, metabolic disorders, and peripheral disorders, particularly eating disorders and hypertension. Methods of treatment of such disorders as well as packaged pharmaceutical compositions are also provided. Compounds of the invention are also useful as probes for the localization of NPY1 receptors and as standards in assays for NPY1 receptor binding. Methods of using the compounds in receptor localization studies are given.

APPLICATION FOR UNITED STATES LETTERS PATENT IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

(Case No. U-012956-1)

(FORMERLY PROVISIONAL U012425-2)

Title: Amino Substituted Pyrazolo[1,5,-a]-1,5-Pyrimidines and Pyrazolo [1,5-a]-1,3,5-Triazines

Amino Substituted Pyrazolo[1,5,-a]-1,5-Pyrimidines and Pyrazolo[1,5-a]-1,3,5-Triazines

Related Application

This application claims priority from provisional application 60/156868 filed on September 30, 1999, which is incorporated herein by reference.

Field of the Invention

This invention relates to certain amino substituted pyrazolo[1,5,-a]-1,5-pyrimidines and pyrazolo[1,5-a]-1,3,5-triazines, preferably those which selectively and/or potently bind mammalian neuropeptide Y (NPY) receptors. This invention also relates to pharmaceutical compositions comprising such compounds. It further relates to the use of such compounds in treating physiological disorders associated with an excess of neuropeptide Y, especially feeding disorders, some psychiatric disorders, and certain cardiovascular diseases.

Background of the Invention

Neuropeptide Y (NPY) is a 36 amino acid peptide first isolated in 1982 and subsequently found to be largely conserved across species. It belongs to a large family of peptides that includes, among others, peptide YY (PYY) and pancreatic peptide (PP). NPY is believed to be the most abundant peptide in the mammalian brain. It is also found in sympathetic neurons, and NPY-containing fibers have been found in peripheral tissues, such as around the arteries in the heart, the respiratory tract, the gastrointestinal tract, and the genitourinary tract. Central injection of NPY elicits a multitude of physiological responses, such as stimulation of feeding, increase in fat storage, elevation of blood sugar and insulin, anxiolytic behaviors, reduction in locomotor activity, hormone release, increase in blood pressure, reduction in body temperature, and catalepsy. In the cardiovascular system, NPY is believed to be involved in the regulation of coronary tone, while in the gastrointestinal tract, PYY is reported to cause inhibition of gastric acid secretion, pancreatic exocrine secretion, and gastroinestinal motility. These effects appear to be selectively mediated by various NPY receptors which currently include the Y_1, Y_2, Y_3, Y_4, Y_5, and Y_6 subtypes, in addition to the hypothetical Y_1-like subtype. Selective peptidic agonists and

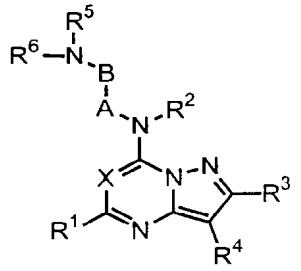
antagonists have been identified for most of the subtypes, but few selective non-peptidic antagonists have been reported. The Y_1 and Y_5 receptor subtypes appear to be involved in appetite regulation, but their relative contribution to the modulation of food intake and energy expenditure remains unclear. The discovery of non-peptidic antagonists of the Y_1 and/or Y_5 receptor provides novel therapeutic agents, that are less prone to the shortcomings of the peptide antagonists, namely, for example, poor metabolic stability, low oral bioavailability, and poor brain permeability, for the treatment of obesity and cardiovascular diseases. Recently, a few of such agents have been reported, some of which having demonstrated pharmacological efficacy in pre-clinical animal models. The present invention provides a novel class of potent non-peptidic antagonists of the NPY receptors, in particular, the Y_1 receptor.

Insofar as is known, aminoalkyl substituted pyrazolo[1,5,-a]-1,5-pyrimidines and pyrazolo[1,5-a]-1,3,5-triazines have not been previously reported as NPY receptor antagonists useful in the treatment of feeding and cardiovascular disorders. However, this general class of compounds has been described for other uses by virtue of different mechanisms of action, e.g., as antagonists of the corticotropin releasing factor (CRF_1). For example International Patent Publication WO 98/03510 describes certain pyraazolotriazines and pyrazolopyrimidines as being of use as corticotropin releasing factors.

Summary of the Invention

Compounds that interact with the Y_1 receptor and inhibit the activity of neuropeptide Y at those receptors are useful in treating physiological disorders associated with an excess of neuropeptide Y, including eating disorders, such as, for example, obesity and bulimia, and certain cardiovascular diseases, for example, hypertension.

This invention relates to a method of treating a physiological disorder associated with an excess of neuropeptide Y, which method comprises administering to a mammal in need of said treatment an effective amount of an amino substituted pyrazolo[1,5,-a]-1,5-pyrimidine or a pyrazolo[1,5-a]-1,3,5-triazine of the formula I:



Formula I

or a pharmaceutically acceptable salt or prodrug thereof, wherein the compound exhibits a K_i of 5 micromolar or less in an assay of NPY receptor binding, and

X is N or CR^{14};

R^1 is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halo, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7, C_1-C_6 cyanoalkyl, NR^8R^9, and C_1-C_6 alkyl-NR^8R^9;

R^2 is H,

C_1-C_6 alkyl which optionally forms a C_3-C_6 aminocarbocycle or a C_2-C_5 aminoheterocycle with A or B, each of which is optionally substituted with

R^7,

C_3-C_{10} cycloalkyl, or

(C_3-C_{10} cycloalkyl) C_1-C_6 alkyl; or

R^2 and R^6 jointly form with the 2 nitrogen atoms to which they are bound a C_2-C_5 aminoheterocycle optionally substituted with R^7;

A is $(CH_2)_m$ where m is 1,2 or 3 and is optionally mono- or di-substituted at each carbon atom with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, cyano, halogen, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7; C_1-C_6 cyanoalkyl, NR^8R^9, or C_1-C_6 alkyl-NR^8R^9, or

A and B jointly form a C_3-C_6 carbocycle, which is optionally substituted at each carbon atom with R^7, or

A and R^2 jointly form a C_3-C_6 aminocarbocycle , which is optionally substituted at each carbon atom with R^7;

B is $(CH_2)_n$ where n is 0, 1, 2, or 3 and is optionally substituted at each carbon atom with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halogen, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7; C_1-C_6 cyanoalkyl, NR^8R^9, or C_1-C_6 alkyl-NR^8R^9, or

B and R^2 jointly form a C_3-C_6 aminocarbocycle , which is optionally substituted at each carbon atom with R^7, or

B and R^6 jointly form a C_3-C_6 aminocarbocycle, which is optionally substituted at each carbon atom with R^7;

R^3 is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halo, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7, C_1-C_6 cyanoalkyl, NR^8R^9, and C_1-C_6 alkyl-NR^8R^9;

R^4 is selected from aryl or heteroaryl, each optionally substituted with 1 to 5 substituents independently selected at each occurrence from C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2 –C_6-alkenyl, C_2 –C_6-alkynyl, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, C_1-C_6 alkyl-$CONR^8R^9$, $COOR^7$, C_1-C_6 alkyl-$COOR^7$, CN, C_1-C_6 alkyl-CN, $SO_2NR^8R^9$, SO_2R^7, aryl, heteroaryl, heterocycloalkyl, and 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), with the proviso that at least one of the positions ortho or para to the point of attachment of the aryl or heteroaryl ring to the pyrazole is substituted;

R^5 and R^6 are independently selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, and C_2-C_6 alkynyl;

R^7 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_3 haloalkyl,

or heterocycloalkyl, C_1-C_8 alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl each of which is optionally substituted with 1 to 5 substituents independently selected at each occurrence from halogen, C_1-C_6 haloalkyl, OR^{13}, NR^8R^9, C_1-C_6 alkyl-OR^{13}, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, $COOR^{13}$, CN, $SO_2NR^8R^9$, SO_2R^{13}, with the proviso that for SO_2R^7, R^7 cannot be H;

R^8 and R^9 are independently selected at each occurrence from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_3-C_{10} cycloalkenyl, C_2-C_6 alkynyl, heterocycloalkyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl, or R^8 and R^9, taken together, can form a C_3-C_6 aminocarbocycle or a C_2-C_5 aminoheterocycle each optionally substituted at each occurrence with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_3 haloalkyl, or heterocycloalkyl, C_1-C_8 alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl;

R^{11} is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl;

R^{12} is selected from H, aryl, heteroaryl, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, optionally substituted with OR^7, NR^8R^9, C_3-C_6 aminocarbocycle, or C_2-C_5 aminoheterocycle;

R^{13} is independently selected at each occurrence from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, with the proviso that when R^7 is SO_2R^{13}, R^{13} cannot be H; and

R^{14} is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, halo, or CN.

Preferred compounds of Formula I exhibit a 20-fold or more greater affinity for the NPY_1 receptor than for the CRF_1 receptor. Preferred compounds of Formula I also do not exhibit high affinity for the CRF_1 receptor.

This invention also encompasses, in additional embodiments, the novel compounds of formula I, and the salts and solvates thereof, as well as pharmaceutical formulations comprising a compound of formula I, or a pharmaceutically acceptable salt or solvate thereof, in combination with one or more pharmaceutically acceptable carriers, excipients, or diluents thereof.

This invention also encompasses methods to treat physiological disorders associated with an excess of neuropeptide Y, such as eating and cardiovascular disorders, which method comprises administering to a mammal in need of said treatment an effective amount of a compound of the formula I.

This invention also encompasses methods of selectively inhibiting binding of NPY to NPY_1 receptors, which comprises contacting a compound of formula I with neuronal cells, wherein the compound is present in an amount effective to produce a concentration sufficient to inhibit binding of NPY to NPY_1 receptors in vitro.

Detailed Description of the Invention

The current invention includes a novel group of aminoalkyl substituted 4-amino pyrazolopyrimidines and 7-amino pyrazolo triazines, those of formula I. Preferred aminoalkyl substituted 4-amino pyrazolopyrimidines and 7-amino pyrazolo triazines bind with high affinity to the NPY_1 receptor and more preferably act as antagonists of NPY binding to the NPY_1 receptor. Preferred compounds of the invention bind with high selectivity to the NPY_1 receptor, particulary such compounds do not bind with high affinity to CRF_1 receptors. Without wishing to be bound to any particular theory, it is believed that the interaction of the compounds of Formula I with the NPY1 receptor results in the pharmaceutical utility of these compounds.

The invention further comprises methods of treating patients in need of such treatment with an amount of a compound of the invention sufficient to alter the symptoms of a eating disorder or cardiovascular disorder.

The present invention also pertains to methods of inhibiting the binding of NPY receptor ligands, such as NPY or PYY, to the NPY_1 receptors which methods involve contacting a compound of the invention with cells expressing NPY_1 receptors, wherein the compound is present at a concentration sufficient to inhibit the binding of NPY receptor Ligands to NPY_1 receptors *in vitro*. This method includes inhibiting the binding of NPY receptor ligands to NPY_1 receptors *in vivo*, e.g., in a patient given an amount of a compound of Formula I that would be sufficient to inhibit the binding of NPY receptor ligands to NPY receptors *in vitro*. The amount of a compound that would be sufficient to inhibit the binding of a NPY receptor ligand to the NPY_1 receptor may be readily determined via an NPY_1 receptor binding assay, such as the assay described in Example 94A. The NPY_1 receptors used to determine *in vitro* binding may be obtained from a variety of sources, for example from preparations of rat brain or from cells expressing cloned human NPY_1 receptors.

The present invention also pertains to methods for altering the signal-transducing activity of NPY1 nreceptors, said method comprising exposing cells expressing such receptors to an effective amount of a compound of the invention. This method includes altering the signal-transducing activity of NPY1 receptors *in vivo*, e.g., in a patient given an amount of a compound of Formula I that would be sufficient to alter the signal-transducing activity of NPY1 receptors *in vitro*. The amount of a compound that would be sufficient to alter the signal-transducing activity of NPY1 receptors may be determined via a NPY1 receptor signal transduction assay, such as the assay described in Example 93B.

The NPY1 receptor ligands provided by this invention and labeled derivatives thereof are also useful as standards and reagents in determining the ability of a potential pharmaceutical to bind to the NPY1 receptor.

Radiolabeled derivatives the NPY1 receptor ligands provided by this invention are also useful for mapping the location of NPY1 receptors (e.g., in tissue sections via autoradiography) and as radiotracers for positron emission tomography (PET) imaging, single photon emission computerized tomography (SPECT), and the like, to characterize such receptors in living subjects.

In addition to compounds of Formula I, the invention also provides as preferred compounds, compounds of Formula I wherein

X is N or CH; and

R^1 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, or (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl. Such compounds will be referred to as compounds of **Formula Ia**.

Other preferred compounds of Formula I are those compounds wherein

X is N or CH; R^1 is C_1-C_6 alkyl; R^2 is H or C_1-C_6 alkyl; and

R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl. Such compounds will be referred to as compounds of **Formula Ib**.

In another embodiment the invention provides compounds of Formula I, wherein

X is N or CH; R^1 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, or (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl; R^2 is H or C_1-C_6 alkyl; R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl; and R^5 is H. Such compounds will be referred to as compounds of **Formula Ic**.

Further provided are compounds wherein:

X is N or CH; R^1 is C_1-C_6 alkyl; R^2 is H or C_1-C_6 alkyl; R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl; R^4 is phenyl, mono-, di-, or tri-substituted C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, C_1-C_6 alkyl-$CONR^8R^9$, $COOR^7$, C_1-C_6 alkyl-$COOR^7$, CN, C_1-C_6 alkyl-CN, $SO_2NR^8R^9$, SO_2R^7, aryl, heteroaryl, heterocycloalkyl, 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), wherein at least one of the positions ortho or para to the point of attachment of the aryl or heteroaryl ring to the pyrazole is substituted; and R^7, R^8, and R^9 are as defined for Formula I. Such compounds will be referred to as compounds of **Formula Id**.

In yet another embodiment the invention provides compounds of Formula I wherein X is N or CH; R^1 is C_1-C_6 alkyl; R^2 is H or C_1-C_6 alkyl; R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl; and R^4 is phenyl, mono-, di-, or trisubstituted with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$,

C_1-C_6 alkyl-$CONR^8R^9$, $COOR^7$, C_1-C_6 alkyl-$COOR^7$, CN, C_1-C_6 alkyl-CN, $SO_2NR^8R^9$, SO_2R^7, aryl, heteroaryl, heterocycloalkyl, 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), wherein at least one of the positions ortho or para to the point of attachment of the aryl or heteroaryl ring to the pyrazole is substituted; R^5 is H; and R^6 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, or (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl. Such compounds will be referred to as compounds of **Formula Ie**.

Additionally the invention provides compounds of Formula I wherein X is N or CH; R^1 is C_1-C_6 alkyl; R^2 is H or C_1-C_6 alkyl; R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl; and R^4 is phenyl, mono-, di-, or trisubstituted with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, C_1-C_6 alkyl-$CONR^8R^9$, $COOR^7$, C_1-C_6 alkyl-$COOR^7$, CN, C_1-C_6 alkyl-CN, $SO_2NR^8R^9$, SO_2R^7, aryl, heteroaryl, heterocycloalkyl, 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), wherein at least one of the positions ortho or para to the point of attachment of the aryl or heteroaryl ring to the pyrazole is substituted; and R^6 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, or (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl. Such compounds will be referred to as compounds of **Formula If.**

Particularly preferred are compounds of Formula I wherein R^5 is H, and R^6 is cycloalkyl or (cycloalkyl)alkyl.(Compounds of **Formula Ig**)

For each of Formula Ia-Ig compounds that exhibits a K_i of 5 micromolar or less in an assay of NPY receptor binding are preferred. Also preferred are compounds of Formula I, and Formula Ia-Ig that do not exhibit a Ki or IC_{50} of 5 micromolar or less, or more preferably that do not exhibit a Ki or IC_{50} of 1 micromolar or less, for the CRF1 receptor in an assay of CRF1 receptor binding. An assay of CRF receptor binding is given in Example 94.

In certain situations, the compounds of formula I may contain one or more asymmetric carbon atoms, so that the compounds can exist in different stereoisomeric forms. These compounds can be, for example, racemates or optically active forms. In these situations, the single enantiomers, i.e., optically active forms, can be obtained by asymmetric synthesis or by resolution of the racemates. Resolution of the racemates can be accomplished, for example, by

conventional methods such as crystallization in the presence of a resolving agent, or chromatography, using, for example a chiral HPLC column.

Representative compounds of the present invention, which are encompassed by formula I, include, but are not limited to the compounds in Examples 1-87 and their pharmaceutically acceptable acid addition salts. In addition, if the compound of the invention is obtained as an acid addition salt, the free base can be obtained by basifying a solution of the acid salt. Conversely, if the product is a free base, an addition salt, particularly a pharmaceutically acceptable addition salt, may be produced by dissolving the free base in a suitable organic solvent and treating the solution with an acid, in accordance with conventional procedures for preparing acid addition salts from base compounds.

Non-toxic pharmaceutical salts include salts of acids such as hydrochloric, phosphoric, hydrobromic, sulfuric, sulfinic, formic, toluenesulfonic, methanesulfonic, nitric, benzoic, citric, tartaric, maleic, hydroiodic, alkanoic such as acetic, HOOC-(CH_2)n-COOH where n is 0-4, and the like. Those skilled in the art will recognize a wide variety of non-toxic pharmaceutically acceptable addition salts.

The present invention also encompasses the acylated prodrugs of the compounds of formula I. "Prodrugs" are considered to be any covalently bonded carriers which release the active parent drug of formula I in vivo when such prodrug is administered to a mammalian subject. Prodrugs of the compounds of the invention are prepared by modifying functional groups present in the compounds in such a way that the modifications are cleaved, either in routine manipulation or in vivo to the parent compounds. Prodrugs include compounds wherein hydroxy, amine, or sulfhydryl groups are bonded to any group that, when administered to a mammalian subject, cleaves to form a free hydroxyl, amino, or sulfhydryl group, respectively. Examples of prodrugs include, but are not limited to, acetate, formate, and benzoate derivatives of alcohol and amine functional groups in the compounds of formula I; and the like. Those skilled in the art will recognize various synthetic methodologies which may be employed to prepare non-toxic pharmaceutically acceptable addition salts and acylated prodrugs of the compounds encompassed by formula I.

Where a compound exists in various tautomeric forms, the invention is not limited to any one of the specific tautomers. The invention includes all tautomeric forms of a compound.

By "heteroatom" in the present invention is meant oxygen or sulfur, or a nitrogen atom optionally substituted by C_1-C_6 lower alkyl, C_1-C_6 arylalkyl, C_1-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_8 alkanoyl, C_1-C_6 sulfonyl.

By "alkyl", "lower alkyl", or "C_1-C_6 alkyl" in the present invention is meant straight or branched chain alkyl groups having 1-6 carbon atoms, such as, for example, methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, pentyl, 2-pentyl, isopentyl, neopentyl, hexyl, 2-hexyl, 3-hexyl, and 3-methylpentyl.

By "cycloalkyl", or "C_3-C_{10} cycloalkyl" in the present invention is meant alkyl groups having 3-10 carbon atoms forming a mono-, bi-, or polycyclic ring system, such as, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, norbornyl, and the like.

By "(cycloalkyl)alkyl", "lower (cycloalkyl)alkyl", or (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl in the present invention is meant a straight or branched alkyl substituent formed of 1 to 6 carbon atoms attached to a mono-, bi, or polycyclic ring system having 3-10 carbon atoms, such as, for example, cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl, cyclohexylmethyl, cycloheptylmethyl, and the like.

The term "C_2-C_6 alkenyl" in the present invention means hydrocarbon chains having 2 to 6 carbons in a straight or branched arrangement and containing one or more unsaturated carbon-carbon double bonds which may occur in any stable point along the chain, such as, for example, ethenyl, allyl, isopropenyl, and the like.

By "cycloalkenyl" or "C_3-C_{10} cycloalkenyl" in the present invention is meant alkyl groups having 3-10 carbon atoms forming a mono-, bi, or polycyclic ring system having 3-10 carbon atoms and containing one or more carbon-carbon double bonds which may occur in any stable point in the ring, such as, for example, cyclopentenyl, cyclohexenyl, or cycloheptenyl.

The term "C_2-C_6 alkynyl" in the present invention means hydrocarbon chains having 2 to 6 carbons in a straight or branched arrangement and containing one or more unsaturated carbon-carbon triple bonds which may occur in any stable point along the chain, such as, for example, ethynyl, propargyl, and the like.

The term "aryl" in the present invention means a monocyclic or bicyclic aromatic group having preferably 6 to 10 carbon atoms, such as, for example, phenyl or naphthyl.

The term "heteroaryl" in the present invention means an aryl group in which one or more of the ring(s) carbon atoms have been replaced with a heteroatom. Such groups preferably have

4 to 10 carbon atoms and 1 to 4 heteroatoms, such as, for example, pyridyl, pyrimidinyl, triazinyl, imidazolyl, oxazolyl, isoxazolyl, indolyl, pyrrolyl, pyrazolyl, quinolinyl, isoquinolinyl, thiazolyl, benzothiadiazolyl, triazolyl, triazinyl, pyrazinyl, furanyl, thienyl, benzothienyl, benzofuranyl, tetrazolyl.

The term "heterocyclyl", "heterocycle"or "heterocycloalkyl" in the present invention means a saturated or partially saturated heteroaryl group.

By "C_1-C_6 arylalkyl" or "C_1-C_6 heteroarylalkyl" in the present invention is meant a branched or straight-chain alkyl group having 1-6 carbon atoms and substituted on one of the carbon atoms by an optionally substituted aryl or heteroaryl ring, such as, for example, benzyl, phenethyl, methylpyridyl, ethylpyridyl, and the like.

By "C_5-C_8 arylcycloalkyl" in the present invention is meant cycloalkyl groups having 5-8 carbon atoms and fused to an aryl group, such as, for example, 1,2,3,4 tetrahydronaphthalenyl, 2,3-dihydrobenzothienyl, or 2,3-dihydobenzofuranyl.

By "C_5-C_8 heteroarylcycloalkyl" in the present invention is meant cycloalkyl groups having 5-8 carbon atoms fused to a heteroaryl group, such as, for example, 1,2,3,4 tetrahydroquinolyl, 2,3-dihydrobenzothienyl, 2,3-dihydobenzofuranyl, or indolinyl.

By "alkoxy", "C_1-C_6 alkoxy", or "C_1-C_6 alkyloxy" in the present invention is meant straight or branched chain alkoxy groups having 1-6 carbon atoms, such as, for example, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, sec-butoxy, tert-butoxy, pentoxy, 2-pentyl, isopentoxy, neopentoxy, hexoxy, 2-hexoxy, 3-hexoxy, and 3-methylpentoxy.

By "cycloalkoxy", "C_3-C_{10} cycloalkoxy", or "C_3-C_{10} cycloalkyloxy" in the present invention is meant a group formed by an oxygen atom attached to a mono-, bi, or polycyclic ring system having 3-10 carbon atoms, such as, for example, cyclopropoxy, cyclobutoxy, cyclopentoxy, cyclohexoxy, or cycloheptoxy.

By "(cycloalkyl)alkyloxy", "(C_3-C_{10} cycloalkyl) C_1-C_6 alkoxy", or "(C_3-C_{10} cycloalkyl) C_1-C_6 alkyloxy" in the present invention is meant a group formed by an oxygen atom attached to a 1-6 carbon chain linked to a mono-, bi, or polycyclic ring system having 3-10 carbon atoms, such as, for example, cyclopropylmethyloxy, cyclobutylmethyloxy, cyclopentylmethyloxy, cyclohexylmethyloxy, cycloheptylmethyloxy, and the like.

By "C_3-C_6 aminocarbocycle" is meant a cyclic amino group formed by a nitrogen contained in a ring having 3 to 6 carbon atoms, such as, for example, azetidino, pyrrolidino, piperidino, perhydroazepino.

By "C_2-C_5 aminoheterocycle" is meant a cyclic amino group formed by a nitrogen contained in a ring having 2 to 5 carbon atoms and one other heteroatom, such as, for example, morpholino, thiomorpholino, piperazino.

By the terms "halo" or "halogen" in the present invention is meant fluoro, chloro, bromo, and iodo.

"Haloalkyl" is intended to include both branched and straight-chain alkyl having the specified number of carbon atoms substituted with 1 or more halogens.

The term "C_2-C_8 alkanoyl" means an acyl group with 2 to 8 carbon atoms in a linear, branched, or C_3-C_{10} cycloalkyl arrangement, optionally substituted with 1 to 5 substituents independently selected at each occurrence from halogen, trifluoromethyl, OR^7, NR^8R^9, $CONR^8R^9$, $COOR^7$, or CN.

The term "C_1-C_6 alkyl sulfonyl" means an alkylsulfonyl group containing 1 to 6 carbon atoms in a linear, branched, or C_3-C_7 cycloalkyl arrangement.

The term "substituted" means that one or more hydrogen on the designated atom is replaced by the specified group, provided that the valence on the designated atom is not exceeded, and that a chemically stable compound results from the substitution.

A stable compound is defined herein as one that can be isolated, characterized, and tested for biological activity.

The term "oxo" (i.e. =O) indicates that two geminal hydrogen atoms are replaced by a double-bond oxygen group.

The term "hydroximino" (i.e. =N-OH)) indicates that two geminal hydrogen atoms are replaced by a double-bond nitrogen atom substituted with a hydroxyl group.

The term "C_1-C_6 alkoximino" (i.e. =N-O-Alkyl) indicates that two geminal hydrogen atoms are replaced by a double-bond nitrogen atom substituted with a C_1-C_2 alkoxy group, such as, for example, methoximino (=N-OMe).

In the present invention, the term "potent" in the context of NPY1 receptor antagonists qualifies a binding affinity with a Ki of less than 10 micromolar, preferably less than 1 micromolar, and more preferably less than 100 nanomolar in the human NPY1 binding assay.

In the present invention, the term "selective" in the context of NPY1 receptor antagonists qualifies a binding affinity with a Ki in the human NPY1 binding assay that is 10-fold or 20-fold, preferably 100-fold, and more preferably 1000-fold, less than the Ki of the same compound measured in another receptor binding assay, in particular the NPY_5 and $CRF1_1$ receptor binding assays. Binding assays for the NPY_5 and $CRF1_1$ receptors have been described, for example, in *J. Clin. Invest.*, 102, 2136 (**1998**) and in *Endocrinology* 116, 1653 (**1985**), respectively. A CRF1 receptor binding assay is also given in Example 94.

As the compounds of formula I are selective antagonists of the Y1 receptor, they are of value in the treatment of a wide variety of clinical conditions which are characterized by the presence of an excess of neuropeptide Y. Thus, the invention provides methods for the treatment or prevention of a physiological disorder associated with an excess of neuropeptide Y, which method comprises administering to a mammal in need of said treatment an effective amount of a compound of formula I or a pharmaceutically acceptable salt, solvate or prodrug thereof. The term "physiological disorder associated with an excess of neuropeptide Y" encompasses those disorders associated with an inappropriate stimulation of neuropeptide Y receptors, regardless of the actual amount of neuropeptide Y present locally. These physiological disorders may include: disorders or diseases pertaining to the heart, blood vessels or the renal system, such as vasospasm, heart failure, shock, cardiac hypertrophy increased blood pressure, angina, myocardial infarction, sudden cardiac death, arrhythmia, peripheral vascular disease, and abnormal renal conditions such as impaired flow of fluid, abnormal mass transport, or renal failure; conditions related to increased sympathetic nerve activity for example, during or after coronary artery surgery, and operations and surgery in the gastrointestinal tract; cerebral diseases and diseases related to the central nervous system, such as cerebral infarction, neurodegeneration, epilepsy, stroke, and conditions related to stroke, cerebral vasospasm and hemorrhage, depression, anxiety, schizophrenia, and dementia; conditions related to pain or nociception; diseases related to abnormal gastrointestinal motility and secretion, such as different forms of ileus, urinary incontinence, and Crohn's disease; abnormal drink and food intake disorders, such as obesity, anorexia, bulimia, and metabolic disorders; diseases related to sexual dysfunction and reproductive disorders; conditions or disorders associated with inflammation; respiratory diseases, such as asthma and conditions related to asthma and

bronchoconstriction; and diseases related to abnormal hormone release, such as leutinizing hormone, growth hormone, insulin, and prolactin.

Pharmaceutical Preparations

The compounds of general Formula I may be administered orally, topically, parenterally, by inhalation or spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In addition, there is provided a pharmaceutical formulation comprising a compound of general Formula I and a pharmaceutically acceptable carrier. One or more compounds of general Formula I may be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants and if desired other active ingredients. The pharmaceutical compositions containing compounds of general Formula I may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene 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, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil, for example arachid oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide palatable oral preparations. 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 provide the active ingredient in admixture 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, for example sweetening, flavoring and coloring agents, may also be present.

Pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachid oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monoleate, and condensation products of the said

partial esters with ethylene oxide, for example polyoxyethylene sorbitan monoleate. The emulsions may also contain sweetening and flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The compounds of general Formula I may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

Compounds of general Formula I may be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.1 mg to about 50 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 3 g per patient per day), although higher amounts , for example up to 140 mg./kg/day may be appropriate in some circumstances. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. Dosage unit forms will generally contain between from about 1 mg to about 500 mg of an active ingredient.

Frequency of dosage may also vary depending on the compound used and the particular disease treated. However, for treatment of most eating disorders, a dosage regimen of 4 times daily or less is preferred. For the treatment of stress and depression a dosage regimen of 1 or 2 times daily is particularly preferred.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

Preferred compounds of the invention will have certain pharmacological properties. Such properties include, but are not limited to oral bioavailability, low toxicity, low serum protein binding and desirable *in vitro* and *in vivo* half-lives. Penetration of the blood brain barrier for compounds used to treat CNS disorders is necessary, while low brain levels of compounds used to treat peripheral disorders are often preferred.

Assays may be used to predict these desirable pharmacological properties. Assays used to predict bioavailability include transport across human intestinal cell monolayers, including Caco-2 cell monolayers. Toxicity to cultured hepatocytes may be used to predict compound toxicity. Penetration of the blood brain barrier of a compound in humans may be predicted from the brain levels of the compound in laboratory animals given the compound intravenously.

Serum protein binding may be predicted from albumin binding assays. Such assays are described in a review by Oravcová, et al. (*Journal of Chromatography B* **1996,** *677*, 1-27).

Compound half-life is inversely proportional to the frequency of dosage of a compound. *In vitro* half-lives of compounds may be predicted from assays of microsomal half-life as described by Kuhnz and Gieschen (*Drug Metabolism and Disposition* **1998,** *26*, 1120-1127).

As discussed above, preferred compounds of the invention exhibit good activity in standard *in vitro* NPY receptor binding assays, specifically the assay as specified in Example 93A, which follows. References herein to "standard *in vitro* NPY receptor binding assay" are intended to refer to that protocol as defined in Example 93A which follows. Generally preferred compounds of the invention exhibit a K_i of about 1 micromolar or less, still more preferably and K_i of about 100 nanomolar or less even more preferably an K_i of about 10 nanomolar or less or even 1 nanomolar or less in such a defined standard *in vitro* NPY receptor binding assay as exemplified by Example 93A which follows.

In appropriate case, the compounds of the invention may be employed in combination with other active agents. The invention therefore also provides pharmaceutical combination compositions comprising a therapeutically effective amount of a composition comprising: (a) first compound, said first compound being a compound as described above a prodrug thereof, or a pharmaceutically acceptable salt of said compound or of said prodrug; and (b) a second compound, said second compound being a β_3 agonist, a thyromimetic, an eating behavior modifying agent or a NPY antagonist; and a pharmaceutical carrier, vehicle or diluent. . To this end therefore the invention also provides a kit comprising: (a) first compound, said first compound being a compound as described above, a prodrug thereof, or a pharmaceutically acceptable salt of said compound or of said prodrug; (b) a second compound, said second compound being a β_3 agonist, a thyromimetic, an eating behavior modifying agent or a NPY antagonist; and a pharmaceutical carrier, vehicle, diluent; and (c) means for containing said first and second unit dosage forms wherein the amounts of the first and second compounds result in a therapeutic effect.

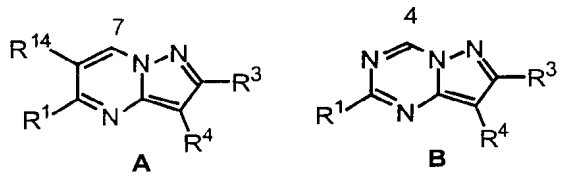
Synthetic Schemes

Preparation of Amino Substituted Pyrazolo[1,5,-a]-1,5-Pyrimidines and Pyrazolo[1,5-a]-1,3,5-Triazines Derivatives

An illustration of preparation methods of compounds of the present invention is given in the Schemes below. In particular displacement of a leaving group Z, as in formula **10** (Scheme 1) by the appropriate substituted amine provides a method to convert the heterocyclic cores of the present invention, i.e. aryl or heteroaryl substituted pyrazolo[1,5,-a]-1,5-pyrimidines and pyrazolo[1,5-a]-1,3,5-triazines, into compounds that potently interact with the NPY1 receptor. Such transformations may require several consecutive chemical steps. Those having skill in the art will recognize that the starting materials may be varied and additional steps employed to produce compounds encompassed by the present invention. The disclosures of all articles and references mentioned in this application, including patents, are incorporated herein by reference.

Unless otherwise specified the variable R^1, R^2, R^3, R^4, R^5, R^6, and X are as defined for Formula I.

One general approach is to convert a heterocyclic core **A** and or a heterocyclic core **B**



A B

to a compound that exhibits a K_i of 5 micromolar or less in an assay of NPY receptor binding, wherein

R^1 is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halo, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7, C_1-C_6 cyanoalkyl, NR^8R^9, and C_1-C_6 alkyl-NR^8R^9;

R^3 is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halo, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7, C_1-C_6 cyanoalkyl, NR^8R^9, and C_1-C_6 alkyl-NR^8R^9;

R^4 is selected from aryl or heteroaryl, each optionally substituted with 1 to 5 substituents independently selected at each occurrence from C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, C_1-C_6 alkyl-$CONR^8R^9$, $COOR^7$, C_1-C_6 alkyl-$COOR^7$, CN, C_1-C_6 alkyl-CN, $SO_2NR^8R^9$, SO_2R^7, aryl, heteroaryl, heterocycloalkyl, and 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), with the proviso that at least one of the positions ortho or para to the point of attachment of the aryl or heteroaryl ring to the pyrazole is substituted;

R^{14} is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, halo, or CN;

by substituting the 7-position of the heterocyclic core **A** or the 4-position of the heterocyclic core **B** with a diamine group $-N[R^2]-A-B-N[R^6]-R^5$

wherein:

R^2 is H,

C_1-C_6 alkyl which optionally forms a C_3-C_6 aminocarbocycle or a C_2-C_5 aminoheterocycle with A or B, each of which is optionally substituted with

R^7,

C_3-C_{10} cycloalkyl, or

(C_3-C_{10} cycloalkyl) C_1-C_6 alkyl; or

R^2 and R^6 jointly form with the 2 nitrogen atoms to which they are bound a C_2-C_5 aminoheterocycle optionally substituted with R^7;

A is $(CH_2)_m$ where m is 1,2 or 3 and is optionally mono- or di-substituted at each carbon atom with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, cyano, halogen, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7; C_1-C_6 cyanoalkyl, NR^8R^9, or C_1-C_6 alkyl-NR^8R^9, or

A and B jointly form a C_3-C_6 carbocycle, which is optionally substituted at each carbon atom with R^7, or

A and R^2 jointly form a C_3-C_6 aminocarbocycle , which is optionally substituted at each carbon atom with R^7;

B is $(CH_2)_n$ where n is 0, 1, 2, or 3 and is optionally substituted at each carbon atom with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halogen, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7; C_1-C_6 cyanoalkyl, NR^8R^9, or C_1-C_6 alkyl-NR^8R^9, or

B and R^2 jointly form a C_3-C_6 aminocarbocycle , which is optionally substituted at each carbon atom with R^7, or

B and R^6 jointly form a C_3-C_6 aminocarbocycle, which is optionally substituted at each carbon atom with R^7;

R^5 and R^6 are independently selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, and C_2-C_6 alkynyl;

R^7 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_3 haloalkyl,
or heterocycloalkyl, C_1-C_8 alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl each of which is optionally substituted with 1 to 5 substituents independently selected at each occurrence from halogen, C_1-C_6 haloalkyl, OR^{13}, NR^8R^9, C_1-C_6 alkyl-OR^{13}, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, $COOR^{13}$, CN, $SO_2NR^8R^9$, SO_2R^{13}, with the proviso that for SO_2R^7, R^7 cannot be H;

R^8 and R^9 are independently selected at each occurrence from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_3-C_{10} cycloalkenyl, C_2-C_6 alkynyl, heterocycloalkyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl, or R^8 and R^9, taken together, can form a C_3-C_6 aminocarbocycle or a C_2-C_5 aminoheterocycle each optionally substituted at each occurrence with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_3 haloalkyl, or heterocycloalkyl, C_1-C_8 alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl;

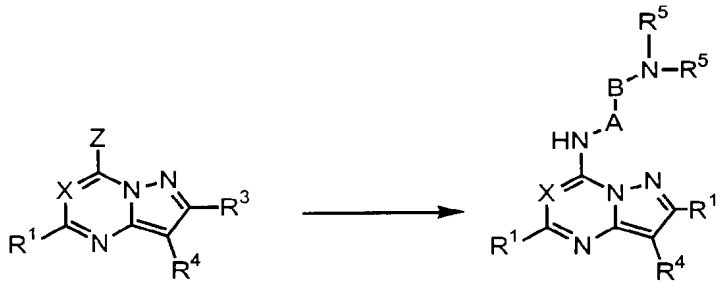
R^{11} is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl;

R^{12} is selected from H, aryl, heteroaryl, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, optionally substituted with OR^7, NR^8R^9, C_3-C_6 aminocarbocycle, or C_2-C_5 aminoheterocycle; and

R^{13} is independently selected at each occurrence from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, with the proviso that when R^7 is SO_2R^{13}, R^{13} cannot be H.

More specifically the following schemes may be used.

SCHEME 1



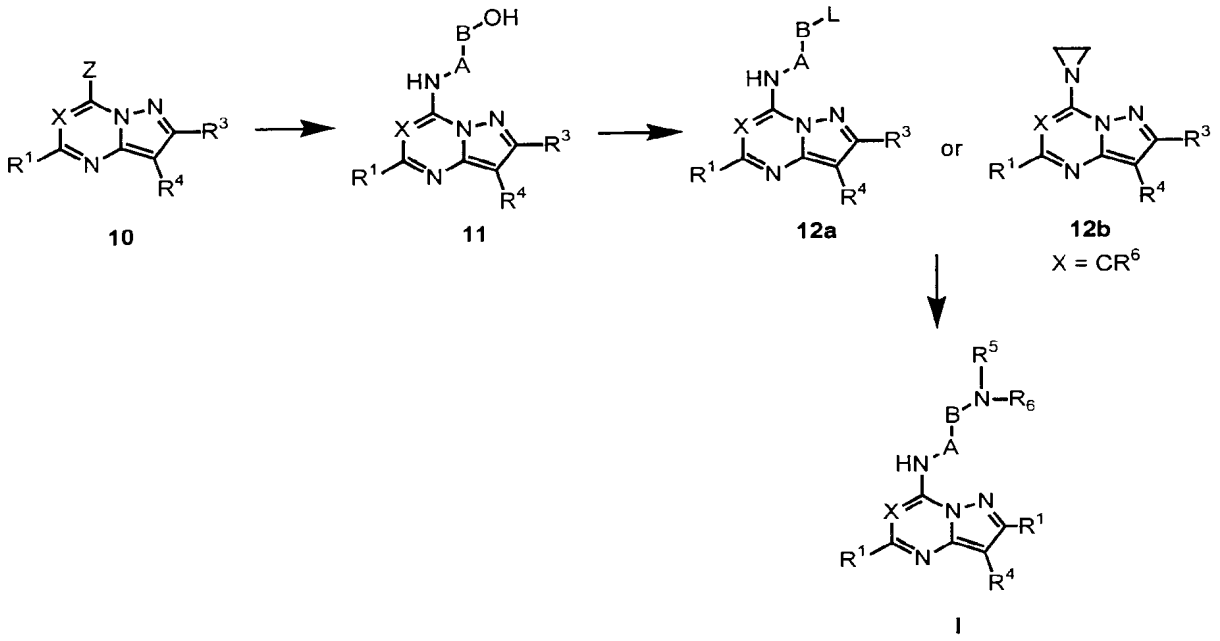
10 I

As illustrated in Scheme 1, compounds of formula I can be prepared from intermediate compounds of formula **10**, where Z is halogen (preferably chloro or bromo), alkane sulfonyloxy, aryl sulfonyloxy or haloalkane sulfonyloxy, and X, R^1, R^3 and R^4 are defined above, using the procedures outlined below.

Compounds of formula **10** react with an amine of formula H_2N-A-B-N[R^6]-R^5, where A,B, R^5 and R^6 are defined as above, in the presence or absence of a base in the presence or absence of an inert solvent at reaction temperatures ranging from -78°C to 250°C to generate compounds of formula I. Bases may include, but are not limited to, alkali metal hydrides (preferably sodium hydride), alkali metal alkoxides (1-6 carbons) (preferably sodium methoxide, sodium ethoxide, or sodium tert-butoxide), alkaline earth metal hydrides, alkali metal dialkylamides (preferably lithium diisopropylamide), alkali metal carbonates, alkali metal bicarbonates, alkali metal bis-(trialkylsilyl)amides (preferably lithium or sodium (trimethylsilyl)amide), trialkylamines (preferably N,N-di-isopropyl-N-ethyl amine or triethylamine), arylamines (preferably 4-dimethyl aniline), or heteroaromatic amines (preferably pyridine). Inert solvents may include, but are not limited to, alkyl alcohols (1-8 carbons) (preferably methanol, ethanol, or tert-butanol), lower alkanenitriles (1-6 carbons) (preferably acetonitrile), dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or

toluene), or haloalkanes (1-10 carbons and 1-10 halogens) (preferably dichloromethane). Preferred reaction temperatures range from 0°C to 140°C.

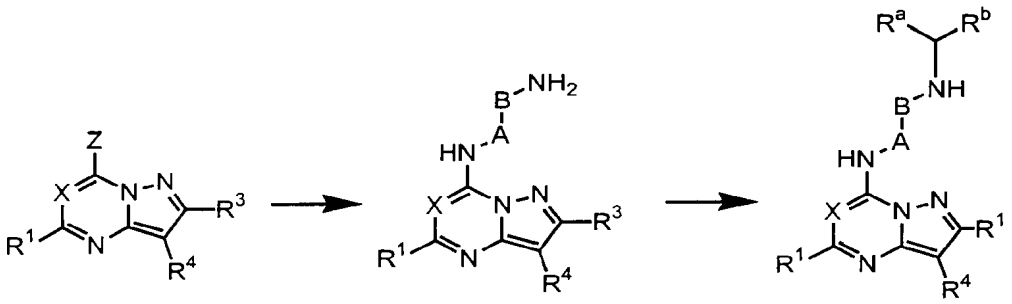
SCHEME 2



Alternatively, as shown in Scheme 2, compounds of formula I can be obtained by first reacting a compound of formula **10** with an amino alcohol of formula H_2N-A-B-OH, where A and B are defined as above, in the presence or absence of a base in the presence or absence of an inert solvent at reaction temperatures ranging from -78°C to 250°C to generate intermediates of formula **11**. Reacting a compound of formula **11** with a halogenating agent or sulfonylating agent in the presence or absence of a base in the presence or absence of an inert solvent at reaction temperatures ranging from –78°C to 250C°C to afford products of formula **12a** (where Z is halogen, alkane sulfonyloxy, aryl sulfonyloxy or haloalkane sulfonyloxy) or **12b** when A and B are both CH_2 and X is CR^{14}. Halogenating agents include, but are not limited to, $SOCl_2$, $POCl_3$, PCl_3, PCl_5, $POBr_3$, PBr_3, PBr_5., CCl_4/PPh_3. Sulfonylating agents include, but are not limited to, alkanesulfonyl halides or anhydrides (preferably methanesulfonyl chloride or methanesulfonic anhydride), aryl sulfonyl halides or anhydrides (such as p-toluenesulfonyl chloride or anhydride), or haloalkylsulfonyl halides or anhydrides (preferably trifluoromethanesulfonic anhydride). Bases may include, but are not limited to, trialkylamines (preferably N,N-di-isopropyl-N-ethyl amine or triethylamine), bicyclic amidines (preferably DBU), anilines (preferably N-dimethyl

aniline), or heteroaromatic amines (preferably pyridine). Inert solvents may include, but are not limited to, lower alkanenitriles (1-6 carbons) (preferably acetonitrile), dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or toluene), or haloalkanes with 1-10 carbons and 1-10 halogens (preferably dichloromethane). Preferred reaction temperatures range from –20°C to 100C°C. Compounds of formula **12a** or **12b** can then be reacted with an amine of formula $HN[R^6]$-R^5, where R^5 and R^6 are defined as above, to give a compound of formula I. Bases may include, but are not limited to, alkali metal hydrides (preferably sodium hydride), alkali metal alkoxides (1-6 carbons) (preferably sodium methoxide, sodium ethoxide, or sodium tert-butoxide), alkaline earth metal hydrides, alkali metal dialkylamides (preferably lithium diisopropylamide), alkali metal carbonates, alkali metal bicarbonates, alkali metal bis-(trialkylsilyl)amides (preferably lithium or sodium (trimethylsilyl)amide), trialkylamines (preferably N,N-di-isopropyl-N-ethyl amine or triethylamine), arylamines (preferably 4-dimethyl aniline), or heteroaromatic amines (preferably pyridine). Inert solvents may include, but are not limited to, alkyl alcohols (1-8 carbons) (preferably methanol, ethanol, or tert-butanol), lower alkanenitriles (1-6 carbons) (preferably acetonitrile), dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or toluene), or haloalkanes (1-10 carbons and 1-10 halogens) (preferably dichloromethane). Preferred reaction temperatures range from 0°C to 140°C.

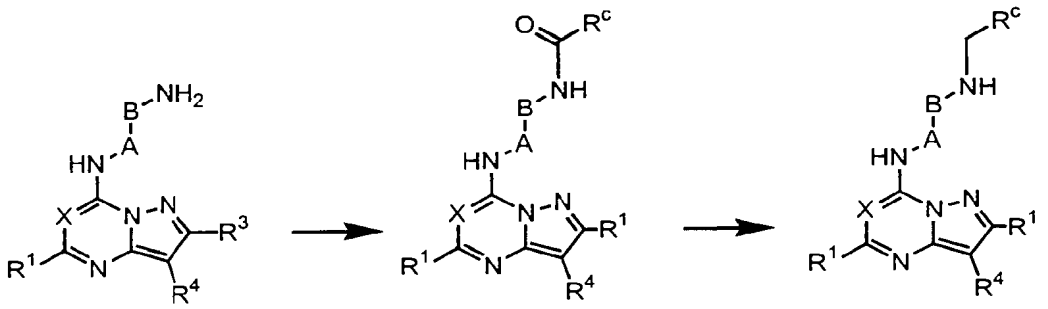
SCHEME 3



10 **13** **Ia**

A subset of compounds of formula I, described under formula Ia, can be obtained by first reacting a compound of formula **10** with a diamine of formula H_2N-A-B-NH_2, where A and B are defined as above, in the presence or absence of a base in the presence or absence of an inert solvent at reaction temperatures ranging from -78°C to 250°C to generate intermediates of formula **13**. Reaction of a compound of formula **13** with an aldehyde or ketone of Formula R^a-C=O-R^b in the presence of a reducing agent provides a compound of formula Ia, where the grouping R^a-CH-R^b corresponds to R^5 in formula I, as defined above. Reducing agents include, but are not limited to, alkali metal or alkaline earth metal borohydrides (preferably lithium or sodium borohydride), borane (preferably complexed with dimethyl sulfide or tetrahydrofuran), dialkylboranes (such as di-isoamylborane), alkali metal aluminum hydrides (preferably lithium aluminum hydride), alkali metal (trialkoxy)aluminum hydrides (such as triethoxyaluminum hydride), dialkyl aluminum hydrides (such as di-isobutyl aluminum hydride), alane (preferably complexed with dimethyl amine). Inert solvents may include, but are not limited to, alkyl alcohols (1-6 carbons) (preferably methanol, ethanol, or tert-butanol), dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), aromatic hydrocarbons (preferably benzene or toluene). Preferred reaction temperatures range from -78C°C to 100°C.

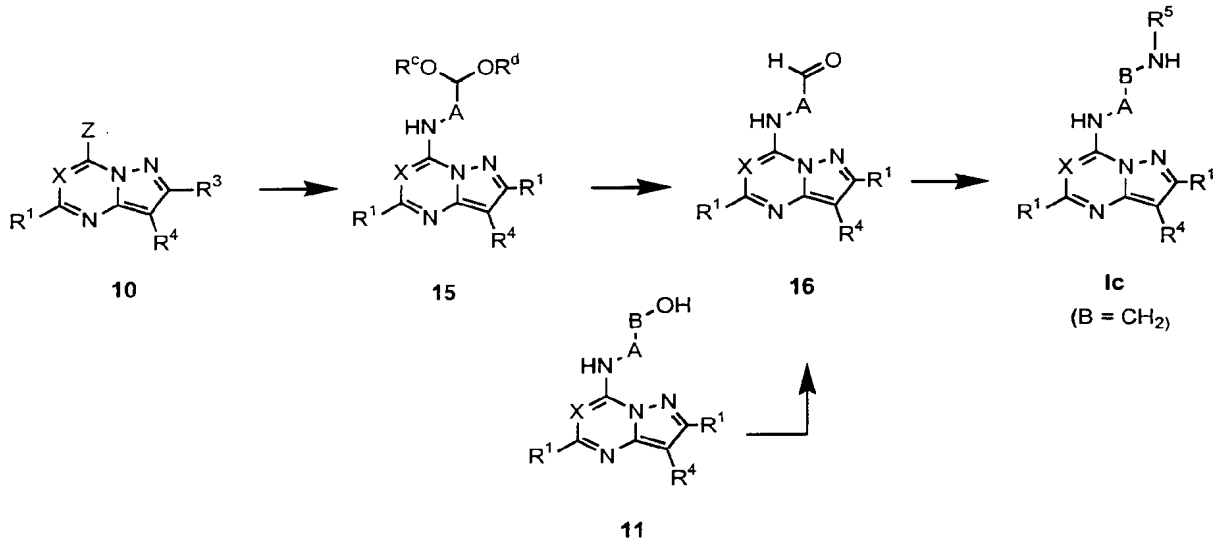
SCHEME 4



13 14 Ib

Alternatively, a subset of compounds of formula I, described under formula Ib, can be obtained by first reacting a compound of formula **13** with an activated acid of formula R^c-C=O-Z, where Z is halo (preferably chloro), O-acyl (preferably O-C=O-R^c), in the presence or absence of a base in the presence or absence of an inert solvent at reaction temperatures ranging from -78 °Cto 250C°C to generate an amide intermediate of formula **14**. Reaction of a compound of formula **14** with a reducing agent provides a compound of formula Ib, where the grouping R^c-CH_2 corresponds to R^5 in formula I, as defined above. Reducing agents include, but are not limited to, alkali metal or alkaline earth metal borohydrides (preferably lithium or sodium borohydride), borane (preferably complexed with dimethyl sulfide or tetrahydrofuran), dialkylboranes (such as di-isoamylborane), alkali metal aluminum hydrides (preferably lithium aluminum hydride), alkali metal (trialkoxy)aluminum hydrides (such as triethoxyaluminum hydride), dialkyl aluminum hydrides (such as di-isobutyl aluminum hydride), alane (preferably complexed with dimethyl amine). Inert solvents may include, but are not limited to, alkyl alcohols (1-6 carbons) (preferably methanol, ethanol, or tert-butanol), dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), aromatic hydrocarbons (preferably benzene or toluene). Preferred reaction temperatures range from -78°C to 100°C.

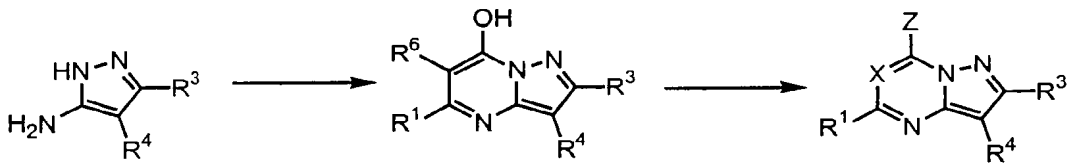
SCHEME 5



Alternatively, a subset of compounds of formula I, described under formula Ic, can be obtained by first reacting a compound of formula **10** with an amine of formula H_2N-A-$CH(OR^c)(OR^d)$, where A is defined above, and R^c and R^d are C_1-C_6 lower alkyls or, taken together, complete a ketal group, such as, for example a dioxane or dioxolane group, in the presence or absence of a base in the presence or absence of an inert solvent at reaction temperatures ranging from -78°C to 250C°C to generate compounds of formula **15**. Bases may include, but are not limited to, alkali metal hydrides (preferably sodium hydride), alkali metal alkoxides (1-6 carbons) (preferably sodium methoxide, sodium ethoxide, or sodium tert-butoxide), alkaline earth metal hydrides, alkali metal dialkylamides (preferably lithium diisopropylamide), alkali metal carbonates, alkali metal bicarbonates, alkali metal bis-(trialkylsilyl)amides (preferably lithium or sodium (trimethylsilyl)amide), trialkylamines (preferably N,N-di-isopropyl-N-ethyl amine or triethylamine), arylamines (preferably 4-dimethyl aniline), or heteroaromatic amines (preferably pyridine). Inert solvents may include, but are not limited to, alkyl alcohols (1-8 carbons) (preferably methanol, ethanol, or tert-butanol), lower alkanenitriles (1-6 carbons) (preferably acetonitrile), dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or

toluene), or haloalkanes (1-10 carbons and 1-10 halogens) (preferably dichloromethane). Compounds of formula **15** react with a protic acid in the presence or absence of an inert solvent at reaction temperatures ranging from -78°C to 250°C, followed by aqueous work-up to generate compounds of formula **16**. Inert solvents may include, but are not limited to dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or toluene), or haloalkanes (1-10 carbons and 1-10 halogens) (preferably dichloromethane). Protic acids include, but are not limited to, formic acid, acetic acid, trifluoroacetic acid, hydrochloric acid, methane sulfonic acid. Alternatively, compounds of formula **16** can be obtained by oxidation of compounds of formula **11** where B= CH_2. Oxidizing agents include, but are not limited to, transition metal oxides, such as CrO_3 or MnO_2, pyridine-chromium complexes, such as $CrO_3.C_5H_5N$, pyridinium dichromate or pyridinium chlorochromate, or an oxalyl chloride-DMSO-triethylamine reagent (Swern oxidation). Compounds of formula **16** react with amines of formula $H_2N\text{-}R^5$, where R^5 is defined above, in the presence of a reducing agent in the presence or absence of an inert solvent in the presence or absence of a protic acid at temperatures ranging from –78°C to 100°C, to give compounds of formula **Ic**. Reducing agents include, but are not limited to, alkali metal or alkaline earth metal borohydrides (preferably lithium or sodium borohydride), borane (preferably complexed with dimethyl sulfide or tetrahydrofuran), dialkylboranes (such as di-isoamylborane), alkali metal aluminum hydrides (preferably lithium aluminum hydride), alkali metal (trialkoxy)aluminum hydrides (such as triethoxyaluminum hydride), dialkyl aluminum hydrides (such as di-isobutyl aluminum hydride), alane (preferably complexed with dimethyl amine). Inert solvents may include, but are not limited to, alkyl alcohols (1-6 carbons) (preferably methanol, ethanol, or tert-butanol), dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), aromatic hydrocarbons (preferably benzene or toluene).

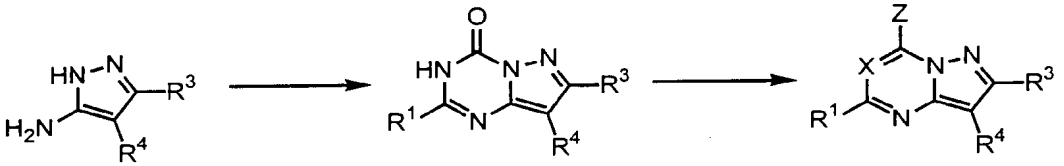
SCHEME 6



When X is CR^{14}, as defined above, compounds of formula **10** may be obtained from compounds of formula **22**. Compounds of formula **22** can be reacted with compounds of formula R^1-C=O-CH(R^{14})-C=O-R^c, where R^1 and R^{14} are defined above, and R^c is halogen, cyano, lower alkoxy (1-6 carbons), or lower alkanoyloxy (1-6 carbons), in the presence or absence of a base in an inert solvent at reaction temperatures ranging from –50C°C to 250C°C to afford compounds of formula **23a**. Bases may include, but are not limited to, alkali metal hydrides (preferably sodium hydride), alkali metal alkoxides (1-6 carbons) (preferably sodium methoxide, sodium ethoxide, or sodium tert-butoxide), alkaline earth metal hydrides, alkali metal dialkylamides (preferably lithium diisopropylamide), alkali metal carbonates, alkali metal hydroxides, alkali metal bis-(trialkylsilyl)amides (preferably lithium or sodium (trimethylsilyl)amide), trialkylamines (preferably N,N-di-isopropyl-N-ethyl amine or triethylamine), bicyclic amidines (preferably DBU), or heteroaromatic amines (preferably pyridine). Inert solvents may include, but are not limited to, alkyl alcohols (1-8 carbons) (preferably methanol, ethanol, or tert-butanol), lower alkanenitriles (1-6 carbons) (preferably acetonitrile), water, dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or toluene). Compounds of formula **23a** can then be reacted with a halogenating agent or sulfonylating agent in the presence or absence of a base in the presence or absence of an inert solvent at reaction temperatures ranging from –78C°C to 250°C to afford products of formula **10** (where Z is halogen, alkane sulfonyloxy, aryl sulfonyloxy or haloalkane sulfonyloxy and X is CR^{14}). Halogenating agents include, but are not limited to, $SOCl_2$, $POCl_3$, PCl_3, PCl_5, $POBr_3$, PBr_3, or PBr_5. Sulfonylating agents include, but are not limited to, alkanesulfonyl halides or anhydrides (preferably methanesulfonyl chloride or methanesulfonic anhydride), aryl sulfonyl halides or

anhydrides (such as p-toluenesulfonyl chloride or anhydride), or haloalkylsulfonyl halides or anhydrides (preferably trifluoromethanesulfonic anhydride). Bases may include, but are not limited to, trialkylamines (preferably N,N-di-isopropyl-N-ethyl amine or triethylamine), bicyclic amidines (preferably DBU), anilines (preferably N-dimethyl aniline), or heteroaromatic amines (preferably pyridine). Inert solvents may include, but are not limited to, lower alkanenitriles (1-6 carbons) (preferably acetonitrile), dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or toluene), or haloalkanes with 1-10 carbons and 1-10 halogens (preferably dichloromethane). Preferred reaction temperatures range from -20°C to 100°C.

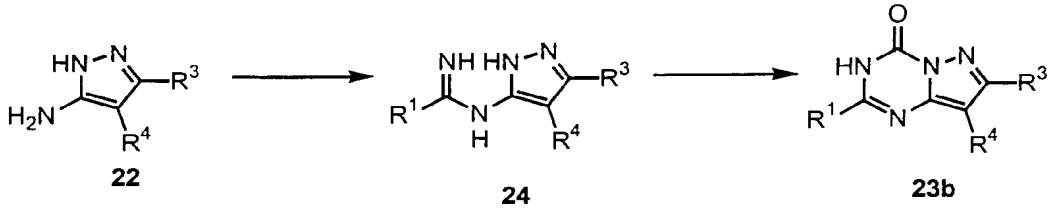
SCHEME 7



When X is N, compounds of formula **22** can be reacted with compounds of formula R^1-C=N($COOR^g$)-OR^f, where R^1 is defined above, and R^g is lower alkyl (1-6 carbons), and R^f is halogen, cyano, lower alkoxy (1-6 carbons), or lower alkanoyloxy (1-6 carbons), in the presence or absence of a base in an inert solvent at reaction temperatures ranging from –50°C to 250°C to afford compounds of formula **23b**. Bases may include, but are not limited to, alkali metal hydrides (preferably sodium hydride), alkali metal alkoxides (1-6 carbons) (preferably sodium methoxide, sodium ethoxide, or sodium tert-butoxide), alkaline earth metal hydrides, alkali metal dialkylamides (preferably lithium diisopropylamide), alkali metal carbonates, alkali metal hydroxides, alkali metal bis-(trialkylsilyl)amides (preferably lithium or sodium (trimethylsilyl)amide), trialkylamines (preferably N,N-di-isopropyl-N-ethyl amine or triethylamine), bicyclic amidines (preferably DBU), or heteroaromatic amines (preferably pyridine). Inert solvents may include, but are not limited to, alkyl alcohols (1-8 carbons) (preferably methanol, ethanol, or tert-butanol), lower alkanenitriles (1-6 carbons) (preferably

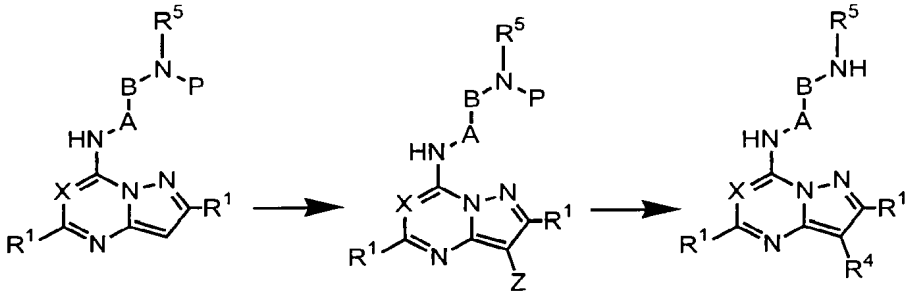
acetonitrile), water, dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or toluene), heteroaromatic hydrocarbons (preferably pyridine). Compounds of formula **23b** can then be reacted with a halogenating agent or sulfonylating agent in the presence or absence of a base in the presence or absence of an inert solvent at reaction temperatures ranging from –78C to 250C to afford products of formula **10** (where Z is halogen, alkane sulfonyloxy, aryl sulfonyloxy or haloalkane sulfonyloxy and X is N). Halogenating agents include, but are not limited to, $SOCl_2$, $POCl_3$, PCl_3, PCl_5, $POBr_3$, PBr_3, or PBr_5. Sulfonylating agents include, but are not limited to, alkanesulfonyl halides or anhydrides (preferably methanesulfonyl chloride or methanesulfonic anhydride), aryl sulfonyl halides or anhydrides (such as p-toluenesulfonyl chloride or anhydride), or haloalkylsulfonyl halides or anhydrides (preferably trifluoromethanesulfonic anhydride). Bases may include, but are not limited to, trialkylamines (preferably N,N-di-isopropyl-N-ethyl amine or triethylamine), bicyclic amidines (preferably DBU), anilines (preferably N-dimethyl aniline), or heteroaromatic amines (preferably pyridine). Inert solvents may include, but are not limited to, lower alkanenitriles (1-6 carbons) (preferably acetonitrile), dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or toluene), or haloalkanes with 1-10 carbons and 1-10 halogens (preferably dichloromethane). Preferred reaction temperatures range from -20°C to 100°C.

SCHEME 8



Alternatively, compounds of formula **23b** can be obtained by first reacting compounds of formula **22** with compounds of the formula R^1-(C=NH)-OR^h, where R^1 is defined above and R^g is a lower alkyl group (preferably methyl or ethyl), in the presence or absence of an acid in an inert solvent to give an intermediate of formula **24**. Compounds of formula **24** react with a compound of formula R^i-C=O-R^j, where R^i and R^j are each or independently lower alkoxy (preferably methoxy or ethoxy), 1-imidazolyl, halo, aryloxy (preferably 4-nitrophenoxy) in the presence or absence of an inert solvent to afford compounds of formula **23b**. Bases may include, but are not limited to, alkali metals (preferably sodium), alkali metal hydrides (preferably sodium hydride), alkali metal alkoxides (1-6 carbons) (preferably sodium methoxide, sodium ethoxide, or sodium tert-butoxide), alkaline earth metal hydrides, alkali metal dialkylamides (preferably lithium diisopropylamide), alkali metal carbonates, alkali metal hydroxides, alkali metal bis-(trialkylsilyl)amides (preferably lithium or sodium (trimethylsilyl)amide), trialkylamines (preferably N,N-di-isopropyl-N-ethyl amine or triethylamine), bicyclic amidines (preferably DBU), or heteroaromatic amines (preferably pyridine). Inert solvents may include, but are not limited to, alkyl alcohols (1-8 carbons) (preferably methanol, ethanol, or tert-butanol), lower alkanenitriles (1-6 carbons) (preferably acetonitrile), water, dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or toluene).

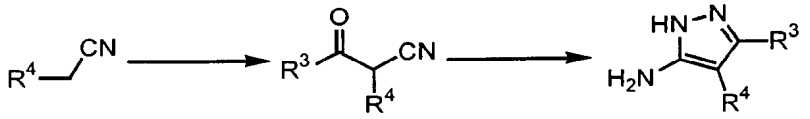
SCHEME 9



17 18 I

Compounds of formula I can also be prepared from compounds of formula **17** (prepared using the methods applicable to the synthesis of compounds of formula I), where P is H or an appropriate amino protecting group. Such groups, known in the art of organic synthesis for the protection of amines, include those listed in "Protective Groups in Organic Synthesis", by Greene and Wuts [John Wiley & Sons, NY, **1991**]. Examples of amine protecting groups include, but are not limited to, acyl types (such as formyl, trifluoroacetyl, phthalyl, and p-toluenesulfonyl), carbamate types (such as benzyloxycarbonyl, t-butoxycarbonyl, 9-fluorenymethyloxycarbonyl, allyloxycarbonyl, and 2,2,2-trichloroethyloxycarbonyl), alkyl types (such as benzyl and triphenylmethyl). Reacting compounds of formula **17** with a halogenating agent provides compounds of formula **18** where X is Br, Cl, or I. Compounds of formula **18** react with a compound of formula R^4M (where M is alkali metal, ZnCl, ZnBr, MgBr, MgCl, MgI, $CeCl_2$, $CeBr_2$, copper halides, $B(OH)_2$, B(O-lower alkyl)$_2$, or Sn(lower alkyl)$_3$) in the presence or absence of an organometallic catalyst in the presence or absence of a base in an inert solvent at temperatures ranging from −100°C to 200°C to give compounds of formula I (or their N-protected forms which can then be deprotected). Similar conditions have been described in WO 98/54093. Those skilled in the art will recognize that the reagents R^4M may be generated in situ. Organometallic catalysts include but are not limited to, palladium phosphine complexes (such as $Pd(PPh_3)_4$), palladium halides or alkanoates (such as $PdCl_2(PPh_3)_2$ or $Pd(OAc)_2$), or nickel complexes (such as $NiCl_2(PPh_3)_2$). Bases may include, but are not limited to, alkali metal alkoxides (1-6 carbons) (preferably sodium methoxide, sodium ethoxide, or sodium tert-butoxide), alkali metal carbonates or bicarbonates, alkali metal hydroxides, alkali metal phosphates, or trialkylamines (preferably N,N-di-isopropyl-N-ethyl amine or triethylamine). Inert solvents may include, but are not limited to, lower alkanenitriles (1-6 carbons) (preferably acetonitrile), water, dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or toluene).

SCHEME 10

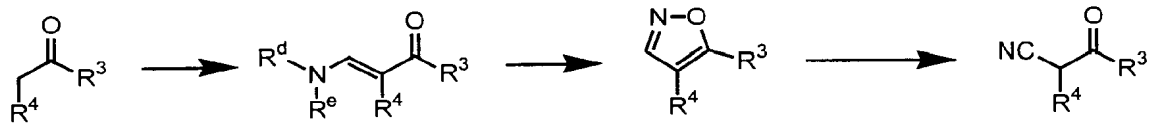


20 **21** **22**

Compounds of formula **22** may be obtained from compounds of formula **20**, where R^4 is defined as above. Compounds of formula **20** are reacted with compounds of formula R^3-C=O-R^c, where R^3 is defined above and R^c is halogen, cyano, lower alkoxy (1-6 carbons), or lower alkanoyloxy (1-6 carbons), in the presence of a base in an inert solvent at reaction temperatures ranging from –78°C to 200°C to afford compounds of formula **21**. Bases may include, but are not limited to, alkali metal hydrides (preferably sodium hydride), alkali metal alkoxides (1-6 carbons) (preferably sodium methoxide, sodium ethoxide, or sodium tert-butoxide), alkaline earth metal hydrides, alkali metal dialkylamides (preferably lithium diisopropylamide), alkali metal carbonates, alkali metal hydroxides, alkali metal bis-(trialkylsilyl)amides (preferably lithium or sodium (trimethylsilyl)amide), trialkylamines (preferably N,N-di-isopropyl-N-ethyl amine or triethylamine), bicyclic amidines (preferably DBU), or heteroaromatic amines (preferably pyridine). Inert solvents may include, but are not limited to, alkyl alcohols (1-8 carbons) (preferably methanol, ethanol, or tert-butanol), lower alkanenitriles (1-6 carbons) (preferably acetonitrile), water, dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or toluene). Alternatively, compounds of formula **20** may be reacted with a solvent of formula R^3-C=O-R^c, where R^3 is defined above and R^c is lower alkoxy (1-6 carbons), in the presence of an alkali metal (preferably sodium) at reaction temperatures ranging from –78°C to 200°C to afford compounds of formula **21**. Compounds of formula **21** may be reacted with hydrazine (hydrate or hydrochloride salt) in an inert solvent, at reaction temperatures ranging from 0°C to 200°C, preferably 70°C to 150°C, to afford compounds of formula **22**. Inert solvents may include, but are not limited to, water, lower alkanoic acids (preferably formic, acetic, or trifluoro acetic acid), alkyl alcohols (1-8 carbons) (preferably methanol or ethanol), lower alkanenitriles (1-6 carbons) (preferably acetonitrile), cyclic ethers

(preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or toluene).

SCHEME 11



24 **25** **26** **21**

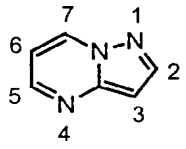
Alternatively, compounds of formula **21** can be obtained by first reacting compounds of formula **24** with dialkyl formamide dialkyl acetal of formula $(R^dR^e)N\text{-}CH(OR^f)_2$ where R^d, R^e, and R^f are each or independently C_1-C_6 lower alkyl (preferably methyl) in the presence or absence of an inert solvent at reaction temperatures ranging from 0°C to 250°C, preferably between 70C°C and 150°C to provide compounds of formula **25**. Inert solvents may include, but are not limited to, lower alkanenitriles (1-6 carbons) (preferably acetonitrile), dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or toluene), or haloalkanes with 1-10 carbons and 1-10 halogens (preferably dichloromethane). Compounds of formula 25 can be reacted with hydroxylamine salt (preferably hydrochloride) in the presence or absence of an inert solvent at reaction temperatures ranging from 0°C to 250°C, preferably between 70C°C and 200°C to provide oxazoles of formula **26**. Inert solvents may include, but are not limited to, alkyl alcohols (1-8 carbons) (preferably methanol, ethanol, or tert-butanol), lower alkanenitriles (1-6 carbons) (preferably acetonitrile), water, dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or toluene). Oxazole intermediates of formula **26**

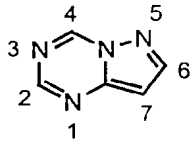
can be reacted with a base in the presence or absence of an inert solvent at reaction temperatures ranging from 0C°C to 200°C. Bases may include, but are not limited to, alkali hydroxides (preferably sodium or potassium hydroxide), alkali metal hydrides (preferably sodium hydride), alkali metal alkoxides (1-6 carbons) (preferably sodium methoxide, sodium ethoxide, or sodium tert-butoxide), alkaline earth metal hydrides, alkali metal dialkylamides (preferably lithium diisopropylamide), alkali metal carbonates, alkali metal hydroxides, alkali metal bis-(trialkylsilyl)amides (preferably lithium or sodium (trimethylsilyl)amide), trialkylamines (preferably N,N-di-isopropyl-N-ethyl amine or triethylamine), bicyclic amidines (preferably DBU), or heteroaromatic amines (preferably pyridine). Inert solvents may include, but are not limited to, alkyl alcohols (1-8 carbons) (preferably methanol, ethanol, or tert-butanol), lower alkanenitriles (1-6 carbons) (preferably acetonitrile), water, dialkyl ethers (preferably diethyl ether), cyclic ethers (preferably tetrahydrofuran or 1,4-dioxane), N,N-dialkylformamides (preferably dimethyl formamide), N,N-dialkylacetamides (preferably dimethyl acetamide), cyclic amides (preferably N-methylpyrrolidin-2-one), dialkylsulfoxides (preferably dimethylsulfoxide), aromatic hydrocarbons (preferably benzene or toluene).

EXAMPLES

The following examples listed in the TABLE are provided to describe the invention in further details. These examples can be prepared by one or more of the above-mentioned method and are intended to illustrate and not to limit the invention.

The numbering system used to describe the compounds of the present invention is as follows:





Commercial reagents were used without further purification. THF refers to tetrahydrofuran. LDA refers to lithium diisopropylamide and DBU refers to 1,8-diazabicyclo[5.4.0]undec-7-ene. Room or ambient temperature refers to 20°C to 25°C. Concentration implies the use of a rotary evaporator. TLC refers to thin layer chromatography.

Mass spectral data were obtained either by CI or APCI methods. Other commonly used abbreviations are: Ph is phenyl, Me is methyl, Et is ethyl, Pr is n-propyl, iPr is isopropyl, Bu is butyl, iBu is isobutyl (CH_2-$CHMe_2$), tBu is tert-butyl, cBu is cyclobutyl, Pent is n-pentyl, cPent is cyclopentyl, cHex is cyclohexyl, Py is pyridyl, MeOH means methanol, EtOH means ethanol, EtOAc means ethyl acetate, Et_2O means diethyl ether, CH_2Cl_2 means methylene chloride, DMSO means dimethyl sulfoxide, NMP means N-methyl pyrrolidone, THF means tetrahydrofuran, DMF means dimethyl formamide, EX means example,

EXAMPLE 1

Preparation of 7-(2-(cyclohexylamino)ethylamino)-2,5-dimethyl-3-(4-chloro-2,6-dimethylphenyl)-pyrazolo[1,5-a] pyrimidine

(formula I where X is CH, R^1 is CH_3, R^2 is H, A is CH_2, B is CH_2, R3 is CH_3, R^4 is 2,6-dimethyl-4-chlorophenyl, R^5 is cyclohexyl)

A. 4-Bromo-3,5-dimethyl chlorobenzene

Slurry 2,6-dimethyl-4-chloroaniline hydrochloride (23g, 193.11 g/mol) in CH_2Cl_2 (100 ml) and wash with saturated $NaHCO_3$ to generate the free base. Dry over Na_2SO_4, filter and evaporate down to a violet oil. Slurry up in 120 mL 6.0 N H_2SO_4 and stir vigorously at ambient temperature to break up larger pieces of solid. Cool to 0°C in an ice/water bath, then portionwise over 15 min add a clear colorless solution of $NaNO_2$ in 50 mL H_2O. Maintain temperature 15°C over course of addition, stirring under dry N_2. After 1 hour, carefully pour the cold reaction solution (solution A) into a second solution (solution B) containing 31.7 g CuBr in 33 mL aqueous HBr (48%) at ambient temperature. Let stand at ambient temperature until gas evolution ceases, then heat to 110°C under N_2 while stirring. Stir for 3 h, then cool to rt. Extract the aqueous layer with a (2:1) mixture of hexanes and Et_20 (2 x 500 mL), then dry the combined organic layers over Na_2SO_4, filter and evaporate down to a brown oil. Triturate the oil with hexanes (100 mL), filter out the remaining solids and wash with copious amounts of hexanes.

Evaporate the hexane layers to concentrate then flush through a pad of silica to remove baseline material, using hexanes as eluent. Evaporate to a clear colorless oil (13.5g).

B. 4-Chloro-2,6-dimethyl benzaldehyde

Dissolve 4-bromo-3,5-dimethyl chlorobenzene (6.5 g) in 50 mL anhydrous THF and cool to –78°C (dry ice/acetone) under N_2. Dropwise over 5 min add a solution of butyllithium (12.50 mL, 2.5M in hexanes) to the stirring solution of aryl bromide at –78°C. After 2 h, dropwise add anhydrous DMF (5.0 mL) to the orange/red reaction solution and allow to warm to ambient temperature overnight while stirring under N_2. Evaporate the yellow solution down to a yellow oil and partition between H_2O (100 mL) and CH_2Cl_2 (100 mL). Extract the aqueous layer once with CH_2Cl_2, then pool the organic layers and dry over Na_2SO_4, filter and evaporate down to 5.0 g of yellow oil. Use without further purification. LCMS = 169.6 (MH^+)

C. 4-Chloro-2,6-dimethyl benzyl alcohol

Dissolve 4-chloro-2,6-dimethyl benzaldehyde (5.0 g, 168.64 g/mol) in 100 mL dry MeOH. Cool to 0°C while stirring under N_2. Portionwise add powdered $NaBH_4$ (0.76g, 37.85 g/mol) over 5 min. Stir at 0°C for 2 h, monitoring by TLC until aldehyde consumed, then evaporate to a yellow oil. Add H_2O (50 mL) and bring to pH 7.0 by addition of saturated NH_4Cl. Extract the neutral aqueous layer with CH_2Cl_2 (3 x 75 mL) and dry the pooled organic layers over Na_2SO_4. Filter and concentrate to a yellow oil. Flush through a pad of silica to remove baseline material, then evaporate to a yellow solid (3.0 g) which can be used without further purification. LCMS = 171.6 (MH^+), 169.6 (M^-)

D. 4-Chloro-2,6-dimethyl phenyl acetonitrile

Dissolve 4-chloro-2,6-dimethyl benzyl alcohol (2.8g, 170.66 g/mol) in CH_2Cl_2 (25 mL) and cool to 0°C under N_2. Dropwise add thionyl chloride (2.4 mL, 3.90 g, 118.9 g/mol) in 10 mL CH_2Cl_2 while stirring under N_2. After 2 h, monitoring by TLC (alcohol Rf=0.35, chloride Rf=1.0; using 20% EtOAc / 80% hexanes as eluent), quench the reaction carefully by addition of

saturated $NaHCO_3$ (100 mL) and stir until gas evolution ceases. Separate layers, then extract the aqueous layer with CH_2Cl_2 (100 mL). Pool the organic layers, dry over Na_2SO_4, filter and evaporate to a pale yellow oil. Take up in DMSO (25 mL), add solid NaCN (1.25 g, 49.011 g/mol) and heat to 60°C while stirring under N_2. Stir 2 h until chloride consumed (TLC; chloride Rf=1.0, nitrile Rf=0.6; using 20% EtOAc / 80% hexanes as eluent), then cool to rt. Add 2.0 N NaOH (150 mL) and stir until orange precipitate forms, then filter and wash solid with H_2O. Dissolve solid in CH_2Cl_2, wash with H_2O, the dry over Na_2SO_4. Filter the organic layer and evaporate to an orange oil which crystallizes upon standing at ambient temperature. (2.3 g). LCMS = 180.2 (MH^+), 178.2 (M^-).

E. 2-(4-Chloro-2,6-dimethylphenyl)-3-oxobutanenitrile

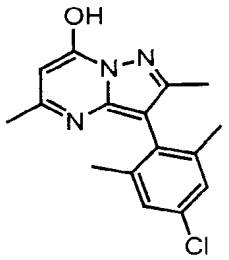
Dissolve 4-chloro-2,6-dimethyl phenyl acetonitrile (2.3 g, 179.2 g/mol) in 15 mL EtOAc and add sodium metal (0.35 g, pea-sized fragments). Heat to reflux (90°C bath temperature) under N_2 overnight. Evaporate down to solid and slurry up in Et_2O (100 mL); stir vigorously to break up fragments. Filter and wash solid with copious amounts of Et_2O. Dissolve solid in H_2O to form a clear yellow solution, and add 1.0 N HCl (100 mL) to pH 1. Extract the resulting cloudy solution with CH_2Cl_2 (3 x 100 mL) until aqueous layer is clear. Pool and dry the organic layers over Na_2SO_4, filter and evaporate to yellow oil (1.8g). TLC: Rf=0.2 using 20% EtOAc / 80% hexanes as eluent. LCMS = 222.3 (MH^+); 220.2 (M^-)

F. 5-Amino-4-(4-chloro-2,6-dimethylphenyl)-3-methylpyrazole

Dissolve anhydrous hydrazine (0.91g, 0.90 mL) in 20 mL toluene. Add glacial acetic acid (2.25 mL) and allow to stand at ambient temperature for 10 min until solution becomes cloudy white. Add a solution of 2-(4-chloro-2,6-dimethylphenyl)-3-oxobutanenitrile in 10 mL toluene, rinsing out the ketonitrile flask with an additional 5 mL toluene. Heat to reflux under N_2 (130°C) with Dean-Stark trap attached. Water will begin to accumulate after 10 min or so. After 2 h, evaporate down and partition between 1.0 N NaOH (100 mL) and EtOAc (100 mL). Extract aqueous layer with EtOAc (2 x 100 mL), then pool the organic layers and dry over Na_2SO_4.

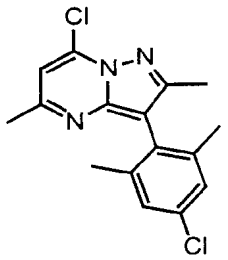
Filter and evaporate to yellow oil (1.75 g). Use without further purification. LCMS = 236.5 (MH^+); 234.5 (M^-).

G. 7-Hydroxy-2,5-dimethyl-3-(4-chloro-2,6-dimethylphenyl)-pyrazolo[1,5-a]pyrimidine



Dissolve 5-amino-4-(4-chloro-2,6-dimethylphenyl)-3-methylpyrazole in 20 mL glacial acetic acid at ambient temperature, and add ethyl acetoacetate (2.0 mL, 1.99 g). Heat to reflux (130°C) under N_2 overnight. Evaporate down to concentrate and add 200 mL Et2O to precipitate out product. Stir at ambient temperature for 1 hour, then filter and wash the resulting white solid (1.25 g) with copious amounts of Et_2O. LCMS = 302.2 (MH^+); 300.2 (M^-).

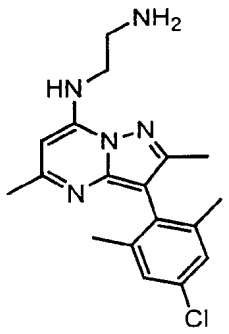
H. 7-Chloro-2,5-dimethyl-3-(4-chloro-2,6-dimethylphenyl)-pyrazolo[1,5-a]pyrimidine



Slurry 7-hydroxy-2,5-dimethyl-3-(4-chloro-2,6-dimethylphenyl)-pyrazolo[1,5-a] pyrimidine in 10 mL $POCl_3$ and reflux at 130°C under N_2. After 2 h, monitoring by TLC (alcohol Rf=0.5, chloride Rf=1.0; EtOAc as eluent), quench the reaction carefully at ambient temperature by diluting with 50 mL CH_2Cl_2 and pouring slowly into non-stirring saturated $NaHCO_3$. Adjust stirring speed to control rate of quenching of residual $POCl_3$ and stir until gas evolution ceases. Separate the layers and extract the aqueous layer with CH_2Cl_2 (2 x 50 mL).

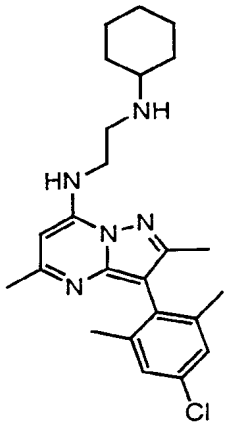
Pool the organic layers and dry over Na_2SO_4. Filter and evaporate to yellow oil, which is used directly without further purification.

I. 7-(2-aminoethylamino)-2,5-dimethyl-3-(4-chloro-2,6-dimethylphenyl)-pyrazolo[1,5-a] pyrimidine



Dissolve 7-chloro-2,5-dimethyl-3-(4-chloro-2,6-dimethylphenyl)-pyrazolo[1,5-a] pyrimidine in 25 mL CH_3CN, then add excess ethylenediamine (5 mL) and heat to 80°C for 3-6 h under N_2 with attached reflux condenser. (TLC; product diamine Rf=0.5, aryl chloride Rf=1.0; [10% (2.0M NH_3 in MeOH) / 90% CH_2Cl_2] as eluent). Cool to ambient temperature and evaporate to yellow oil. Partition between CH_2Cl_2 (50 mL) and 1.0 N NaOH (50 mL), and extract aqueous layer 2 x 30 mL CH_2Cl_2. Pool organic layers, dry over Na_2SO_4, filter and evaporate to yellow-white foam (1.25 g). Use without further purification. LCMS = 344.4 (MH^+); 342.3 (M^-).

J. 7-(2-(Cyclohexylamino)ethylamino)-2,5-dimethyl-3-(4-chloro-2,6-dimethylphenyl)-pyrazolo[1,5-a] pyrimidine



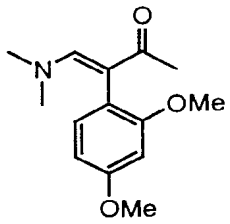
Dissolve 7-(2-aminoethylamino)-2,5-dimethyl-3-(4-chloro-2,6-dimethylphenyl)-pyrazolo [1,5-a] pyrimidine (0.183g, 5.4 x 10^{-4} mol, 339.2 g/mol) in dichloroethane (5 mL) and add cyclohexanone (100.12 g/mol) and sodium triacetoxyborohydride (0.172 g, 211.94 g/mol). To the resultant slurry, add glacial acetic acid (0.032 mL, 5.4 x 10^{-4} mol) and stir at ambient temperature under N_2 for 3 h. Partition between CH_2Cl_2 (3 mL) and 1.0 N NaOH (10 mL), then separate the layers and chromatograph the CH_2Cl_2 layer using [10% (2.0M NH_3 in MeOH) / 90% CH_2Cl_2] as eluent. Obtained 0.16 g white solid-foam upon evaporation.

EXAMPLE 2

Preparation of 7-(2-cyclopentylamino)ethylamino)-3-(2,4-dimethoxyphenyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine

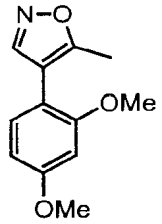
(formula I where X is CH, R^1 is CH_3, R^2 is H, A is CH_2, B is CH_2, R^3 is CH_3, R^4 is 2,4-dimethoxyphenyl, R^5 is cyclopentyl)

A. (3E)-3-(2,4-dimethoxyphenyl)-4-(dimethylamino)but-3-en-2-one



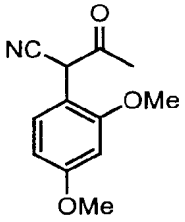
Dissolve 1-(2,4-dimethoxyphenyl)acetone (1.0 g, 5.15 mmol, 194.23 g/mol) in DMF-diethyl acetal (4.5 mL, 25.7 mmol, 147.22 g/mol) and stir under N_2 at 100°C overnight. TLC using 20% EtOAc / 80% hexanes; (ketone Rf = 0.25, product Rf = 0.0). Evaporate to thick oil, dissolve in EtOAc (25 mL) and wash with H_2O (3 x 25 mL). Extract pooled H_2O layers with EtOAc. Dry pooled organic layers over Na_2SO_4, filter and evaporate to thick oil which solidifies upon standing at ambient temperature (0.98 g). Use without further purification. LCMS = 250.2 (MH^+); 248.2 (M^-).

B. 4-(2,4-Dimethoxyphenyl)-5-methyl-isoxazole



Dissolve (3E)-3-(2,4-dimethoxyphenyl)-4-(dimethylamino)but-3-en-2-one (5.1 g, 20.6 mmol) in EtOH (50 mL) and add NH_2OH•HCl (3.05 g, 44.0 mmol). Heat to reflux under N_2 for 20 min. Cool and evaporate to red-brown oil. Dissolve in CH_2Cl_2, dry over Na_2SO_4, filter and concentrate to red-brown oil (4.4 g). Use without further purification. LCMS = 220.2 (MH^+); 218.2 (M^-).

C. 2-(2,4-Dimethoxyphenyl)-3-oxobutanenitrile.



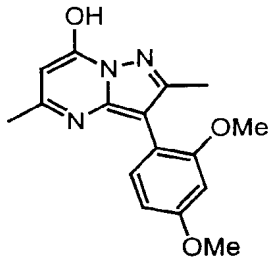
Slurry 4-(2,4-dimethoxyphenyl)-5-methyl-isoxazole (4.4 g) in 1.0 N NaOH (35 mL) and add 35 mL MeOH to dissolve. Heat at 60°C under N_2 for 1 hour, then cool to clear brown solution. Add 1.0 N HCl to acidify to pH 1, then filter the resulting white solid precipitate.

Dissolve solid in EtOAc, dry over Na_2SO_4, filter and concentrate to red oil. Use without further purification. LCMS = 220.2 (MH^+); 218.2 (M^-).

D. 5-Amino-4-(2,4-dimethoxylphenyl)-3-methylpyrazole

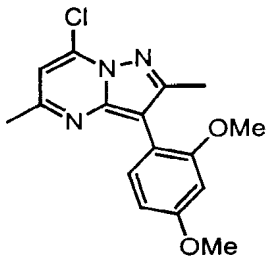
This compound was prepared as described in Example 1F.

E. 7-Hydroxy-2,5-dimethyl-3-(2,4-dimethoxyphenyl)-pyrazolo[1,5-a]pyrimidine



This compound was prepared as described in Example 1G.

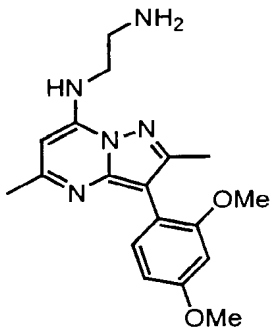
F. 7-Chloro-2,5-dimethyl-3-(2,4-dimethoxyphenyl)-pyrazolo[1,5-a]pyrimidine



Slurry 7-hydroxy-2,5-dimethyl-3-(2,4-dimethoxyphenyl)-pyrazolo[1,5-a] pyrimidine in 10 mL $POCl_3$ and reflux at 130°C under N_2. After 2 h, monitoring by TLC (alcohol Rf=0.5, chloride Rf=1.0; EtOAc as eluent), quench the reaction carefully at ambient temperature by diluting with 50 mL CH_2Cl_2 and pouring slowly into non-stirring saturated $NaHCO_3$. Adjust stirring speed to control rate of quenching of residual $POCl_3$ and stir until gas evolution ceases. Separate the layers and extract the aqueous layer with CH_2Cl_2 (2 x 50 mL). Pool the organic

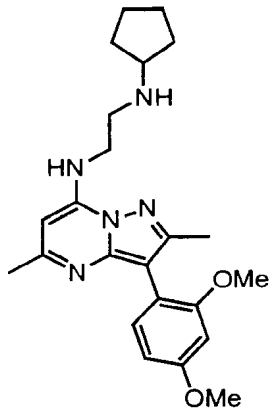
layers and dry over Na_2SO_4. Filter and evaporate to yellow oil, which is used directly without further purification.

G. 7-(2-aminoethylamino)-2,5-dimethyl-3-(2,4-dimethoxyphenyl)-pyrazolo[1,5-a] pyrimidine



Dissolve 7-chloro-2,5-dimethyl-3-(2,4-dimethoxyphenyl)-pyrazolo[1,5-a] pyrimidine in 25 mL CH_3CN, then add excess ethylenediamine (5 mL) and heat to 80°C for 3-6 h under N_2 with attached reflux condenser. (TLC: [10% (2.0M NH_3 in MeOH) / 90% CH_2Cl_2] as eluent). Cool to ambient temperature and evaporate to yellow oil. Partition between CH_2Cl_2 (50 mL) and 1.0 N NaOH (50 mL), and extract aqueous layer 2 x 30 mL CH_2Cl_2. Pool organic layers, dry over Na_2SO_4, filter and evaporate to yellow-white foam. Use without further purification.

H. 7-(2-cyclopentylamino)ethylamino)-3-(2,4-dimethoxyphenyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine.



This compound was prepared as described in Example 1J.

EXAMPLE 3

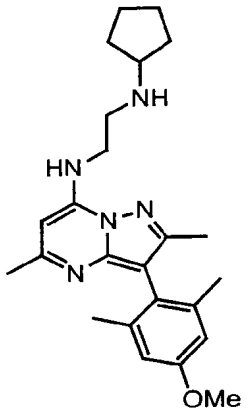
Preparation of 7-(2-(cyclopentylamino)ethylamino)-2,5-dimethyl-3-(2,6-dimethyl-4-methoxyphenyl)-pyrazolo[1,5-a] pyrimidine

(formula I where X is CH, R^1 is CH_3, R^2 is H, A is CH_2, B is CH_2, R^3 is CH_3, R^4 is 2,4-dimethyl-4-methoxyphenyl, R^5 is cyclopentyl)

A. 4-Methoxy-2,6-dimethyl phenyl acetonitrile

Dropwise add a solution of chlorotrimethylsilane (20 mL) in CH_2Cl_2 (40 mL) to a stirred solution cooled to 0°C of 4-methoxy-2,6-dimethyl benzyl alcohol (approx. 74 mmol) in 300 mL CH_2Cl_2. Solution changes color from colorless to yellow and then to purple over the course of the reaction. After 2 h, monitoring by TLC (alcohol Rf=0.25, chloride Rf=0.95; using 20% EtOAc / 80% hexanes as eluent), evaporate down to a yellow oil. Dissolve in dry DMF (50 mL) and cool to 0°C under N_2. Add freshly ground NaCN (7.0 g) portionwise over 5 minute (exothermic) to the stirring reaction, forming a yellow/white slurry. Stir for 5-8 h at 0°C until no starting material remains, as determined by TLC (nitrile Rf=0.5; using 20% EtOAc / 80% hexanes as eluent). Partition the reaction solution between EtOAc (100 mL) and 0.1 N NaOH (300 mL). Dry the EtOAc layer over Na2SO4, filter and evaporate to yellow oil. Chromatograph in 10% EtOAC / 90% hexanes on silica to remove residual chloride and evaporate to 2.1 g yellow solid; clean by TLC. LCMS = 176.5 (MH^+), 174.4 (M^-).

B. 7-(2-(Cyclopentylamino)ethylamino)-2,5-dimethyl-3-(2,6-dimethyl-4-methoxyphenyl)-pyrazolo[1,5-a] pyrimidine



7-(2-(cyclopentylamino)ethylamino)-2,5-dimethyl-3-(2,6-dimethyl-4-methoxyphenyl)-pyrazolo[1,5-a] pyrimidine is obtained from 4-methoxy-2,6-dimethyl phenyl acetonitrile using the procedures described in EXAMPLE 1 E, F, G, H, I, and J.

EXAMPLE 4

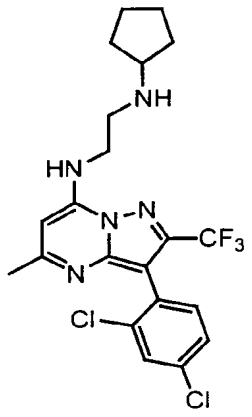
Preparation of 7-(2-(cyclopentylamino)ethylamino)-2-trifluoromethyl-5-methyl-3-(2,4-dichlorophenyl)-pyrazolo[1,5-a] pyrimidine

(formula I where X is CH, R^1 is CH_3, R^2 is H, A is CH_2, B is CH_2, R^3 is CF_3, R^4 is 2,4-dichlorophenyl, R^5 is cyclopentyl)

A. 2-(2,4-Dichlorophenyl)-4,4,4-trifluoro-3-oxobutanenitrile

Slurry 2,4-dichlorophenylacetonitrile (I) (5.0 g, 26.9 mmol, 186.04 g/mol) in ethyl trifluoroacetate (6.4 mL, 7.6 g, 142.08 g/mol) and add 20 mL anhydrous THF. Portionwise at ambient temperature add NaH (1.88g, 47.1 mmol, 60% in mineral oil) over 5 min. Heat reaction to reflux (90°C bath temperature) overnight. Evaporate to thick red-brown oil and partition between Et_2O (100 mL) and H_2O (60 mL). Separate layers and extract H_2O with Et_2O (2 x 75 mL). Acidify the aqueous layer with 1.0 N HCl to pH 1 (becomes cloudy white suspension) and extract aqueous layer with CH_2Cl_2 (3 x 100 mL). Dry pooled CH_2Cl_2 layers over Na_2SO_4, filter and concentrate to yellow oil (7.5 g, 26.5 mmol). Use without further purification. LCMS = 281.9 (MH^+); 279.8 (M^-)

B. 7-(2-(Cyclopentylamino)ethylamino)-2-trifluoromethyl-5-methyl-3-(2,4-dichlorophenyl)-pyrazolo[1,5-a] pyrimidine



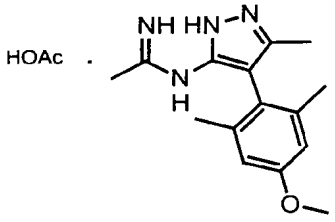
7-(2-(Cyclopentylamino)ethylamino)-2-trifluoromethyl-5-methyl-3-(2,4-dichlorophenyl)-pyrazolo[1,5-a] pyrimidine is obtained from 2-(2,4-dichlorophenyl)-4,4,4-trifluoro-3-oxobutanenitrile using the procedures described in EXAMPLE 1 F, G, H, I, and J.

EXAMPLE 5

Preparation of 7-(2-(cyclopentylamino)ethylamino)-2,5-dimethyl-3-(4-methoxy-2,6-dimethylphenyl)-[1,5-a]-pyrazolo-1,3,5-triazine.

(formula I where X is N, R^1 is CH_3, R^2 is H, A is CH_2, B is CH_2, R^3 is CH_3, R^4 is 2,6-dimethyl-4-methoxyphenyl, R^5 is cyclopentyl)

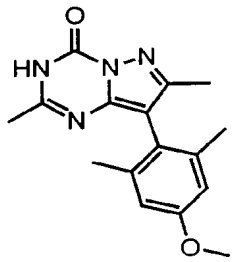
A. (Iminoethyl)[4-(4-methoxy-2,6-dimethylphenyl)-3-methylpyrazol-5-yl]amine acetate salt.



To a solution of 5-amino-4-(4-methoxy-2,6-dimethylphenyl)-3-methylpyrazole (1.89 g) in acetonitrile (30 mL) add ethylacetimidate (free base, 1.8 mL) followed by acetic acid (0.47

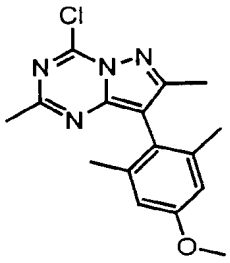
mL). Collect the precipitate that formed upon stirring overnight by filtration. Wash the solid with dry ether and dry to afford 2.61 g of (iminoethyl)[4-(4-methoxy-2,6-dimethylphenyl)-3-methylpyrazol-5-yl]amine acetate salt as a white powder.

B. 2,6-Dimethyl-7-(2,6-dimethyl-4-methoxyphenyl)-3H-[1,5-c]-pyrazolo-1,3,5-triazin-4-one.



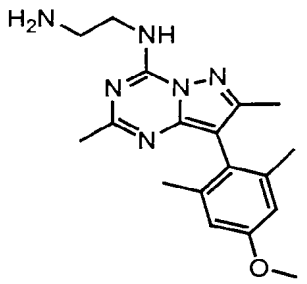
Add sodium pieces (1.81 g) to a flask containing anhydrous ethanol and equipped with a reflux condenser. Allow the mixture to stir until all the sodium is consumed and then add the amidine (2.61 g as the acetate salt) from step A in one portion. Add diethyl carbonate (7.6 mL) and reflux the mixture overnight. Concentrate the mixture under reduced pressure, dissolve the residue in water (75 mL) and adjust the pH to 5 with 3N HCl. Extract the aqueous mixture with EtOAc and wash the extracts with brine, dry over anhydrous sodium sulfate, and concentrate in vacuo to obtain a foam. Stir the residue with hexanes for 20 min and collect the solid by filtration, then wash with hexanes to obtain 2.01 g of 2,6-dimethyl-7-(2,6-dimethyl-4-methoxyphenyl)-3H-[1,5-c]-pyrazolo-1,3,5-triazin-4-one as a yellow powder: MS 299 (M+H).

C. 4-Chloro-2,6-dimethyl-7-(2,6-dimethyl-4-methoxyphenyl) [1,5-a]-pyrazolo-1,3,5-triazine.



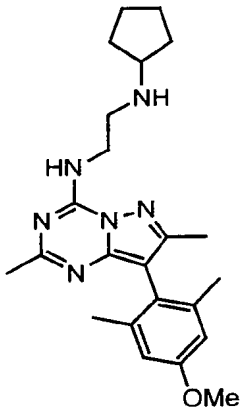
Dissolve 2,6-dimethyl-7-(2,6-dimethyl-4-methoxyphenyl)-3H-[1,5-c]-pyrazolo-1,3,5-triazin-4-one from step B (1 g) in $POCl_3$ (50 mL) and add N,N-dimethylaniline (0.55 mL). Reflux the reaction mixture under a dry nitrogen atmosphere for 18h at which time concentrate the mixture under reduced pressure. Dissolve the residue in EtOAc and wash with a saturated aqueous $NaHCO_3$ solution, then with brine. Dry the organic layer over anhydrous sodium sulfate, filter and concentrate under reduced pressure to obtain 4-chloro-2,6-dimethyl-7-(2,6-dimethyl-4-methoxyphenyl) [1,5-a]-pyrazolo-1,3,5-triazine as a dark oil. MS 317 (M+H).

D. 2,6-Dimethyl-7-(2,6-dimethyl-4-methoxyphenyl)-4-(2-aminoethyl)amino-[1,5-a]-pyrazolo-1,3,5-triazine.



Dissolve 4-chloro-2,6-dimethyl-7-(2,6-dimethyl-4-methoxyphenyl) [1,5-a]-pyrazolo-1,3,5-triazine in dry toluene (10 mL) to form a stock solution of the chloride (~0.34 M). Add a portion of this solution (8 mL) dropwise into a stirring solution of ethylenediamine (3.6 mL) in acetonitrile (50 mL) which is heated to 60°C. After 3h at 60°C, cool the solution, concentrate under reduced pressure, dilute with 10% NaOH and extract with EtOAc. Wash the combined extracts with brine, dry over anhydrous sodium sulfate and concentrate under reduced pressure to obtain a yellow residue. Triturate the residue with 20% EtOAc/hexanes and collect the resulting solid by filtration to obtain 0.72 g of 2,6-dimethyl-7-(2,6-dimethyl-4-methoxyphenyl)-4-(2-aminoethyl)amino-[1,5-a]-pyrazolo-1,3,5-triazine as a yellow solid: MS 341 (M+H).

E. 7-(2-(Cyclopentylamino)ethylamino)-2,5-dimethyl-3-(4-methoxy-2,6-dimethylphenyl)-[1,5-a]-pyrazolo-1,3,5-triazine.



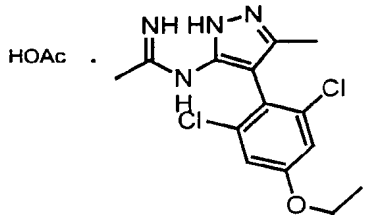
Dissolve 2,6-dimethyl-7-(2,6-dimethyl-4-methoxyphenyl)-4-(2-aminoethyl)amino-[1,5-a]-pyrazolo-1,3,5-triazine from step D (0.211 g) in dry dichloroethane (15 mL) and add cyclopentanone (1 equiv). Add acetic acid (35 μL) followed by sodium triacetoxyborohydride (0.184 g) and stir the resulting homogeneous mixture overnight at ambient temperature. Dilute the reaction mixture with 4 volumes of CH_2Cl_2, wash with brine, dry over anhydrous sodium sulfate, and concentrate under reduced pressure to obtain a yellow solid. Purify using preparative thin-layer chromatography [10% MeOH(2N NH_3)/CH_2Cl_2] to obtain 7-(2-(cyclopentylamino)ethylamino)-2,5-dimethyl-3-(4-methoxy-2,6-dimethylphenyl)-[1,5-a]-pyrazolo-1,3,5-triazine.

EXAMPLE 6

Preparation of 7-(2-(cyclopentylamino)ethylamino)-2,5-dimethyl-3-(4-ethoxy-2,6-dichlorophenyl)-[1,5-a]-pyrazolo-1,3,5-triazine.

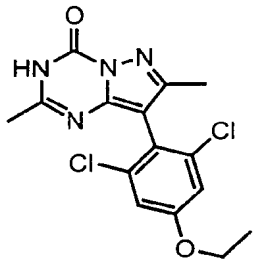
formula I where X is N, R^1 is CH_3, R^2 is H, A is CH_2, B is CH_2, R^3 is CH_3, R^4 is 2,6-dichloro-4-ethoxyphenyl, R^5 is cyclopentyl.

A. (Iminoethyl)[4-(4-ethoxy-2,6-dichlorophenyl)-3-methylpyrazol-5-yl]amine acetate salt.



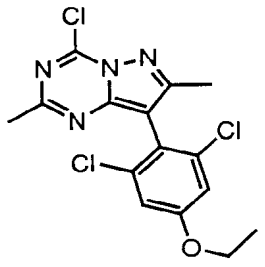
To a solution of 5-amino-4-(4-ethoxy-2,6-dichlorophenyl)-3-methylpyrazole (4.8 g) in acetonitrile (50 mL) add ethylacetimidate (free base, 2.3 mL) followed by acetic acid (0.96 mL). Collect the precipitate that formed upon stirring overnight by filtration. Wash the solid with dry ether and dry to afford 5.02 g of (iminoethyl)[4-(4-ethoxy-2,6-dichlorophenyl)-3-methylpyrazol-5-yl]amine acetate salt as a white powder.

B. 2,6-Dimethyl-7-(2,6-dichloro-4-ethoxyphenyl)-3H-[1,5-c]-pyrazolo-1,3,5-triazin-4-one.



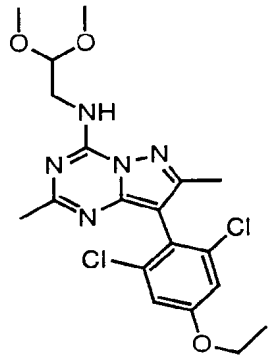
Add sodium pieces (2.98 g) to a flask containing anhydrous ethanol and equipped with a reflux condenser. Allow the mixture to stir until all the sodium is consumed and then add the amidine (5.02 g as the acetate salt) from step A in one portion. Add diethyl carbonate (12.6 mL) and reflux the mixture for four h. Concentrate the mixture under reduced pressure, dissolve the residue in water (75 mL) and adjust the pH to 5 with 3N HCl. Extract the aqueous mixture with EtOAc and wash the extracts with brine, dry over anhydrous sodium sulfate, and concentrate in vacuo to obtain a foam. Stir the residue with hexanes for 20 min and collect the solid by filtration, then wash with hexanes to obtain 4.41 g of 2,6-dimethyl-7-(2,6-dichloro-4-ethoxyphenyl)-3H-[1,5-c]-pyrazolo-1,3,5-triazin-4-one as a beige solid: MS 353 (M+H).

C. 4-Chloro-2,6-dimethyl-7-(2,6-dichloro-4-ethoxyphenyl) [1,5-a]-pyrazolo-1,3,5-triazine.



Dissolve 2,6-dimethyl-7-(2,6-dichloro-4-ethoxyphenyl)-3H-[1,5-c]-pyrazolo-1,3,5-triazin-4-one from step B (1.05 g) in $POCl_3$ (50 mL) and add 2,6-lutidine (0.45 mL). Reflux the reaction mixture under a dry nitrogen atmosphere for 48h and then concentrate the mixture under reduced pressure. Dissolve the residue in EtOAc and wash with a saturated aqueous $NaHCO_3$ solution, then with brine. Dry the organic layer over anhydrous sodium sulfate, filter and concentrate under reduced pressure to obtain an oil which crystallizes upon standing. Wash the solid with hexanes to remove residual 2,6-lutidine and collect the solid on a sintered glass funnel yielding 4-chloro-2,6-dimethyl-7-(2,6-dichloro-4-ethoxyphenyl) [1,5-a]-pyrazolo-1,3,5-triazine. MS 372 (M+H).

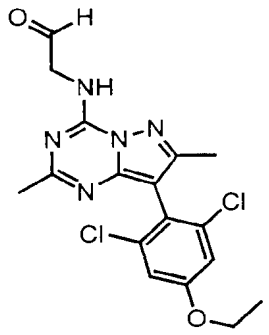
D. 2,6-Dimethyl-7-(2,6-dichloro-4-ethoxyphenyl)-4-(2,2-dimethoxyethyl)amino-[1,5-a]-pyrazolo-1,3,5-triazine.



Dissolve the product from step C in dry acetonitrile and then add 2.1 equivalents of aminoacetaldehyde dimethyl acetal. Heat the solution to 60°C and stir under a dry nitrogen

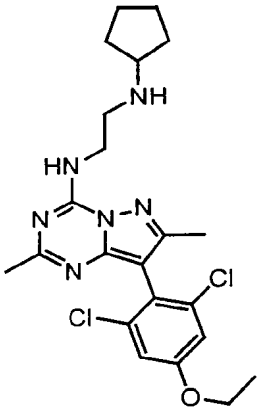
atmosphere for 2-6 h. Remove the solvent under reduced pressure, dilute with 10% NaOH and extract with EtOAc. Wash the combined extracts with brine, dry over anhydrous sodium sulfate and concentrate under reduced pressure to obtain a yellow oil which crystallizes upon standing. The product, 2,6-dimethyl-7-(2,6-dichloro-4-ethoxyphenyl)-4-(2,2-dimethoxyethyl)amino-[1,5-a]-pyrazolo-1,3,5-triazine, is used without further purification. MS (M+H).

E. 2-{[7-(2,6-dichloro-4-ethoxyphenyl)-2,5,6-trimethyl-3-pyrazolino[2,3-a]1,3,5-triazin-4-yl]amino}ethanal.



Dissolve the product obtained from step D in neat trifluoroacetic acid (25mL). After allowing the mixture to stand at ambient temperature for 0.5h, concentrate the mixture under reduced pressure. Add saturated aqueous sodium bicarbonate and stir the resulting heterogeneous mixture for 0.5h. Extract the aqueous solution with EtOAc, wash the EtOAc extracts with brine and then dry over anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure yields the aldehyde as an off-white foam. ^{1}H NMR (CDCl3): δ 9.79 (s, 1H, CHO).

F. 7-(2-(Cyclopentylamino)ethylamino)-2,5-dimethyl-3-(4-ethoxy-2,6-dichlorophenyl)-[1,5-a]-pyrazolo-1,3,5-triazine.



Dissolve the aldehyde (62 mg, 0.16 mmol) obtained from step E in dry dichloroethane (4 mL). Add 1.1 equivalents of cyclopentylamine followed by 1 equivalent of acetic acid. After the addition of sodium triacetoxyborohydride (1.4 eq), stir the solution at ambient temperature for several h. Dilute the reaction mixture with 4 volumes of CH_2Cl_2 then wash the mixture with brine (1x), dry over anhydrous Na2SO4. Concentrate under reduced pressure. Preparative thin layer chromatography [10% MeOH(2N NH_3)/CH_2Cl_2)] of the oily residue yields7-(2-(cyclopentylamino)ethylamino)-2,5-dimethyl-3-(4-ethoxy-2,6-dichlorophenyl)-[1,5-a]-pyrazolo-1,3,5-triazine.

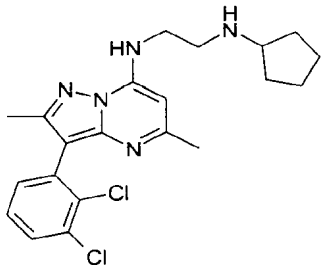
The preparation of the compounds of the present invention by the above-mentioned methods is illustrated further by the following examples, delineated in the TABLE which are not to be construed as limiting the invention in scope or spirit to the specific procedures and compounds described in them. Commonly used abbreviations are: Ph is phenyl, Me is methyl, Et is ethyl, Pr is n-propyl, iPr is isopropyl, cPr is cyclopropyl, Bu is butyl, iBu is isobutyl (CH_2-$CHMe_2$), tBu is tert-butyl, cBu is cyclobutyl, Pent is n-pentyl, cPent is cyclopentyl, cHex is cyclohexyl, Py is pyridyl, Bn is benzyl (CH_2Ph), Ac is acetyl (CH_3-(C=O)), tBOC is tert-butyloxycarbonyl (tBuO-(C=O)). EX means example.

By way of further exemplification, details of the preparation used in Example 14 which is included in the Table are as follows:

EXAMPLE 14

Preparation of N-cyclopentyl-N'-[3-(2,3-dichloro-phenyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-yl]-ethane-1,2-diamine hydrochloride salt

formula I where X is CH, R^1 is CH_3, R^2 is H, A is CH_2, B is CH_2, R^3 is CH_3, R^4 is 2,3-dichloro-4-ethoxyphenyl, R^5 is cyclopentyl, R^6 is H.



A. 7-Chloro-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine

2,5-Dimethyl-4H-pyrazolo[1,5-a]pyrimidin-7-one (3.5 g, 21 mmol) in phosphorus oxychloride (20 mL) was stirred at reflux. After 3 hours the dark purple reaction was cooled and then extracted from ice water with ethyl acetate. The combined organic layers were washed with water and saturated aqueous sodium bicarbonate, dried (Na_2SO_4) and then concentrated under reduced pressure to give the title compound (3.0 g, 77%) as a brown solid: +APcI MS $(M+1)^+$ 182; ^{1}H NMR ($CDCl_3$) δ: 6.74 (s, 1H), 6.42 (s, 1H), 2.55 (s, 3H), 2.53 (s, 3H).

B. N-(2,5-Dimethyl-pyrazolo[1,5-a]pyrimidin-7-yl)-ethane-1,2-diamine

To a stirred solution of 7-chloro-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine (3.0 g, 16.5 mmol) in ethanol (60 mL) was added ethylene diamine (4 mL, 60 mmol). After refluxing 18 hours the reaction was cooled, poured into saturated aqueous sodium bicarbonate, and then washed with ethyl acetate. The aqueous layer was then extracted with 3:7 isopropyl alcohol/chloroform, the combined extracts were dried (Na_2SO_4) and then concentrated under reduced pressure to give the title compound as an amorphous brown solid (3.2 g, 94%): +APcI MS $(M+1)^+$ 206; ^{1}H NMR (methanol-d_4) δ: 6.05 (s, 1H), 6.00 (s, 1H), 3.45 (m, 2H), 2.91 (m, 2H).

C. N-Cyclopentyl-N'-(2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-yl)-ethane-1,2-diamine

To a stirred solution of N-(2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-yl)-ethane-1,2-diamine (3.2 g, 16 mmol) and cyclopentanone (2.7 mL, 30 mmol) in methanol (30 mL)/acetic acid (3 mL) was added sodium cyanoborohydride (0.94 g, 15 mmol), portionwise. After stirring 1 hour the reaction was concentrated under reduced pressure and then extracted from saturated aqueous sodium bicarbonate with ethyl acetate. The combined extracts were dried (Na_2SO_4), concentrated under reduced pressure to give the crude product as a brown oil (5.6 g): +APcI MS $(M+1)^+$ 274; ^1H NMR ($CDCl_3$) δ: 6.11 (s, 1H), 6.76 (s, 1H), 3.21 (t, 2H), 3.21 (m, 1H), 3.04 (t, 1H), 2.42 (s, 3H), 2.40 (s, 3H).

D. Cyclopentyl-[2-(2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-ylamino)-ethyl]-carbamic acid tert-butyl ester

To a stirred solution of the crude N-cyclopentyl-N'-(2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-yl)-ethane-1,2-diamine (5.6 g) in methylene chloride (24 mL)/methanol (2 mL) was added di-tert-butyl dicarbonate (5.1 g, 23 mmol) and then 4- dimethylaminopyridine (0.6 g, 4.9 mmol). After stirring 5 hours the reaction was concentrated under reduced pressure and then chromatographed (1:1 ethyl acetate/hexanes) to afford the title compound as an orange glass (2.3 g, 39% from B)): +APcI MS $(M+1)^+$ 374; ^1H NMR ($CDCl_3$) δ: 6.16 (s, 1H), 5.78 (s, 1H), 4.25-4.15 (br s, 1H), 3.47 (m, 4H), 2.47 (s, 3H), 2.41 (s, 3H), 1.51 (s, 9H).

E. Cyclopentyl-[2-(3-iodo-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-ylamino)-ethyl]-carbamic acid tert-butyl ester

To a 0 °C stirred solution of cyclopentyl-[2-(2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-ylamino)-ethyl]-carbamic acid tert-butyl ester (89 mg, 0.24 mmol) in chloroform (5 mL) was added N-iodosuccinimide (54 mg, 0.24 mmol). After stirring 30 minutes the reaction was extracted from saturated aqueous sodium thiosulfate with chloroform. The combined extracts were dried (Na_2SO_4), concentrated under reduced pressure to give the product as a slightly yellowed glass

(102 mg, 85%): +APcI MS $(M+1)^+$ 500; ^{1}H NMR ($CDCl_3$) δ: 5.82 (s, 1H), 4.25-4.10 (br s, 1H), 3.46 (s, 4H), 2.51 (s, 3H), 2.41 (s, 3H), 1.50 (s, 9H).

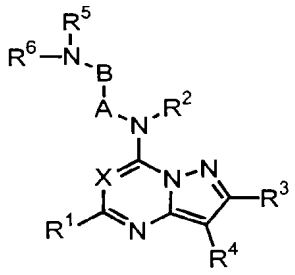
F. Cyclopentyl-{2-[3-(2,3-dichloro-phenyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-ylamino]-ethyl}-carbamic acid tert-butyl ester

A suspension of cyclopentyl-[2-(3-iodo-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-ylamino)-ethyl]-carbamic acid tert-butyl ester (38 mg, 0.076 mmol) and 2,3-dichlorobenzeneboronic acid in toluene (0.5 mL)/ethanol (0.5 mL)/2 M aqueous sodium carbonate (0.22 mL) was degassed (3X) by alternately pulling a vacuum followed by repressurization with nitrogen. Tetrakis(triphenylphosphine)palladium (10 mg, 0.009 mmol) was added, and the mixture was degassed (3X) again. The mixture was stirred for 2.5 hours at 100 °C, concentrated under reduced pressure, and then chromatographed (4:1 hexanes/ethyl acetate) to give the product as colorless oil (24 mg, 60%): +APcI MS $(M+1)^+$ 518; ^{1}H NMR ($CDCl_3$) δ: 7.42 (dd, 1H), 7.30 (dd, 1H), 7.22 (t, 1H), 5.84 (s, 1H), 4.25-4.10 (br s, 1H), 3.49 (s, 4H), 2.44 (s, 3H), 2.32 (s, 3H), 1.52 (s, 9H).

G. N-Cyclopentyl-N'-[3-(2,3-dichloro-phenyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-yl]-ethane-1,2-diamine hydrochloride salt

The cyclopentyl-{2-[3-(2,3-dichloro-phenyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-ylamino]-ethyl}-carbamic acid tert-butyl ester (21mg, 0.040 mmol) was stirred for 1 hour in 2:1 ethanol/concentrated aqueous hydrochloric acid (1.5 mL), concentrated under reduced pressure, and then concentrated 2 additional times from ethanol to give the title compound (17 mg, 94%): +APcI MS $(M+1)^+$ 418; ^{1}H NMR (methanol-d_4) δ: 7.69 (d, 1H), 7.50-7.40 (m, 2H), 6.78 (s, 1H), 4.06 (br s, 2H), 3.63 (br m, 1H), 3.42 (br s, 2H), 2.60 (s, 3H), 2.34 (s, 3H).

Table of Examples



EX	X	R^1	R^2	R^3	R^4	A-B-N[R^6]-R^5	MW
7.	CH	Me	H	Me	2-Cl-4-F-Ph	(CH2)2-NH-cHex	415.93
8.	CH	Me	H	Me	2-Cl-4-F-Ph	(CH2)2-NH-cPent	401.92
9.	CH	Me	H	Me	2-Cl-4-OEt-Ph	(CH2)2-NH-cPent	427.98
10.	CH	Me	H	Me	2-Cl-6-F-Ph	(CH2)2-NH-cPent	401.91
11.	CH	Me	H	Me	2-Cl-Ph	(CH2)2-NH-cPent	383.93
12.	CH	Me	H	Me	2-F-4-Br-Ph	(CH2)2-NH-cPent	482.83
13.	CH	Me	H	Me	2-F-Ph	(CH2)2-NH-cPent	367.47
14.	CH	Me	H	Me	2,3-diCl-Ph	(CH2)2-NH-cPent	418.37
15.	CH	Me	H	Me	2,4-diCl-6-OMe-Ph	(CH2)2-NH-cPent	448.4
16.	CH	Me	H	Me	2,4-diCl-Ph	(CH2)2-NH-cBu	404.24
17.	CH	Me	H	Me	2,4-diCl-Ph	(CH2)2-NH-CH2-cPr	404.34
18.	CH	Me	H	Me	2,4-diCl-Ph	(CH2)2-NH-cHex	432.39
19.	N	Me	H	Me	2,4-diCl-Ph	(CH2)2-NH-cHex	433.4
20.	CH	Me	H	Me	2,4-diCl-Ph	(CH2)2-NH-cPent	418.36
21.	N	Me	H	Me	2,4-diCl-Ph	(CH2)2-NH-cPent	419.4
22.	CH	Me	H	Me	2,4-diF-6-OEt-Ph	(CH2)2-NH-cPent	465.98
23.	CH	Me	H	Me	2,4-diF-Ph	(CH2)2-NH-cHex	399.48
24.	CH	Me	H	Me	2,4-diF-Ph	(CH2)2-NH-cPent	385.45
25.	CH	Me	H	Me	2,4-diOMe-6Cl-Ph	(CH2)2-NH-cPent	443.98
26.	CH	Me	H	Me	2,4-diOMe-6Cl-Ph	(CH2)2-NH2	375.9
27.	CH	Me	H	Me	2,4-diOMe-Ph	(CH2)2-NH-cHex	423.55
28.	CH	Me	H	Me	2,4,6-triF-Ph	(CH2)2-NH-cPent	439.91
29.	CH	Me	H	Me	2,4,6-triMe-Ph	CH2-(2-Me-piperidin-6-yl)	391.6
30.	CH	Me	H	Me	2,4,6-triMe-Ph	(CH2)2-NH-cBu	377.53
31.	CH	Me	H	Me	2,4,6-triMe-Ph	(CH2)2-NH-CH2-cPr	377.53

32.	CH	Me	H	Me	2,4,6-triMe-Ph	(CH2)2-NH-cHex	405.59
33.	CH	Et	H	Me	2,4,6-triMe-Ph	(CH2)2-NH-cHex	419.61
34.	CH	Me	H	Me	2,4,6-triMe-Ph	(CH2)2-NH-cPent	391.55
35.	CH	Me	H	CF3	2,4,6-triMe-Ph	(CH2)2-NH-cPent	445.52
36.	N	Me	H	Me	2,4,6-triMe-Ph	(CH2)2-NH-cPent	392.5
37.	CH	Et	H	Me	2,4,6-triMe-Ph	(CH2)2-NH-cPent	405.59
38.	CH	Me	H	Me	2,4,6-triMe-Ph	(CH2)2-NH-iBu	379.55
39.	CH	Me	H	Me	2,4,6-triMe-Ph	(CH2)2-NH-iPr	365.52
40.	CH	Me	H	Me	2,4,6-triMe-Ph	(CH2)2-NH-tBu	379.54
41.	CH	Me	H	Me	2,4,6-triMe-Ph	(CH2)3-NH-cPent	405.59
42.	CH	Me	H	Me	2,5-diCl-Ph	(CH2)2-NH-cPent	418.37
43.	CH	Me	H	Me	2,5-diF-Ph	(CH2)2-NH-cHex	385.46
44.	N	Me	H	Me	2,5-diMe-4-OMe-Ph	(CH2)2-NH-cHex	422.6
45.	CH	Me	H	Me	2,5-diMe-4-OMe-Ph	(CH2)2-NH-cHex	421.59
46.	N	Me	H	Me	2,5-diMe-4-OMe-Ph	(CH2)2-NH-cPent	408.5
47.	CH	Me	H	Me	2,6-diCl-4-OEt-Ph	(CH2)2-NH-(CH2)2-NH2	394.31
48.	CH	Me	H	Me	2,6-diCl-4-OEt-Ph	(CH2)2-NH-CH2-CMe2-NH2	422.36
49.	N	Me	H	Me	2,6-diCl-4-OEt-Ph	(CH2)2-NH2	395.3
50.	CH	Me	H	Me	2,6-diCl-4-OEt-Ph	CH2-CHMe-NH2	408.33
51.	CH	Me	H	Me	2,6-diCl-4-OH-Ph	(CH2)2-NH-cPent	434.37
52.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	4-NH2-cHex	434.37
53.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	4-NH2-cHex	434.37
54.	CH	iPr	H	Me	2,6-diCl-4-OMe-Ph	(CH2)2-CHEt-NH2	450.4
55.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	CH2-(2-Me-piperidin-6-yl)	448.4
56.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	(CH2)2-N(CH2-cPr)2	488.46
57.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	(CH2)2-NH-(3-NH2-cHex)	434.37
58.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	(CH2)2-NH-(cis-2-NH2-cHex)	434.37
59.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	(CH2)2-NH-(trans-2-NH2-cHex)	434.37
60.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	(CH2)2-NH-CH2-cPr	434.37
61.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	(CH2)2-NH-CHMe-Et	436.39
62.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	(CH2)2-NH-cPent	448.4
63.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	(CH2)2-NH-Et	408.33
64.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	(CH2)2-NH2	380.28
65.	CH	iPr	H	Me	2,6-diCl-4-OMe-Ph	(CH2)2-NH2	408.33
66.	CH	tBu	H	Me	2,6-diCl-4-OMe-Ph	(CH2)2-NH2	422.4
67.	CH	nPr	H	Me	2,6-diCl-4-OMe-Ph	(CH2)2-NH2	408.3
68.	CH	CF3	H	Me	2,6-diCl-4-OMe-Ph	(CH2)2-NH2	434.3

69.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	(CH2)3-NH-Et	422.36
70.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	(CH2)3-NH2	394.31
71.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	CH2-(piperidin-2-yl)	434.3
72.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	CH2-CHMe-NH2	394.3
73.	CH	Et	H	Me	2,6-diCl-4-OMe-Ph	CH2-CHMe-NH2	408.33
74.	CH	iPr	H	Me	2,6-diCl-4-OMe-Ph	CH2-CHMe-NH2	422.36
75.	CH	nPr	H	Me	2,6-diCl-4-OMe-Ph	CH2-CHMe-NH2	422.36
76.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	CH2-CMe2-CH2-NH2	422.36
77.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	CH2-CMe2-CH2-NH2	477.44
78.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	CH2-CMe2-NH2	408.33
79.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	CHMe-CHMe-NH2	408.33
80.	CH	Et	H	Me	2,6-diCl-4-OMe-Ph	CHMe-CHMe-NH2	422.4
81.	CH	Me	H	Me	2,6-diCl-4-OMe-Ph	pyrrolidin-3-yl	406.3
82.	CH	Me	H	Me	2,6-diCl-Ph	(CH2)2-NH-cPent	418.37
83.	N	Me	H	Me	2,6-diCl-Ph	(CH2)2-NH-cPent	419.4
84.	CH	Me	H	Me	2,6-diCl-Ph	(CH2)2-NH-Et	378.30
85.	CH	Me	H	Me	2,6-diF-4-OEt-Ph	(CH2)2-NH-cPent	465.98
86.	CH	Me	H	Me	2,6-diF-Ph	(CH2)2-NH-cPent	385.46
87.	CH	Me	H	Me	2,6-diMe-4-OCF3-Ph	(CH2)2-NH-cPent	461.52
88.	N	Me	H	Me	2,6-diMe-4-OMe-Ph	(CH2)2-NH2	340.4
89.	CH	Me	H	Me	2,6-diMe-4-tBu-Ph	(CH2)2-NH-cPent	433.65
90.	N	Me	H	Me	2Cl-4F-Ph	(CH2)2-NH-cHex	416.9
91.	N	Me	H	Me	2Cl-4F-Ph	(CH2)2-NH-cPent	402.9

EXAMPLE 92

Cyclopentyl-{1-[3-(2,6-dichloro-4-methoxy-phenyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-yl]-piperidin-3-yl}-amine (MW 488.5)

EXAMPLE 93

7-piperazino-2,5-dimethyl-3-(2,4-dichlorophenyl)-pyrazolo[1,5-a] pyrimidine (MW 376.28)

EXAMPLE 94

Characterization of NPY receptor interactions and in vivo function

A. Assay for Human NPY_1 Receptor Binding Activity: Compounds are assayed for activity using the following method: A cDNA encoding human NPY1 (SEQ ID NO:1) is ligated in the appropriate orientation for expression into the commercial expression vector pBacPAK9 (Clontech, Palo Alto, CA) for expression in SF9 cells. Each Baculoviral expression vector is co-transfected along with BACULOGOLD DNA (BD PharMingen, San Diego, CA) into S*f*9 cells. The S*f*9 cell culture supernatant is harvested three days post-transfection. The recombinant virus-containing supernatant is serially diluted in Hink's TNM-FH insect medium (JRH Biosciences, Kansas City) supplemented Grace's salts and with 4.1mM L-Gln, 3.3 g/L LAH, 3.3 g/L ultrafiltered yeastolate and 10% heat-inactivated fetal bovine serum (hereinafter "insect medium") and plaque assayed for recombinant plaques. After four days, recombinant plaques are selected and harvested into 1 ml of insect medium for amplification. Each 1 ml volume of recombinant baculovirus (at passage 0) is used to infect a separate T25 flask containing 2 x 10^6 S*f*9 cells in 5 mls of insect medium. After five days of incubation at 27°C, supernatant medium is harvested from each of the T25 infections for use as passage 1 inoculum. Recombinant baculoviral clones are then subjected to a second round of amplification, using 1 ml of passage 1 stock to infect 1 x 10^8 cells in 100 ml of insect medium divided into 2 T175 flasks. Forty-eight hours post infection, passage 2 medium is harvested from each 100ml prep and plaque assayed for titer. The cell pellets from the second round of amplification are assayed for affinity binding of radiolabeled ligand (see below) to verify recombinant receptor expression. A third round of amplification is then initiated using an M.O.I. of 0.1 to infect a liter of S*f*9 cells. Forty hours post-infection the supernatant medium is harvested to yield passage 3 baculoviral stock and the cell pellet assayed for affinity binding. Titer of the passage 3 baculoviral stock is determined by plaque assay and an M.O.I. and Incubation Time Course experiment is carried out to determine conditions for optimal receptor expression.

Log-phase S*f*9 cells are infected with stocks of recombinant baculovirus encoding the proteins of interest (e.g., human NPY1 and three g-proteins), followed by culturing in insect medium at 27°C. 72 hours post-infection, a sample of cell suspension is analyzed for viability by

trypan blue dye exclusion, and the remaining S*f*9 cells are harvested via centrifugation (3000 rpm/ 10 min/ 4°C).

Preparation of purified membranes: S*f*9 cell pellets are resuspended in homogenization buffer (10 mM HEPES, 250 mM sucrose, 0.5 □g/ml leupeptin, 2 □g/ml Aprotinin, 200 □M PMSF, and 2.5 mM EDTA, pH 7.4) and homogenized using a POLYTRON homogenizer (setting 5 for 30 seconds). The homogenate is centrifuged (536 x g/ 10 min/ 4°C) to pellet the nuclei. The supernatant containing isolated membranes is decanted to a clean centrifuge tube, centrifuged (48,000 X g/ 30 min, 4°C) and resuspended in 30 ml, or preferably 20 ml of homogenization buffer. This centrifugation and resuspension step is repeated twice. The final pellet is resuspended in ice cold Dulbecco's PBS containing 5 mM EDTA and stored in frozen aliquots until needed at -80°C. The protein concentration of the resulting membrane preparation is measured using the Bradford protein assay (Bio-Rad Laboratories, Hercules, CA). By this measure, a 1-liter culture of cells typically yields 50-100 mg of total membrane protein.

CO-infection for GTPγ^{35}S binding assay: Four baculoviral expression vector stocks are used to infect a culture of *Sf*9 cells with an MOI of 1:1:1:1. These four consisted of one vector encoding the human NPY1 receptor and a different commercially obtained baculoviral expression vector stock encoding each of the three subunits of a heterotrimeric G-protein, in particular, the G-protein-encoding virus stocks are obtained from BIOSIGNAL Inc., Montreal, and are **1)** a Gα G-protein subunit-encoding virus stock (either the rat $G\alpha_{i2}$ G-protein-encoding virus stock BIOSIGNAL #V5J008 or the rat $G\alpha_O$ G-protein-encoding virus stock BIOSIGNAL #V5H010), **2)** a bovine β1 G-protein-encoding virus stock (BIOSIGNAL #V5H012), and **3)** a human γ2 G-protein-encoding virus stock (BIOSIGNAL #V6B003). Agonist-stimulated GTPγ^{35}S binding on purified membranes is assessed using hNPY 1-36 (American Peptide Co., Sunnyvale, CA) as agonist in order to ascertain functional activity as measured by GTPγ^{35}S binding.

B. GTPγ^{35}S binding assay: Purified S*f*9 cell membranes are resuspended by Dounce homogenization (tight pestle) in GTPγ^{35}S binding assay buffer (50 mM Tris pH 7.0, 120 mM NaCl, 2 mM MgCl2, 2 mM EGTA, 0.1% BSA, 0.1 mM bacitracin, 100KIU/mL Aprotinin, 5 μM GDP) and added to reaction tubes at a concentration of 30 μg/reaction tube. After adding increasing doses of the agonist hNPY 1-36 (American Peptide Co., Sunnyvale, CA), reactions

are initiated by the addition of 100 pM GTPγ^{35}S. Following a 30-minute incubation at ambient temperature, the reactions are terminated by vacuum filtration over GF/C filters (pre-soaked in wash buffer, 0.1% BSA) followed by washing with ice-cold wash buffer (50 mM Tris pH 7.0, 120mM NaCl).

Bound GTPγ^{35}S is determined by liquid scintillation spectrometry of the washed filters. Non-specific binding is determined using 10 mM GTPγS. Data are generally expressed as % maximal response and are derived by determining the maximal agonist stimulated % above basal stimulation. Computer analysis may be conveniently used to calculate estimated EC_{50}, IC_{50} and K_i values from GTPγ^{35}S binding experiment data, e.g., using SigmaPlot software. The binding affinity for the preferred compounds of the invention, expressed as K_i values, ranges from about 0.1 nanomolar to about 5 micromolar. Particularly preferred compounds yield a K_i value of less than 100 nanomolar, most preferably less than 10 nanomolar .

Assay for affinity binding of radiolabeled ligand: Purified membranes are washed with PBS and re-suspended by gentle pipetting in binding buffer (50 mM Tris(HCl), 5 mM KCl, 120 mM NaCl, 2 mM CaCl2, 1 mM MgCl2, 0.1% bovine serum albumin (BSA), pH 7.4). Membranes (5μg) are added to siliconized (Sigmacote, Sigma) polypropylene tubes in addition to 0.050 nM [125I]NPY (porcine, New England Nuclear Corp., Boston, MA) for competition analysis or 0.010-0.500 nM [125I]NPY (porcine) for saturation analysis. For evaluation of guanine nucleotide effects on receptor affinity, GTP is added at a final concentration of 100 μM. Cold displacers are added at concentrations ranging from 10-12 M to 10-6 M to yield a final volume of 0.250 mL. Nonspecific binding is determined in the presence of 1 μM NPY (human, American Peptide Co., Sunnyvale, CA) and accounts for less than 10% of total binding. Following a 2-hour incubation at ambient temperature, the reaction is terminated by rapid vacuum filtration. Samples are filtered over presoaked GF/C Whatman filters (1.0% polyethyleneimine for 2 hours) and rinsed 2 times with 5 mL cold binding buffer lacking BSA. Remaining bound radioactivity is measured by gamma counting. To estimate the Bmax, Kd and Ki, the results of binding experiments are analyzed using SigmaPlot software (SPSS Science, Chicago, IL). The binding affinity for the compounds of the invention, expressed as a Ki value, ranges from about 0.1 nanomolar to about 10 micromolar. The most preferred compounds of the

invention have a Ki of less than 100 nanomolar and a binding selectivity of >100-fold relative to other G-protein coupled receptors, including NPY_5 and $CRF1_1$ receptors.

C. *In vivo* analysis - Food Deprivation

Subjects. Experimentally naive and experienced male Sprague-Dawley rats (Sasco, St. Louis, MO) weighing 210-300g at the beginning of the experiment are used. Animals are triple-housed in stainless steel hanging cages in a temperature (22 C ± 2) and humidity (40-70% RH) controlled animal facility with a 12:12 hour light-dark cycle. Food (Standard Rat Chow, PMI Feeds Inc., #5012) and water are available ad libitum.

Apparatus. Consumption data is collected while the animals are housed in Nalgene Metabolic cages (Model #650-0100). Each cage is comprised of subassemblies made of clear polymethlypentene (PMP), polycarbonate (PC), or stainless steel (SS). All parts disassemble for quick and accurate data collection and for cleaning. The entire cylinder-shaped plastic and SS cage rests on a SS stand and houses one animal.

The animal is contained in the round Upper Chamber (PC) assembly (12cm high and 20cm in diameter) and rests on a SS floor. Two subassemblies are attached to the Upper Chamber. The first assembly consists of a SS feeding chamber (10cm long, 5cm high and 5cm wide) with a PC feeding drawer attached to the bottom. The feeding drawer has two compartments: a food storage compartment with the capacity for approximately 50g of pulverized rat chow, and a food spillage compartment. The animal is allowed access to the pulverized chow by an opening in the SS floor of the feeding chamber. The floor of the feeding chamber does not allow access to the food dropped into the spillage compartment.
The second assembly includes a water bottle support, a PC water bottle (100ml capacity) and a graduated water spillage collection tube. The water bottle support funnels any spilled water into the water spillage collection tube.

The lower chamber consists of a PMP separating cone, PMP collection funnel, PMP fluid (urine) collection tube, and a PMP solid (feces) collection tube. The separating cone is attached to the

top of the collection funnel, which in turn is attached to the bottom of the Upper Chamber. The urine runs off the separating cone onto the walls of the collection funnel and into the urine collection tube. The separating cone also separates the feces and funnels it into the feces collection tube.

Food consumption, water consumption, and body weight may be measured with an Ohaus Portable Advanced scale (±0.1g accuracy).

Procedure. Prior to the day of testing, animals are habituated to the testing apparatus by placing each animal in a Metabolic cage for 1 hour. On the day of the experiment, animals that have been food deprived the previous night are weighed and assigned to treatment groups. Assignments are made using a quasi-random method utilizing the body weights to assure that the treatment groups had similar average body weight. Animals are then administered either vehicle (0.5% methyl cellulose) or drug (a compound of the invention). At that time, the feeding drawer is filled with pulverized chow, the filled water bottle, and the empty urine and feces collection tubes are weighed. Two hours after drug treatment, each animal is weighed and placed in a Metabolic Cage. Following a one-hour test session, animals are removed and body weight obtained. The food and water containers are then weighed and the food and water consumption data recorded.

Drugs. Drug suspended in vehicle, or vehicle alone as a control, is administered orally (PO) using a gavage tube connected to a 3 or 5ml syringe at a volume of 10ml/kg. Drug is made into a homogenous suspension by stirring and ultrasonicating for at least 1 hour prior to dosing.

Statistical Analyses. The means and standard errors of the mean (SEM) for food consumption, water consumption, and body weight change are obtained. One-way analysis of variance using Systat (5.2.1) is used to test for group differences. A significant effect is defined as having a p value of $<.05$.

The following parameters are defined: Body weight change is the difference between the body weight of the animal immediately prior to placement in the metabolic cage and its body weight at

the end of the one hour test session. Food consumption is the difference in the weight of the food drawer prior to testing and the weight following the 1-hour test session. Water consumption is the difference in the weight of the water bottle prior to testing and the weight following the 1-hour test session. Preferred compounds of the invention reduce food intake and body weight gain, preferably to a statistically significant degree as determined by standard parametric analysis such as a student's T-test.

Example 95

Assay for CRF Receptor Binding Activity

As discussed above, the following assay is defined herein as a standard in vitro CRF receptor binding assay. The assay may be used to demonstrate CRF1 receptor-binding activity. The CRF receptor binding is performed using a modified version of the assay described by Grigoriadis and De Souza (*Methods in Neurosciences*, Vol. 5, 1991). IMR-32 human neuroblastoma cells, a cell-line that naturally expresses the CRF1 receptor, are grown to confluency in DMEM containing FBS.

To prepare receptor-containing membranes, cells are homogenized in wash buffer (50 mM Tris HCl, 10 mM $MgCl_2$, 2 mM EGTA, pH 7.4) and centrifuged at 48,000g for 10 min at 4°C. The pellet is re-suspended in wash buffer and the homogenization and centrifugation steps are performed two additional times.

Membrane pellets containing CRF receptors are re-suspended in 50 mM Tris buffer pH 7.7 containing 10 mM $MgCl_2$ and 2 mM EDTA and centrifuged for 10 min at 48000 x g. Membranes are washed again and brought to a final concentration of 1500 mg/ml in binding buffer (Tris buffer above with 0.1 % BSA, 15 mM bacitracin and 0.01 mg/ml aprotinin.). For the binding assay, 100 ml of the membrane preparation are added to 96 well microtube plates containing 100 ml of ^{125}I-CRF (SA 2200 Ci/mmol, final concentration of 100 pM) and 50 ml of test compound. Binding is carried out at ambient temperature for 2 h. Plates are then harvested on a Brandel 96 well cell harvester and filters are counted for gamma emissions on a Wallac 1205 Betaplate liquid scintillation counter. Non specific binding is defined by 1 mM cold CRF. IC_{50} values are calculated with the non-linear curve fitting program RS/1 (BBN Software Products Corp., Cambridge, MA). The binding affinity for a compound of Formula I expressed as an IC_{50} value generally ranges from about 0.5 nanomolar to about 10 micromolar.

Example 96

Preparation of radiolabeled probe compounds of the invention

The compounds of the invention are prepared as radiolabeled probes by carrying out their synthesis using precursors comprising at least one atom that is a radioisotope. The radioisotope is preferably selected from of at least one of carbon (preferably ^{14}C), hydrogen (preferably ^{3}H), sulfur (preferably ^{35}S), or iodine (preferably ^{125}I). Such radiolabeled probes are conveniently synthesized by a radioisotope supplier specializing in custom synthesis of radiolabeled probe compounds. Such suppliers include Amersham Corporation, Arlington Heights, IL; Cambridge Isotope Laboratories, Inc. Andover, MA; SRI International, Menlo Park, CA; Wizard Laboratories, West Sacramento, CA; ChemSyn Laboratories, Lexena, KS;

Example 97

Preparation of radiolabeled probe compounds of the invention

The compounds of the invention are prepared as radiolabeled probes by carrying out their synthesis using precursors comprising at least one atom that is a radioisotope. The radioisotope is preferably selected from of at least one of carbon (preferably ^{14}C), hydrogen (preferably ^{3}H), sulfur (preferably ^{35}S), or iodine (preferably ^{125}I). Such radiolabeled probes are conveniently synthesized by a radioisotope supplier specializing in custom synthesis of radiolabeled probe compounds. Such suppliers include Amersham Corporation, Arlington Heights, IL; Cambridge Isotope Laboratories, Inc. Andover, MA; SRI International, Menlo Park, CA; Wizard Laboratories, West Sacramento, CA; ChemSyn Laboratories, Lexena, KS; American Radiolabeled Chemicals, Inc., St. Louis, MO; and Moravek Biochemicals Inc., Brea, CA.

Tritium labeled probe compounds are also conveniently prepared catalytically via platinum-catalyzed exchange in tritiated acetic acid, acid-catalyzed exchange in tritiated trifluoroacetic acid, or heterogeneous-catalyzed exchange with tritium gas. Such preparations are also conveniently carried out as a custom radiolabeling by any of the suppliers listed in the preceding paragraph using the compound of the invention as substrate. In addition, certain precursors may be subjected to tritium-halogen exchange with tritium gas, tritium gas reduction of unsaturated bonds, or reduction using sodium borotritide, as appropriate.

Example 98

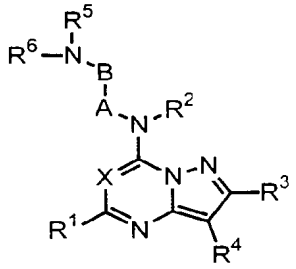
Receptor autoradiography

Receptor autoradiography (receptor mapping) is carried out in vitro as described by Kuhar in sections 8.1.1 to 8.1.9 of Current Protocols in Pharmacology (1998) John Wiley & Sons, New York, using radiolabeled compounds of the invention prepared as described in the preceding Example.

The invention and the manner and process of making and using it, are now described in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, to make and use the same. It is to be understood that the foregoing describes preferred embodiments of the present invention and that modifications may be made therein without departing from the spirit or scope of the present invention as set forth in the claims. To particularly point out and distinctly claim the subject matter regarded as invention, the following claims conclude this specification.

WHAT IS CLAIMED IS:

1. A compound of the formula



I

or a pharmaceutically acceptable salt or prodrug thereof, wherein the compound exhibits a K_i of 5 micromolar or less in an assay of NPY receptor binding, and

X is N or CR^{14};

R^1 is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halo, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7, C_1-C_6 cyanoalkyl, NR^8R^9, and C_1-C_6 alkyl-NR^8R^9;

R^2 is H,

C_1-C_6 alkyl which optionally forms a C_3-C_6 aminocarbocycle or a C_2-C_5 aminoheterocycle with A or B, each of which is optionally substituted with

R^7,

C_3-C_{10} cycloalkyl, or

(C_3-C_{10} cycloalkyl) C_1-C_6 alkyl; or

R^2 and R^6 jointly form with the 2 nitrogen atoms to which they are bound a C_2-C_5 aminoheterocycle optionally substituted with R^7;

A is $(CH_2)_m$ where m is 1,2 or 3 and is optionally mono- or di-substituted at each carbon atom with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, cyano, halogen, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7; C_1-C_6 cyanoalkyl, NR^8R^9, or C_1-C_6 alkyl-NR^8R^9, or

A and B jointly form a C_3-C_6 carbocycle, which is optionally substituted at each carbon atom with R^7, or

A and R^2 jointly form a C_3-C_6 aminocarbocycle , which is optionally substituted at each carbon atom with R^7;

B is $(CH_2)_n$ where n is 0, 1, 2, or 3 and is optionally substituted at each carbon atom with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halogen, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7; C_1-C_6 cyanoalkyl, NR^8R^9, or C_1-C_6 alkyl-NR^8R^9, or

B and R^2 jointly form a C_3-C_6 aminocarbocycle , which is optionally substituted at each carbon atom with R^7, or

B and R^6 jointly form a C_3-C_6 aminocarbocycle, which is optionally substituted at each carbon atom with R^7;

R^3 is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halo, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7, C_1-C_6 cyanoalkyl, NR^8R^9, and C_1-C_6 alkyl-NR^8R^9;

R^4 is selected from aryl or heteroaryl, each optionally substituted with 1 to 5 substituents independently selected at each occurrence from C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, C_1-C_6 alkyl-$CONR^8R^9$, $COOR^7$, C_1-C_6 alkyl-$COOR^7$, CN, C_1-C_6 alkyl-CN, $SO_2NR^8R^9$, SO_2R^7, aryl, heteroaryl, heterocycloalkyl, and 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), with the proviso that at least one of the positions ortho or para to the point of attachment of the aryl or heteroaryl ring to the pyrazole is substituted;

R^5 and R^6 are independently selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, and C_2-C_6 alkynyl;

R^7 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_3 haloalkyl, or heterocycloalkyl, C_1-C_8 alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl each of which is optionally substituted with 1 to 5 substituents independently selected at each occurrence from halogen, C_1-C_6 haloalkyl, OR^{13}, NR^8R^9, C_1-C_6 alkyl-OR^{13}, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, $COOR^{13}$, CN, $SO_2NR^8R^9$, SO_2R^{13}, with the proviso that for SO_2R^7, R^7 cannot be H;

R^8 and R^9 are independently selected at each occurrence from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_3-C_{10} cycloalkenyl, C_2-C_6 alkynyl, heterocycloalkyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl, or R^8 and R^9, taken together, can form a C_3-C_6 aminocarbocycle or a C_2-C_5 aminoheterocycle each optionally substituted at each occurrence with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_3 haloalkyl, or heterocycloalkyl, C_1-C_8 alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl;

R^{11} is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl;

R^{12} is selected from H, aryl, heteroaryl, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, optionally substituted with OR^7, NR^8R^9, C_3-C_6 aminocarbocycle, or C_2-C_5 aminoheterocycle;

R^{13} is independently selected at each occurrence from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, with the proviso that when R^7 is SO_2R^{13}, R^{13} cannot be H; and

R^{14} is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, halo, or CN.

2. A compound according to Claim 1, wherein X is N.

3. A compound according to Claim 1, wherein

X is N; and

R^1 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, or (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl.

4. A compound according to Claim 1, wherein

X is N;

R^1 is C_1-C_6 alkyl;

R^2 is H or C_1-C_6 alkyl; and

R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl.

5. A compound according to Claim 1, wherein

X is N;

R^1 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, or (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl;

R^2 is H or C_1-C_6 alkyl;

R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl; and

R^5 is H.

6. A compound according to Claim 1, wherein;

X is N;

R^1 is C_1-C_6 alkyl;

R^2 is H or C_1-C_6 alkyl;

R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl;

R^4 is phenyl, mono-, di-, or tri-substituted C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, C_1-C_6 alkyl-$CONR^8R^9$, $COOR^7$, C_1-C_6 alkyl-$COOR^7$, CN, C_1-C_6 alkyl-CN, $SO_2NR^8R^9$, SO_2R^7, aryl, heteroaryl, heterocycloalkyl, 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), wherein at least one of the positions ortho or para to the point of attachment of the aryl or heteroaryl ring to the pyrazole is substituted; and

R^7, R^8, and R^9 are as defined in Claim 1.

7. A compound according to Claim 1, wherein:

X is N;

R^1 is C_1-C_6 alkyl;

R^2 is H or C_1-C_6 alkyl;

R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl; and

R^4 is phenyl, mono-, di-, or trisubstituted with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, C_1-C_6 alkyl-$CONR^8R^9$, $COOR^7$, C_1-C_6 alkyl-$COOR^7$, CN, C_1-C_6 alkyl-CN, $SO_2NR^8R^9$, SO_2R^7, aryl, heteroaryl, heterocycloalkyl, 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), wherein at least one of the positions ortho or para to the point of attachment of the aryl or heteroaryl ring to the pyrazole is substituted;

R^5 is H; and

R^6 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, or (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl.

8. A compound according to Claim 1, wherein:

X is N;

R^1 is C_1-C_6 alkyl;

R^2 is H or C_1-C_6 alkyl;

R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl; and

R^4 is phenyl, mono-, di-, or trisubstituted with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, C_1-C_6 alkyl-$CONR^8R^9$, $COOR^7$, C_1-C_6 alkyl-$COOR^7$, CN, C_1-C_6 alkyl-CN, $SO_2NR^8R^9$, SO_2R^7, aryl, heteroaryl, heterocycloalkyl, 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), wherein at least one of the positions ortho or para to the point of attachment of the aryl or heteroaryl ring to the pyrazole is substituted; and

R^6 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, or (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl.

9. A compound according to Claim 1, wherein X is CH.

10. A compound according to Claim 1, wherein

X is CH; and

R^1 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, or (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl.

11. A compound according to Claim 1, wherein

X is CH;

R^1 is C_1-C_6 alkyl;

R^2 is H or C_1-C_6 alkyl; and

R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl.

12. A compound according to Claim 1, wherein

X is N;

R^1 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, or (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl;

R^2 is H or C_1-C_6 alkyl;

R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl; and

R^5 is H.

13. A compound according to Claim 1, wherein;

X is N;

R^1 is C_1-C_6 alkyl;

R^2 is H or C_1-C_6 alkyl;

R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl;

R^4 is phenyl, mono-, di-, or tri-substituted C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, C_1-C_6 alkyl-$CONR^8R^9$, $COOR^7$, C_1-C_6 alkyl-$COOR^7$, CN, C_1-C_6 alkyl-CN, $SO_2NR^8R^9$, SO_2R^7, aryl, heteroaryl, heterocycloalkyl, 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), wherein at least one of the positions ortho or para to the point of attachment of the aryl or heteroaryl ring to the pyrazole is substituted; and

R^7, R^8, and R^9 are as defined in Claim 1.

14. A compound according to Claim 1, wherein:

X is N;

R^1 is C_1-C_6 alkyl;

R^2 is H or C_1-C_6 alkyl;

R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl; and

R^4 is phenyl, mono-, di-, or trisubstituted with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, C_1-C_6 alkyl-$CONR^8R^9$, $COOR^7$, C_1-C_6 alkyl-$COOR^7$, CN, C_1-C_6 alkyl-CN, $SO_2NR^8R^9$, SO_2R^7, aryl, heteroaryl, heterocycloalkyl, 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), wherein at least one of the positions ortho or para to the point of attachment of the aryl or heteroaryl ring to the pyrazole is substituted;

R^5 is H; and

R^6 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, or (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl.

15. A compound according to Claim 1, wherein:

X is N;

R^1 is C_1-C_6 alkyl;

R^2 is H or C_1-C_6 alkyl;

R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl; and

R^4 is phenyl, mono-, di-, or trisubstituted with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, C_1-C_6 alkyl-$CONR^8R^9$, $COOR^7$, C_1-C_6 alkyl-$COOR^7$, CN, C_1-C_6 alkyl-CN, $SO_2NR^8R^9$, SO_2R^7, aryl, heteroaryl, heterocycloalkyl, 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), wherein at least one of the positions ortho or para to the point of attachment of the aryl or heteroaryl ring to the pyrazole is substituted; and

R^6 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, or (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl.

16. A compound according to Claim 1 wherein the compound exhibits a 20-fold greater affinity for the NPY1 receptor in a assay of NPY1 receptor binding than for the CRF1 receptor in an assay of CRF1 receptor binding.

17. A compound according to Claim 1 wherein the compound exhibits a 100-fold greater affinity for the NPY1 receptor in a assay of NPY1 receptor binding than for the CRF1 receptor in an assay of CRF1 receptor binding.

18. A method for treating eating disorders and cardiovascular disorders comprising administering to a patient suffering from an eating disorder or cardiovascular disorder a compound according to Claim 1.

19. A pharmaceutical composition comprising a compound according to Claim 1 and a pharmaceutically acceptable carrier.

20. A packaged pharmaceutical composition comprising the pharmaceutical composition of Claim 19 in a container and comprising instructions for using the composition to treat a patient suffering from an eating disorder or hypertension.

21. A method for localizing NPY receptors in tissue section samples comprising: contacting with a sample of tissue a detectably-labeled compound of Claim 1 under conditions that permit binding of the compound to the sample of tissue; washing the tissue sample to remove unbound compound; and detecting the bound compound.

22. The method of Claim 21, wherein the compound is radiolabeled.

23. A method of inhibiting the binding of NPY to the NPY1 receptor, which method comprises contacting, in the presence of NPY, a solution comprising a compound of Claim 1 with cells expressing the NPY1 receptor, wherein the compound is present in the solution at a

concentration sufficient to reduce levels of NPY binding to cells expressing the NPY1 receptor in vitro.

24. A method for altering the signal-transducing activity of a cell surface NPY1 receptor, said method comprising contacting cells expressing such a receptor with a solution comprising a compound according to Claim 1, wherein the compound is present in the solution at a concentration sufficient to reduce levels of NPY binding to cells expressing the NPY1 receptor in vitro.

25. A compound according to any one of Claim 1 wherein in an assay of NPY binding the compound exhibits an K_i of 1 micromolar or less.

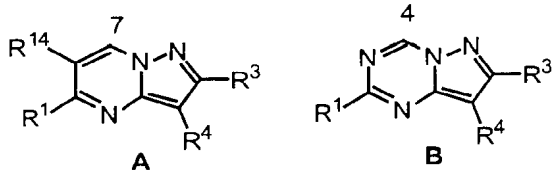
26. A compound according to any one of Claim 1 wherein in an assay of NPY binding the compound exhibits an K_i of 100 nanomolar or less.

27. A compound according to any one of Claim 1 wherein in an assay of NPY binding the compound exhibits an K_i of 100 nanomolar 10 nanomolar or less.

28. A method for treating obesity or bulimia nervosa which comprises administering an effective amount of a compound according to Claim 1 to a patient in need thereof.

29. A method for treating hypertension which comprises administering an effective amount of a compound according to Claim 1 to a patient in need thereof.

30. A method of converting a heterocyclic cores **A** and or a heterocyclic core **B**



to a compound that exhibits a K_i of 5 micromolar or less in an assay of NPY receptor binding,

wherein

R^1 is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halo, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7, C_1-C_6 cyanoalkyl, NR^8R^9, and C_1-C_6 alkyl-NR^8R^9;

R^3 is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halo, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7, C_1-C_6 cyanoalkyl, NR^8R^9, and C_1-C_6 alkyl-NR^8R^9;

R^4 is selected from aryl or heteroaryl, each optionally substituted with 1 to 5 substituents independently selected at each occurrence from C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, C_1-C_6 alkyl-$CONR^8R^9$, $COOR^7$, C_1-C_6 alkyl-$COOR^7$, CN, C_1-C_6 alkyl-CN, $SO_2NR^8R^9$, SO_2R^7, aryl, heteroaryl, heterocycloalkyl, and 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), with the proviso that at least one of the positions ortho or para to the point of attachment of the aryl or heteroaryl ring to the pyrazole is substituted;

R^{14} is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, halo, or CN;

by substituted the 7-position of the heterocyclic core **A** or the 4-position of the heterocyclic core **B** with a diamine group $-N[R^2]$-A-B-$N[R^6]$-R^5

wherein:

R^2 is H,

C_1-C_6 alkyl which optionally forms a C_3-C_6 aminocarbocycle or a C_2-C_5 aminoheterocycle with A or B, each of which is optionally substituted with

R^7,

C_3-C_{10} cycloalkyl, or

(C_3-C_{10} cycloalkyl) C_1-C_6 alkyl; or

R^2 and R^6 jointly form with the 2 nitrogen atoms to which they are bound a C_2-C_5 aminoheterocycle optionally substituted with R^7;

A is $(CH_2)_m$ where m is 1,2 or 3 and is optionally mono- or di-substituted at each carbon atom with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, cyano, halogen, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7; C_1-C_6 cyanoalkyl, NR^8R^9, or C_1-C_6 alkyl-NR^8R^9, or

A and B jointly form a C_3-C_6 carbocycle, which is optionally substituted at each carbon atom with R^7, or

A and R^2 jointly form a C_3-C_6 aminocarbocycle , which is optionally substituted at each carbon atom with R^7;

B is $(CH_2)_n$ where n is 0, 1, 2, or 3 and is optionally substituted at each carbon atom with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halogen, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7; C_1-C_6 cyanoalkyl, NR^8R^9, or C_1-C_6 alkyl-NR^8R^9, or

B and R^2 jointly form a C_3-C_6 aminocarbocycle , which is optionally substituted at each carbon atom with R^7, or

B and R^6 jointly form a C_3-C_6 aminocarbocycle, which is optionally substituted at each carbon atom with R^7;

R^5 and R^6 are independently selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, and C_2-C_6 alkynyl;

R^7 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_3 haloalkyl,

or heterocycloalkyl, C_1-C_8 alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl each of which is optionally substituted with 1 to 5 substituents independently selected at each occurrence from halogen, C_1-C_6 haloalkyl, OR^{13}, NR^8R^9, C_1-C_6 alkyl-OR^{13}, C_1-C_6

alkyl-NR^8R^9, $CONR^8R^9$, $COOR^{13}$, CN, $SO_2NR^8R^9$, SO_2R^{13}, with the proviso that for SO_2R^7, R^7 cannot be H;

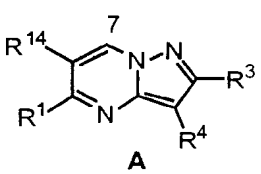
R^8 and R^9 are independently selected at each occurrence from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_3-C_{10} cycloalkenyl, C_2-C_6 alkynyl, heterocycloalkyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl, or R^8 and R^9, taken together, can form a C_3-C_6 aminocarbocycle or a C_2-C_5 aminoheterocycle each optionally substituted at each occurrence with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_3 haloalkyl, or heterocycloalkyl, C_1-C_8 alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl;

R^{11} is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl;

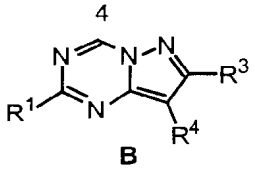
R^{12} is selected from H, aryl, heteroaryl, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, optionally substituted with OR^7, NR^8R^9, C_3-C_6 aminocarbocycle, or C_2-C_5 aminoheterocycle; and

R^{13} is independently selected at each occurrence from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, with the proviso that when R^7 is SO_2R^{13}, R^{13} cannot be H.

31. A method of converting a heterocyclic cores **A** and or a heterocyclic core **B**



A

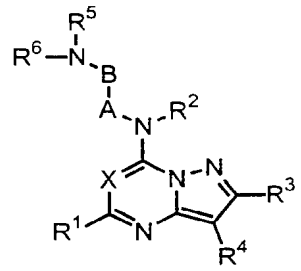


B

to a compound that exhibits a K_i of 5 micromolar or less in an assay of NPY receptor binding, and

exhibits a 20-fold greater affinity for the NPY1 receptor in a assay of NPY1 receptor binding than for the CRF1 receptor in an assay of CRF1 receptor binding.

32. A compound of the formula



I

or a pharmaceutically acceptable salt or prodrug thereof, wherein the compound exhibits a K_i of 5 micromolar or less in an assay of NPY receptor binding, and

X is N or CR^{14};

R^1 is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halo, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7, C_1-C_6 cyanoalkyl, NR^8R^9, and C_1-C_6 alkyl-NR^8R^9;

R^2 is H,

C_1-C_6 alkyl which optionally forms a C_3-C_6 aminocarbocycle or a C_2-C_5 aminoheterocycle with A or B, each of which is optionally substituted with

R^7,

C_3-C_{10} cycloalkyl, or

(C_3-C_{10} cycloalkyl) C_1-C_6 alkyl; or

R^2 and R^6 jointly form with the 2 nitrogen atoms to which they are bound a C_2-C_5 aminoheterocycle optionally substituted with R^7;

A is $(CH_2)_m$ where m is 1,2 or 3 and is optionally mono- or di-substituted at each carbon atom with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-

C_6 alkynyl, cyano, halogen, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7; C_1-C_6 cyanoalkyl, NR^8R^9, or C_1-C_6 alkyl-NR^8R^9, or

A and B jointly form a C_3-C_6 carbocycle, which is optionally substituted at each carbon atom with R^7, or

A and R^2 jointly form a C_3-C_6 aminocarbocycle , which is optionally substituted at each carbon atom with R^7;

B is $(CH_2)_n$ where n is 0, 1, 2, or 3 and is optionally substituted at each carbon atom with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halogen, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7; C_1-C_6 cyanoalkyl, NR^8R^9, or C_1-C_6 alkyl-NR^8R^9, or

B and R^2 jointly form a C_3-C_6 aminocarbocycle , which is optionally substituted at each carbon atom with R^7, or

B and R^6 jointly form a C_3-C_6 aminocarbocycle, which is optionally substituted at each carbon atom with R^7;

R^3 is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halo, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7, C_1-C_6 cyanoalkyl, NR^8R^9, and C_1-C_6 alkyl-NR^8R^9;

R^4 is selected from aryl or heteroaryl, each optionally substituted with 1 to 5 substituents independently selected at each occurrence from C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1 – C_6, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, C_1-C_6 alkyl-$CONR^8R^9$, $COOR^7$, C_1-C_6 alkyl-$COOR^7$, CN, C_1-C_6 alkyl-CN, $SO_2NR^8R^9$, SO_2R^7, aryl, heteroaryl, heterocycloalkyl, and 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), with the proviso that at least one of the positions ortho or para to the point of attachment of the aryl or heteroaryl ring to the pyrazole is substituted;

R^5 is H;

R^6 and C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl;

R^7 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_3 haloalkyl, or heterocycloalkyl, C_1-C_8 alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl each of which is optionally substituted with 1 to 5 substituents independently selected at each occurrence from halogen, C_1-C_6 haloalkyl, OR^{13}, NR^8R^9, C_1-C_6 alkyl-OR^{13}, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, $COOR^{13}$, CN, $SO_2NR^8R^9$, SO_2R^{13}, with the proviso that for SO_2R^{13}, R^{13} cannot be H;

R^8 and R^9 are independently selected at each occurrence from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_3-C_{10} cycloalkenyl, C_2-C_6 alkynyl, heterocycloalkyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl, or R^8 and R^9, taken together, can form a C_3-C_6 aminocarbocycle or a C_2-C_5 aminoheterocycle each optionally substituted at each occurrence with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_3 haloalkyl, or heterocycloalkyl, C_1-C_8 alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl;

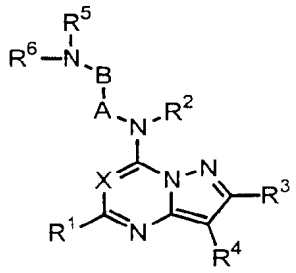
R^{11} is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl;

R^{12} is selected from H, aryl, heteroaryl, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, optionally substituted with OR^7, NR^8R^9, C_3-C_6 aminocarbocycle, or C_2-C_5 aminoheterocycle;

R^{13} is independently selected at each occurrence from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, with the proviso that when R^7 is SO_2R^{13}, R^{13} cannot be H; and

R^{14} is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, halo, or CN.

33. A compound of the formula



I

or a pharmaceutically acceptable salt or prodrug thereof, wherein the compound exhibits a K_i of 5 micromolar or less in an assay of NPY receptor binding, and

X is N or CR^{14};

R^1 is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halo, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7, C_1-C_6 cyanoalkyl, NR^8R^9, and C_1-C_6 alkyl-NR^8R^9;

R^2 is H,
C_1-C_6 alkyl which optionally forms a C_3-C_6 aminocarbocycle or a C_2-C_5 aminoheterocycle with A or B, each of which is optionally substituted with
R^7,
C_3-C_{10} cycloalkyl, or
(C_3-C_{10} cycloalkyl) C_1-C_6 alkyl; or
R^2 and R^6 jointly form with the 2 nitrogen atoms to which they are bound a C_2-C_5 aminoheterocycle optionally substituted with R^7;

A is $(CH_2)_m$ where m is 1,2 or 3 and is optionally mono- or di-substituted at each carbon atom with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, cyano, halogen, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7; C_1-C_6 cyanoalkyl, NR^8R^9, or C_1-C_6 alkyl-NR^8R^9, or

A and B jointly form a C_3-C_6 carbocycle, which is optionally substituted at each carbon atom with R^7, or

A and R^2 jointly form a C_3-C_6 aminocarbocycle , which is optionally substituted at each carbon atom with R^7;

B is $(CH_2)_n$ where n is 0, 1, 2, or 3 and is optionally substituted at each carbon atom with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halogen, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7; C_1-C_6 cyanoalkyl, NR^8R^9, or C_1-C_6 alkyl-NR^8R^9, or

B and R^2 jointly form a C_3-C_6 aminocarbocycle , which is optionally substituted at each carbon atom with R^7,

B and R^6 jointly form a C_3-C_6 aminocarbocycle, which is optionally substituted at each carbon atom with R^7;

R^3 is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halo, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7, C_1-C_6 cyanoalkyl, NR^8R^9, and C_1-C_6 alkyl-NR^8R^9;

R^4 is selected from aryl or heteroaryl, optionally substituted with 1 to 5 substituents independently selected at each occurrence from C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, halogen, C_1-C_6 haloalkyl, CN, alkylsulfonyl, OR^7, NR^8R^9, $CONR^8R^9$, $COOR^7$, $SO_2NR^8R^9$, COR^7, SO_2R^7.

R^5 and R^6 are independently selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl;

R^7 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_3 haloalkyl, or heterocycloalkyl, C_1-C_8 alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl each of which is optionally substituted with 1 to 5 substituents

independently selected at each occurrence from halogen, C_1-C_6 haloalkyl, OR^{13}, NR^8R^9, C_1-C_6 alkyl-OR^{13}, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, $COOR^{13}$, CN, $SO_2NR^8R^9$, SO_2R^{13}, with the proviso that when R^7 is SO_2R^{13}, R^{13} cannot be H;

R^8 and R^9 are independently selected at each occurrence from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_3-C_{10} cycloalkenyl, C_2-C_6 alkynyl, heterocycloalkyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl, or R^8 and R^9, taken together, can form a C_3-C_6 aminocarbocycle or a C_2-C_5 aminoheterocycle each optionally substituted at each occurrence with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_3 haloalkyl, or heterocycloalkyl, C_1-C_8 alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl;

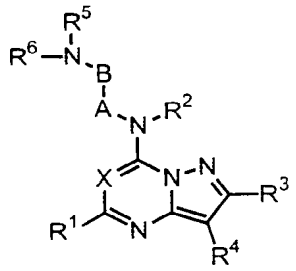
R^{11} is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl;

R^{12} is selected from H, aryl, heteroaryl, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, optionally substituted with OR^7, NR^8R^9, C_3-C_6 aminocarbocycle, or C_2-C_5 aminoheterocycle;

R^{13} is independently selected at each occurrence from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, with the proviso that when R^7 is SO_2R^{13}, R^{13} cannot be H; and

R^{14} is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, halo, or CN.

34. A compound of formula



I

or a pharmaceutically acceptable salt, hydrate, or prodrug wherein:

X is N or CR^{14};

R^1 is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halo, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7, C_1-C_6 cyanoalkyl, NR^8R^9, and C_1-C_6 alkyl-NR^8R^9;

R^2 is H,

C_1-C_6 alkyl which optionally forms a C_3-C_6 aminocarbocycle or a C_2-C_5 aminoheterocycle with A or B, each of which is optionally substituted with

R^7,

C_3-C_{10} cycloalkyl, or

(C_3-C_{10} cycloalkyl) C_1-C_6 alkyl; or

R^2 and R^6 jointly form with the 2 nitrogen atoms to which they are bound a C_2-C_5 aminoheterocycle optionally substituted with R^7;

A is $(CH_2)_m$ where m is 1,2 or 3 and is optionally mono- or di-substituted at each carbon atom with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, cyano, halogen, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7; C_1-C_6 cyanoalkyl, NR^8R^9, or C_1-C_6 alkyl-NR^8R^9, or

A and B jointly form a C_3-C_6 carbocycle, which is optionally substituted at each carbon atom with R^7, or

A and R^2 jointly form a C_3-C_6 aminocarbocycle , which is optionally substituted at each carbon atom with R^7;

B is $(CH_2)_n$ where n is 0, 1, 2, or 3 and is optionally substituted at each carbon atom with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halogen, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7; C_1-C_6 cyanoalkyl, NR^8R^9, or C_1-C_6 alkyl-NR^8R^9, or

B and R^2 jointly form a C_3-C_6 aminocarbocycle , which is optionally substituted at each carbon atom with R^7, or

B and R^6 jointly form a C_3-C_6 aminocarbocycle, which is optionally substituted at each carbon atom with R^7;

R^3 is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cyano, halo, C_1-C_6 haloalkyl, OR^7, C_1-C_6 alkyl-OR^7, C_1-C_6 cyanoalkyl, NR^8R^9, and C_1-C_6 alkyl-NR^8R^9;

R^4 is selected from aryl or heteroaryl, each substituted with aryl, heteroaryl, heterocycloalkyl, and 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), heterocycloalkyl, cyclopentenyl, cyclohexenyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl , trifluoromethylsulfonyl, C_1-C_6 alkyl-NR^8R^9, C_1-C_6 alkyl-CN , C_1-C_6 alkyl-$CONR^8R^9$, (C_1-C_4)alkyl-OR^7, C_1-C_6 alkyl-$COOR^7$and optionally substituted with 1 to 4 substituents independently selected at each occurrence from C_1-C_6 alkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, C_1-C_6 alkyl-$CONR^8R^9$, $COOR^7$, C_1-C_6 alkyl-$COOR^7$, CN, C_1-C_6 alkyl-CN, $SO_2NR^8R^9$, SO_2R^7.

R^5 and R^6 are independently selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl;

R^7 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_3 haloalkyl,

or heterocycloalkyl, C_1-C_8 alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl each of which is optionally substituted with 1 to 5 substituents independently selected at each occurrence from halogen, C_1-C_6 haloalkyl, OR^{13}, NR^8R^9, C_1-C_6 alkyl-OR^{13}, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, $COOR^{13}$, CN, $SO_2NR^8R^9$, SO_2R^{13}, with the proviso that when R^7 is SO_2R^{13}, R^{13} cannot be H;

R^8 and R^9 are independently selected at each occurrence from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_3-C_{10} cycloalkenyl, C_2-C_6 alkynyl, heterocycloalkyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl, or R^8 and R^9, taken together, can form a C_3-C_6 aminocarbocycle or a C_2-C_5 aminoheterocycle each optionally substituted at each occurrence with C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_1-C_3 haloalkyl, or heterocycloalkyl, C_1-C_8 alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, C_1-C_8 alkanoyl, aroyl, heteroaroyl, aryl, heteroaryl, C_1-C_6 arylalkyl or C_1-C_6 heteroarylalkyl;

R^{11} is selected from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl;

R^{12} is selected from H, aryl, heteroaryl, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, optionally substituted with OR^7, NR^8R^9, C_3-C_6 aminocarbocycle, or C_2-C_5 aminoheterocycle;

R^{13} is independently selected at each occurrence from H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, with the proviso that when R^7 is SO_2R^{13}, R^{13} cannot be H; and

R^{14} is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, halo, or CN.

35. A compound according to Claim 33, wherein

X is N, CH.

R^1 is H, C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, or (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl

R^2 is H

R^3 is C_1-C_6 alkyl, trifluoromethyl, or C_1-C_6alkyl-O C_1-C_6alkyl.

R^4 is phenyl, mono-, di-, or tri-substituted C_1-C_6 alkyl, halogen, C_1-C_6 haloalkyl, OR^7

R^5 is H

R^7 is methyl, ethyl, isopropyl, propyl.

36 A compound of claim 35 wherein the compound has the structure N-cyclohexyl-N'-[3-(2,6-dichloro-4-methoxy-phenyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-yl]-ethane-1,2-diamine; N-Cyclohexyl-N'-[8-(2,6-dichloro-4-methoxy-phenyl)-2,7-dimethyl-pyrazolo[1,5-a][1,3,5]triazin-4-yl]-ethane-1,2-diamine; N-cyclohexyl-N'-[2,7-dimethyl-8-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a][1,3,5]triazin-4-yl]-ethane-1,2-diamine; N-cyclohexyl-N'-[2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-ethane-1,2-diamine; N-cCyclopentyl-N'-[2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-ethane-1,2-diamine; N-cyclopentyl-N'-[2,7-dimethyl-8-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a][1,3,5]triazin-4-yl]-ethane-1,2-diamine; N-cyclopentyl-N'-[8-(2,6-dichloro-4-methoxy-phenyl)-2,7-dimethyl-pyrazolo[1,5-a][1,3,5]triazin-4-yl]-ethane-1,2-diamine; N-cyclopentyl-N'-[3-(2,6-dichloro-4-methoxy-phenyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-yl]-ethane-1,2-diamine.

37 A compound according to Claim 34, wherein R^{14} is H, C_1-C_4 alkyl.

38 A compound according to claim 37 wherein

R^2 is H; R^6 is H;

X is N, CH;

A is CH_2, optionally substituted with one or two of the following: F, CF_3, or methyl, ethyl, isopropyl;

B is CH_2, optionally substituted with one or two of the following: F, CF_3, or methyl, ethyl, isopropyl.

39 A compound according to claim 38 wherein

R^5 is C_1-C_7 alkyl, C_1-C_6 cycloalkyl, or C_1-C_6 cycloalkyl C_1-C_2 alkyl;

A, B is CH_2.

40 A compound according to claim 38 wherein
R^4 is selected from aryl or heteroaryl, each substituted with aryl, heteroaryl, heterocycloalkyl, and 3-, 4-, or 5-(2-oxo-1,3-oxazolidinyl), heterocycloalkyl, cyclopentenyl, cyclohexenyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl , trifluoromethylsulfonyl, C_1-C_6 alkyl-NR^8R^9, C_1-C_6 alkyl-CN , C_1-C_6 alkyl-$CONR^8R^9$, (C_1-C_4)alkyl-OR^7, C_1-C_6 alkyl-$COOR^7$and optionally substituted with 1 to 4 substituents independently selected at each occurrence from C_1-C_6 alkyl, (C_3-C_{10} cycloalkyl) C_1-C_6 alkyl, halogen, C_1-C_6 haloalkyl, trifluromethylsulfonyl, OR^7, C_1-C_6 alkyl-OR^7, NR^8R^9, C_1-C_6 alkyl-NR^8R^9, $CONR^8R^9$, C_1-C_6 alkyl.

41 A compound of claim 40 wherein
R^4 is phenyl substituted with acetylene, cyclopentenyl, cyclohexenyl, (C_1-C_4)alkyl-OR^7, C_1-C_3 alkenyl, and optionally substituted with 1 to 2 substituents independently selected at each occurrence from methyl, F, Cl, CF_3, OR^7;
R^7 is H, methyl, ethyl.

42 A compound of claim 41 where the structure is N-cyclohexyl-N'-[3-(2,6-dichloro-4-ethynyl-phenyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-yl]-ethane-1,2-diamine; N-cyclopentyl-N'-[3-(2,6-dichloro-4-ethynyl-phenyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-yl]-ethane-1,2-diamine; N-cyclopentyl-N'-[3-(2,6-dichloro-4-cyclopent-1-enyl-phenyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-yl]-ethane-1,2-diamine; N-cyclohexyl-N'-[3-(2,6-dichloro-4-cyclopent-1-enyl-phenyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-yl]-ethane-1,2-diamine; N-Cyclohexyl-N'-[8-(2,6-dichloro-4-cyclopent-1-enyl-phenyl)-2,7-dimethyl-pyrazolo[1,5-a][1,3,5]triazin-4-yl]-ethane-1,2-diamine; N-cyclohexyl-N'-[8-(2,6-dichloro-4-propenyl-phenyl)-2,7-dimethyl-pyrazolo[1,5-a][1,3,5]triazin-4-yl]-ethane-1,2-diamine.

43 A compound according to Claim 33 wherein the compound exhibits a 20-fold greater affinity for the NPY1 receptor in a assay of NPY1 receptor binding than for the CRF receptor in an assay of CRF receptor binding.

44 A compound according to Claim 33 wherein the compound exhibits a 100-fold greater affinity for the NPY1 receptor in a assay of NPY1 receptor binding than for the CRF receptor in an assay of CRF receptor binding.

45 A method for treating eating disorders and cardiovascular disorders comprising administering to a patient suffering from an eating disorder or cardiovascular disorder a compound according to Claim 33.

46 A pharmaceutical composition comprising a compound according to Claim 33 and a pharmaceutically acceptable carrier.

47 A packaged pharmaceutical composition comprising the pharmaceutical composition of Claim 33 in a container and comprising instructions for using the composition to treat a patient suffering from an eating disorder or hypertension.

48 A method for localizing NPY receptors in tissue section samples comprising: contacting with a sample of tissue a detectably-labeled compound of Claim 33 under conditions that permit binding of the compound to the sample of tissue; washing the tissue sample to remove unbound compound; and detecting the bound compound.

49 The method of Claim 48, wherein the compound is radiolabeled.

50 A method of inhibiting the binding of NPY to the NPY1 receptor, which method comprises contacting, in the presence of NPY, a solution comprising a compound of Claim 33 with cells expressing the NPY1 receptor, wherein the compound is present in the solution at a concentration sufficient to reduce levels of NPY binding to cells expressing the NPY1 receptor in vitro.

51 A method for altering the signal-transducing activity of a cell surface NPY1 receptor, said method comprising contacting cells expressing such a receptor with a solution comprising a compound according to Claim 33, wherein the compound is present in the solution at a

concentration sufficient to reduce levels of NPY binding to cells expressing the NPY1 receptor in vitro.

52 A compound according to Claim 33 wherein in an assay of NPY binding the compound exhibits an K_i of 1 micromolar or less.

53 A compound according to any one of Claim 33 wherein in an assay of NPY binding the compound exhibits an K_i of 1 micromolar or less.

54 A compound according to any one of Claim 33 wherein in an assay of NPY binding the compound exhibits an K_i of 100 nanomolar or less.

55 A method for treating obesity or bulimia nervosa which comprises administering an effective amount of a compound according to Claim 33 to a patient in need thereof.

56 A method for treating hypertension which comprises administering an effective amount of a compound according to Claim 33 to a patient in need thereof.

57 A method of converting core heterocyles of formula A and B to a compound that exhibits a Ki of 5 micromolar or less in an assay of NPY receptor binding.

58 A method of treating obesity comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound of formula as defined in claim 34 or a prodrug thereof or a pharmaceutically acceptable salt of said compound or of said prodrug.

59 A method as recited in claim 58 wherein the amount of the compound in FormulaI as defined in claim 34 is about 0.01 mg/kg/day to about 50 mg/kg/day.

60 A method as recited in claim 59 wherein the mammal is female or male human.

61 A pharmaceutical composition which comprises a therapeutically effective amount of compound of claim 34 or a prodrug thereof or a pharmaceutically acceptable salt of said compound or of said prodrug and a pharmaceutically acceptable carrier, vehicle or diluent.

62 A pharmaceutical composition for the treatment of obesity which comprises a therapeutically effective amount of compound of claim 34 or a prodrug thereof or a pharmaceutically acceptable salt of said compound or of said prodrug and a pharmaceutically acceptable carrier, vehicle or diluent.

63 A pharmaceutical combination composition comprising a therapeutically effective amount of a composition comprising: (a) first compound, said first compound being a compound of claim 34, a prodrug thereof, or a pharmaceutically acceptable salt of said compound or of said prodrug; and (b) a second compound, said second compound being a β_3 agonist, a thyromimetic, an eating behavior modifying agent or a NPY antagonist; and a pharmaceutical carrier, vehicle, diluent.

64 A method of treating obesity comprising administering to a mammal in need of such treatment: (a) first compound, said first compound being a compound of claim 34, a prodrug thereof, or a pharmaceutically acceptable salt of said compound or of said prodrug; and (b) a second compound, said second compound being a β_3 agonist, a thyromimetic, an eating behavior modifying agent or a NPY antagonist; and a pharmaceutical carrier, vehicle, diluent; (and (c) wherein the amounts of the first and second compounds result in a therapeutic effect.

65 A kit comprising: (a) first compound, said first compound being a compound of claim 34, a prodrug thereof, or a pharmaceutically acceptable salt of said compound or of said prodrug; (b) a second compound, said second compound being a β_3 agonist, a thyromimetic, an eating behavior modifying agent or a NPY antagonist; and a pharmaceutical carrier, vehicle, diluent; and (c) means for containing said first and second unit dosage forms wherein the amounts of the first and second compounds result in a therapeutic effect.

66 A pharmaceutical combination composition comprising a therapeutically effective amount of a composition comprising (a) first compound, said first compound being a compound of claim 34, a prodrug thereof, or a pharmaceutically acceptable salt of said compound or of said prodrug; (b) a second compound, said second compound being an aldose reductase inhibitor, a glycogen phosphorylase inhibitor, a sorbitol dehydrogenase inhibitor, insulin, metformin, acarbose, a thiazolidinedione, a glitazone, rezulin, trogitalazone, a sulfonylurea, glipazide, glyburide, or chlorpropamide; (c) a pharmaceutical carrier, vehicle, or diluent.

67 A pharmaceutical composition comprising a compound as defined in claim 34 for the treatment of disorders or disease states caused by eating disorders, of obesity, bulimia nervosa, diabetes, dislipidemia, hypertension, memory loss, epileptic seizures, migraine, sleep disorders, pain, sexual/reproductive disorders, depression, anxiety, cerebral hemorrhage, shock, congestive heart failure, nasal congestion or diarrhea.

68 A method of selectively inhibiting binding of NPY_1 receptors, which comprises contacting a compound of claim 33 with neuronal cells, wherein the compound is present in an amount effective to produce a concentration sufficient to selectively inhibit binding of NPY peptides to NPY_1 receptors in vitro.

SEQUENCE LISTING

<110> Darrow, James W.
Horvath, Raymond F.
Blum, Charles
Tran, Jennifer
De Lombaert, Stephane
Hodgetts, Kevin J.
Carpino, Philip A.

<120> Amino Substituted Pyrazolo [1,5,-a]-1,5-Pyrimidines and Pyrazolo [1,5-a]-1,3,5-Triazines

<130> 0129561

<140>
<141>

<160> 1

<170> PatentIn Ver. 2.0

<210> 1
<211> 1605
<212> DNA
<213> Homo sapiens

<400> 1
ccttctttaa tgaagcagga gcgaaaaaga caaattccaa agaggattgt tcagttcaag 60
ggaatgaaga attcagaata attttggtaa atggattcca atatggggaa taagaataag 120
ctgaacagtt gacctgcttt gaagaaacat actgtccatt tgtctaaaat aatctataac 180
aaccaaacca atcaaaatga attcaacatt attttcccag gttgaaaatc attcagtcca 240
ctctaatttc tcagagaaga atgcccagct tctggctttt gaaaatgatg attgtcatct 300
gcccttggcc atgatattta ccttagctct tgcttatgga gctgtgatca ttcttggtgt 360
ctctggaaac ctggccttga tcataatcat cttgaaacaa aaggagatga gaaatgttac 420
caacatcctg attgtgaacc tttccttctc agacttgctt gttgccatca tgtgtctccc 480
ctttacattt gtctacacat taatggacca ctgggtcttt ggtgaggcga tgtgtaagtt 540
gaatcctttt gtgcaatgtg tttcaatcac tgtgtccatt ttctctctgg ttctcattgc 600
tgtggaacga catcagctga taatcaaccc tcgagggtgg agaccaaata atagacatgc 660
ttatgtaggt attgctgtga tttgggtcct tgctgtggct tcttctttgc ctttcctgat 720
ctaccaagta atgactgatg agccgttcca aaatgtaaca cttgatgcgt acaaagacaa 780
atacgtgtgc tttgatcaat ttccatcgga ctctcatagg ttgtcttata ccactctcct 840
cttggtgctg cagtattttg gtccactttg ttttatattt atttgctact tcaagatata 900
tatacgccta aaaaggagaa acaacatgat ggacaagatg agagacaata agtacaggtc 960
cagtgaaacc aaaagaatca atatcatgct gctctccatt gtggtagcat ttgcagtctg 1020
ctggctccct cttaccatct ttaacactgt gtttgattgg aatcatcaga tcattgctac 1080
ctgcaaccac aatctgttat tcctgctctg ccacctcaca gcaatgatat ccacttgtgt 1140
caaccccata ttttatgggt tcctgaacaa aaacttccag agagacttgc agttcttctt 1200

caacttttgt gatttccggt ctcgggatga tgattatgaa acaatagcca tgtccacgat 1260
gcacacagat gtttccaaaa cttctttgaa gcaagcaagc ccagtcgcat ttaaaaaaat 1320
caacaacaat gatgataatg aaaaaatctg aaactactta tagcctatgg tcccggatga 1380
catctgttta aaaacaagca caacctgcaa catactttga ttacctgttc tcccaaggaa 1440
tggggttgaa atcatttgaa aatgactaag attttcttgt cttgcttttt actgcttttg 1500
ttgtagttgt cataattaca tttggaacaa aaggtgtggg ctttggggtc ttctggaaat 1560
agttttgacc agacatcttt gaagtgcttt ttgtgaattt accag 1605

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) **International Patent Classification** 5 :
C07D 487/04, A61K 31/505, C07D 207/34 // (C07D 487/04, 239:00, 209:00)

A1

(11) **International Publication Number:** **WO 94/13676**

(43) **International Publication Date:** 23 June 1994 (23.06.94)

(21) **International Application Number:** PCT/US93/10715

(22) **International Filing Date:** 12 November 1993 (12.11.93)

(30) **Priority Data:**
07/991,764 17 December 1992 (17.12.92) US

(60) **Parent Application or Grant**
(63) Related by Continuation
US 07/991,764 (CIP)
Filed on 17 December 1992 (17.12.92)

(71) **Applicant** *(for all designated States except US)*: PFIZER INC. [US/US]; 235 East 42nd Street, New York, NY 10017 (US).

(72) **Inventor; and**
(75) **Inventor/Applicant** *(for US only)*: CHEN, Yuhpyng, L. [US/US]; 8 Waterview Drive, Waterford, CT 06385 (US).

(74) **Agents:** RICHARDSON, Peter, C. et al.; Pfizer Inc., 235 East 42nd Street, New York, NY 10017 (US).

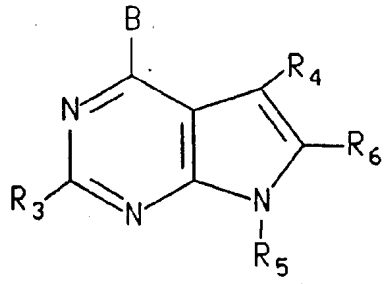
(81) **Designated States:** AU, BR, CA, CZ, JP, KR, NO, NZ, PL, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) **Title:** PYRROLOPYRIMIDINES AS CRF ANTAGONISTS

(57) **Abstract**

The compounds of formula (I), wherein B, R_3, R_4, R_5 and R_6 are as defined herein, are useful in the treatment of stress-related and other diseases. These compounds have corticotropin-releasing factor antagonist activity and as such are of use in the treatment of depression and anxiety related, and other disorders.



(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.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

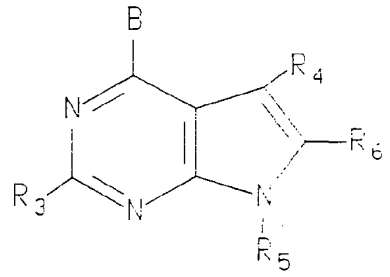
PYRROLOPYRIMIDINES AS CRF ANTAGONISTS

This invention relates to pyrrolopyrimidines, pharmaceutical compositions containing them, and their use in the treatment of stress-related and other diseases. The compounds have corticotropin-releasing factor (CRF) antagonist activity.

CRF antagonists are mentioned in U.S. Patents 4,605,642 and 5,063,245 referring to peptides and pyrazolinones, respectively. The importance of CRF antagonists is set out in the literature, e.g. as discussed in U.S. Patent 5,063,245, which is incorporated herein by reference. A recent outline of the different activities possessed by CRF antagonists is found in M.J. Owens et al., Pharm. Rev., Vol. 43, pages 425 to 473 (1991), also incorporated herein by reference. Based on the research described in these two and other references, CRF antagonists are considered effective in the treatment of a wide range of diseases including stress-related illnesses, such as stress-induced depression, anxiety, and headache; abdominal bowel syndrome; irritable colon syndrome; spastic colon; irritable colon; inflammatory diseases; immune suppression; human immunodeficiency virus (HIV) infections; Alzheimer's disease; gastrointestinal diseases; anorexia nervosa; hemorrhagic stress; drug and alcohol withdrawal symptoms; drug addiction, and fertility problems.

Certain substituted pyrrolopyrimidines have been described in the prior art. U.S. Patent 4,229,453 describes 4-amino substituted pyrrolopyrimidines for treating CNS illnesses or inflammations. Robins, Can. J. Chem., 55, 1251 (1977) describes the antibiotic tubercidin having a 7-ribofuranosyl group attached to 4-aminopyrrolopyrimidine. German Patent Publication 3145287 refers to three 7-bromophenyl-5,6-dimethyl-pyrrolopyrimidines as having analgesic, sedative, anti-convulsant and anti-inflammatory activity.

The present invention relates to a compound of the formula



I

and the pharmaceutically acceptable acid addition salts thereof, wherein

B is NR_1R_2, $CR_1R_2R_{11}$, $C(=CR_2R_{12})R_1$, $NHCR_1R_2R_{11}$, $OCR_1R_2R_{11}$, $SCR_1R_2R_{11}$, $NHNR_1R_2$, $CR_2R_{11}NHR_1$, $CR_2R_{11}OR_1$, $CR_2R_{11}SR_1$, or $C(O)R_2$;

R_1 is hydrogen, or C_1-C_6 alkyl which may be substituted by one or two substituents R_7 independently selected from the group consisting of hydroxy, fluoro, chloro, bromo, iodo, C_1-C_8 alkoxy, O-C -(C_1-C_6 alkyl), O-C NH(C_1-C_4 alkyl), O-C -N(C_1-C_4 alkyl)(C_1-C_2 alkyl), amino, NH(C_1-C_4 alkyl), N(C_1-C_2 alkyl)(C_1-C_4 alkyl), S(C_1-C_6 alkyl), N(C_1-C_4 alkyl)C (C_1-C_4 alkyl), NHC (C_1-C_4 alkyl), COOH, C O(C_1-C_4 alkyl), C NH(C_1-C_4 alkyl), C N(C_1-C_4 alkyl)(C_1-C_2 alkyl), SH, CN, NO_2, SO(C_1-C_4 alkyl), SO_2(C_1-C_4 alkyl), SO_2NH(C_1-C_4 alkyl), SO_2N(C_1-C_4 alkyl)(C_1-C_2 alkyl), and said C_1-C_6 alkyl may contain one or two double or triple bonds;

R_2 is C_1-C_{12} alkyl, aryl or (C_1-C_{10} alkylene)aryl wherein said aryl is phenyl, naphthyl, thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidyl, imidazolyl, furanyl, benzofuranyl, benzothiazolyl, isothiazolyl, benzisothiazolyl, thiazolyl, isoxazolyl, benzisoxazolyl, benzimidazolyl, triazolyl, pyrazolyl, pyrrolyl, indolyl, pyrrolopyridyl, oxazolyl, or benzoxazolyl; 3- to 8-membered cycloalkyl or (C_1-C_6 alkylene) cycloalkyl, wherein said cycloalkyl may contain one or two of O, S or N-Z wherein Z is hydrogen, C_1-C_4 alkyl, benzyl or C_1-C_4 alkanoyl, wherein R_2 may be substituted independently by from one to three of chloro, fluoro, or C_1-C_4 alkyl, or one of hydroxy, bromo, iodo, C_1-C_6 alkoxy, O-C -(C_1-C_6 alkyl), O-C -N(C_1-C_4 alkyl)(C_1-C_2 alkyl), S(C_1-C_6 alkyl), NH_2, NH(C_1-C_2 alkyl), N(C_1-C_2 alkyl) (C_1-C_4 alkyl), N(C_1-C_4 alkyl)-C (C_1-C_4 alkyl), NHC (C_1-C_4 alkyl), COOH, C O(C_1-C_4 alkyl), C NH(C_1-C_4 alkyl), C N(C_1-C_4 alkyl)(C_1-C_2 alkyl), SH, CN, NO_2, SO(C_1-C_4 alkyl), SO_2(C_1-C_4 alkyl), SO_2NH(C_1-C_4 alkyl), SO_2N(C_1-C_4 alkyl)(C_1-

C_2 alkyl), and wherein said C_1-C_{12} alkyl or C_1-C_{10} alkylene may contain one to three double or triple bonds; or

NR_1R_2 or $CR_1R_2R_{11}$ then R_1 and R_2 taken together with the atom to which they are attached may form a saturated 3- to 8-membered ring of which the 5- to 8-membered ring may contain one or two double bonds or one or two of O, S or N-Z wherein Z is hydrogen, C_1-C_4 alkyl, C_1-C_4 alkanoyl or benzyl;

R_3 is hydrogen, C_1-C_6 alkyl, fluoro, chloro, bromo, iodo, hydroxy, amino, $O(C_1$-C_6 alkyl), $NH(C_1$-C_6 alkyl), $N(C_1$-C_4 alkyl)(C_1-C_2 alkyl), SH, $S(C_1$-C_4 alkyl), $SO(C_1$-C_4 alkyl), or $SO_2(C_1$-C_4 alkyl), wherein said C_1-C_4 alkyl and C_1-C_6 alkyl may contain one double or triple bond and may be substituted by from 1 to 3 substituents R_8 independently selected from the group consisting of hydroxy, C_1-C_3 alkoxy, fluoro, chloro or C_1-C_3 thioalkyl;

R_4 is hydrogen, C_1-C_6 alkyl, fluoro, chloro, bromo, iodo, C_1-C_6 alkoxy, formyl, $NH(C_1$-C_6 alkyl), $N(C_1$-C_6 alkyl)(C_1-C_2 alkyl), $SO_n(C_1$-C_6 alkyl), wherein n is 0, 1 or 2, cyano, hydroxy, carboxy, or amido, wherein said C_1-C_6 alkyls may be substituted by one hydroxy, trifluoromethyl, amino, carboxy, amido, $NHC(=O)(C_1$-C_4 alkyl), $NH(C_1$-C_4 alkyl), $N(C_1$-C_4 alkyl)(C_1-C_2 alkyl), $C(=O)O(C_1$-C_4 alkyl), C_1-C_3 alkoxy, C_1-C_3 thioalkyl, fluoro, bromo, chloro, iodo, cyano or nitro;

R_5 is phenyl, naphthyl, thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidyl, imidazolyl, furanyl, benzofuranyl, benzothiazolyl, isothiazolyl, benzoisothiazolyl, thiazolyl, isoxazolyl, benzisoxazolyl, benzimidazolyl, triazolyl, pyrazolyl, pyrrolyl, indolyl, pyrrolopyridyl, benzoxazolyl, oxazolyl, pyrrolidinyl, thiazolidinyl, morpholinyl, piperidinyl, piperazinyl, tetrazolyl, or 3- to 8-membered cycloalkyl or 9- to 12-membered bicycloalkyl, optionally containing one or two of O, S or N-Z wherein Z is hydrogen, C_1-C_4 alkyl, C_1-C_4 alkanoyl, phenyl or benzyl, wherein each one of the above groups may be substituted independently by from one to three of fluoro, chloro, bromo, formyl, C_1-C_6 alkyl, C_1-C_6 alkoxy or trifluoromethyl, or one of hydroxy, iodo, cyano, nitro, amino, $NH(C_1$-C_4 alkyl), $N(C_1$-C_4 alkyl)(C_1-C_2 alkyl), $COO(C_1$-C_4 alkyl), $CO(C_1$-C_4 alkyl), $SO_2NH(C_1$-C_4 alkyl), $SO_2N(C_1$-C_4 alkyl)(C_1-C_2 alkyl), SO_2NH_2, $NHSO_2(C_1$-C_4 alkyl), $S(C_1$-C_6 alkyl), $SO_2(C_1$-C_6 alkyl), wherein said C_1-C_4 alkyl

and C_1-C_6 alkyl may be substituted by one or two of fluoro, chloro, hydroxy, C_1-C_4 alkoxy, amino, methylamino, dimethylamino or acetyl wherein said C_1-C_4 alkyl and C_1-C_6 alkyl may contain one double or triple bond; with the proviso that R_5 is not unsubstituted phenyl;

R_6 is hydrogen, C_1-C_6 alkyl, fluoro, chloro, bromo, iodo, C_1-C_6 alkoxy, formyl, amino, $NH(C_1$-C_6 alkyl), $N(C_1$-C_6 alkyl)(C_1-C_2 alkyl), $SO_n(C_1$-C_6 alkyl), wherein n is 0, 1 or 2, cyano, carboxy, or amido, wherein said C_1-C_6 alkyls may be substituted by one hydroxy, trifluoromethyl, amino, carboxy, amido, NHC(C_1-C_4 alkyl), $NH(C_1$-C_4 alkyl), $N(C_1$-C_4 alkyl)(C_1-C_2 alkyl), C O(C_1-C_4 alkyl), C_1-C_3 alkoxy, C_1-C_3 thioalkyl, fluoro, bromo, chloro, iodo, cyano or nitro;

R_{11} is hydrogen, hydroxy, fluoro, chloro, $COO(C_1$-C_2 alkyl), cyano, or $CO(C_1$-C_2 alkyl); and

R_{12} is hydrogen or C_1-C_4 alkyl; with the proviso that (1) B is not straight chain alkyl, (2) when R_5 is unsubstituted cycloalkyl, R_3 and R_4 are hydrogen, and R_6 is hydrogen or methyl, then B is not NHR_2 wherein R_2 is benzyl or thienylmethyl, and (3) when R_5 is p-bromophenyl, and R_3, R_4 and R_6 are methyl, then B is not methylamino or hydroxyethylamino.

Preferred compounds of the formula I of the invention are those wherein B is NR_1R_2, $NHCHR_1R_2$, or $OCHR_1R_2$, wherein R_1 is C_1-C_6 alkyl, which may be substituted by one of hydroxy, fluoro or C_1-C_2 alkoxy, and may contain one double or triple bond; those wherein R_2 is benzyl or C_1-C_6 alkyl which may contain one double or triple bond, wherein said C_1-C_6 alkyl or the phenyl in said benzyl may be substituted by fluoro, C_1-C_6 alkyl, or C_1-C_6 alkoxy; those wherein R_3 is methyl, ethyl, fluoro, chloro or methoxy; those wherein R_4 and R_6 are independently hydrogen, methyl, or ethyl; and those wherein R_5 is phenyl substituted by two or three substituents, said substituent being independently fluoro, chloro, bromo, iodo, C_1-C_4 alkoxy, trifluoromethyl, C_1-C_6 alkyl which may be substituted by one of hydroxy, C_1-C_4 alkoxy or fluoro and may have one double or triple bond, -(C_1-C_2 alkylene)O(C_1-C_2 alkyl), C_1-C_3 hydroxyalkyl, hydroxy, formyl, $COO(C_1$-C_2 alkyl), -(C_1-C_2 alkylene)amino, or -C(O)(C_1-C_2 alkyl).

Specific preferred compounds include:

n-butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amine;

di-n-propyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amine;

ethyl-n-propyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amine;

diethyl-2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amine;

n-butyl-ethyl-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amine;

2-{N-n-butyl-N-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amino}-ethanol;

4-(1-ethyl-propyl)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine;

n-butyl-ethyl-[2,5-dimethyl-7-(2,4-dimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amine;

2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidyl-4-yl]-(1-ethyl-propyl)amine;

2-[7-(4-bromo-2,6-dimethylphenyl)-2,5-dimethyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamino]-butan-1-ol;

2-(S)-[7-(4-bromo-2,6-dimethylphenyl)-2,5-dimethyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamino]-butan-1-ol;

4-(1-ethyl-propoxy)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine;

4-(1-methoxymethyl-propoxy)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine;

4-(1-ethyl-butyl)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo-[2,3-d]pyrimidine;

[7-(4-bromo-2,6-dimethyl-phenyl)-2,5-dimethyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-(1-methoxymethyl-propyl)-amine;

2-[7-(2-bromo-4,6-dimethyl-phenyl)-2,5-dimethyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamino]-butan-1-ol;

2-[7-(4-ethyl-2,6-dimethyl-phenyl)-2,5-dimethyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamino]-butan-1-ol;

2-[7-(2-ethyl-4,6-dimethylphenyl)-2,5-dimethyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamino]-butan-1-ol; and

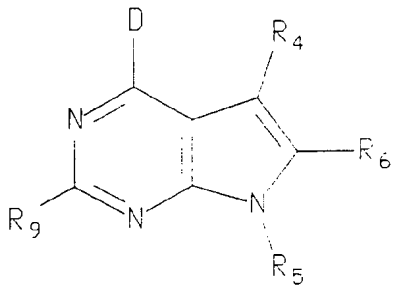
2-[7-(2-fluoromethyl-4,6-dimethylphenyl)-2,5-dimethyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamino]-butan-1-ol.

The invention also relates to a pharmaceutical composition for the treatment of illnesses induced or facilitated by corticotropin releasing factor which comprises a compound of the formula I as defined above in an amount effective in the treatment of said illnesses, and a pharmaceutically acceptable carrier, and a pharmaceutical composition for the treatment of inflammatory disorders, such as arthritis, asthma and allergies; anxiety; depression; fatigue syndrome; headache; pain; cancer; irritable bowel syndrome, including Crohn's disease, spastic colon and irritable colon; immune dysfunction; human immunodeficiency virus (HIV) infections; neurodegenerative diseases such as Alzheimer's disease; gastrointestinal diseases; eating disorders such as anorexia nervosa; hemorrhagic stress; drug and alcohol withdrawal symptoms; drug addiction; stress-induced psychotic episodes; and fertility problems, which comprises a compound of the formula I as defined above in an amount effective in the treatment of said disorders, and a pharmaceutically acceptable carrier. Preferred compositions of the invention are those containing preferred compounds of formula I as described above.

The invention further relates to a method for the treatment of illnesses induced or facilitated by corticotropin releasing factor by administering to a subject in need of such treatment a compound of formula I as defined above in an amount effective in such treatment, and a method for the treatment of inflammatory disorders, such as arthritis, asthma and allergies; anxiety; depression; fatigue syndrome; headache; pain; cancer; irritable bowel syndrome, including Crohn's disease, spastic colon and irritable colon; immune dysfunction; human immunodeficiency virus (HIV) infections; neurodegenerative diseases such as Alzheimer's disease; gastrointestinal diseases; eating disorders such as anorexia nervosa; hemorrhagic stress; drug and alcohol withdrawal symptoms; drug addiction; stress-induced psychotic episodes; and fertility problems, particularly depression and anxiety, by administering to a subject in need of such treatment a compound of formula I as defined above in an amount effective in

such treatment. Preferred methods of the invention are those administering a preferred compound of the formula I as described above.

The invention also relates to an intermediate compound of the formula



I

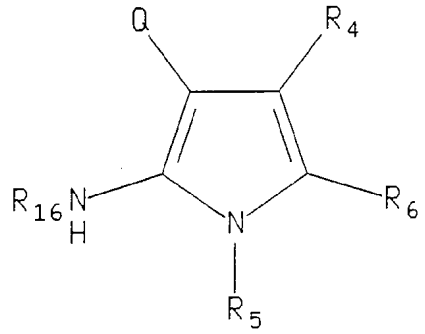
wherein

D is hydroxy, chloro, or cyano,

R_4 and R_6 are each independently hydrogen, C_1-C_6 alkyl, fluoro, chloro, bromo, iodo, C_1-C_6 alkoxy, $SO_n(C_1$-C_6 alkyl), wherein n is 0, 1 or 2, or cyano, wherein said C_1-C_6 alkyls may be substituted by one to three of hydroxy, amino, carboxy, amido, $NH\overset{O}{\overset{\|}{C}}(C_1$-$C_4$ alkyl), $NH(C_1$-C_4 alkyl), $N(C_1$-C_4 alkyl)$(C_1$-C_2 alkyl), $\overset{O}{\overset{\|}{C}}O(C_1$-$C_4$ alkyl), C_1-C_3 alkoxy, C_1-C_3 thioalkyl, fluoro, bromo, chloro, iodo, cyano or nitro;

R_5 is phenyl, naphthyl, thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidyl, imidazolyl, furanyl, benzofuranyl, benzothiazolyl, isothiazolyl, benzoisothiazolyl, thiazolyl, isoxazolyl, benzisoxazolyl, benzimidazolyl, triazolyl, pyrazolyl, pyrrolyl, indolyl, azaindolyl, benzoxazolyl, oxazolyl, pyrrolidinyl, thiazolidinyl, morpholinyl, piperdinyl, piperazinyl, tetrazolyl, or 3- to 8-membered cycloalkyl or 9- to 12-membered bicycloalkyl, optionally containing one to three of O, S or N-Z wherein Z is hydrogen, C_1-C_4 alkyl, C_1-C_4 alkanoyl, phenyl or phenylmethyl, wherein each of the above groups may be substituted independently by from one to three of fluoro, chloro, C_1-C_6 alkyl, C_1-C_6 alkoxy or trifluoromethyl, or one of bromo, iodo, cyano, nitro, amino, $NH(C_1$-C_4 alkyl), $N(C_1$-$C_4)(C_1$-C_2 alkyl), $COO(C_1$-C_4 alkyl), $CO(C_1$-C_4 alkyl), $SO_2NH(C_1$-C_4 alkyl), $SO_2N(C_1$-C_4 alkyl)$(C_1$-C_2 alkyl), SO_2NH_2, $NHSO_2(C_1$-$C_4)$, $S(C_1$-C_6 alkyl), $SO_2(C_1$-C_6 alkyl), wherein said C_1-C_4 alkyl and C_1-C_6 alkyl may be substituted by one or two of fluoro, chloro, hydroxy, amino, methylamino, dimethylamino or acetyl; with the proviso that R_5 is not unsubstituted phenyl; and

R_9 is hydrogen, C_1-C_6 alkyl or chloro; with the proviso that when (a) R_4 and R_6 are methyl, R_9 is hydrogen and D is hydroxy, then R_5 is not phenyl (1) substituted by one of halogen, nitro, C_1-C_6 alkyl, C_1-C_6 alkoxy, or trifluoromethyl, and optionally in addition substituted by one or two of halogen, C_1-C_6 alkyl or C_1-C_6 alkoxy, or (2) di-or trisubstituted by one of nitro or trifluoromethyl and one or two of halogen, C_1-C_6 alkyl or C_1-C_6 alkoxy, and (b) when D is chloro, R_4 and R_9 are hydrogen, and R_6 is C_1-C_6 alkyl, then R_5 is not unsubstituted cyclohexyl; and a compound of the formula



VII

wherein

Q is $C(O)CHR_1R_2$ or cyano;

R_1 is hydrogen, or C_1-C_6 alkyl which may be substituted by one or two substituents R_7 independently selected from the group consisting of hydroxy, fluoro, chloro, bromo, iodo, C_1-C_8 alkoxy, $S(C_1$-C_6 alkyl), or nitro, and said C_1-C_6 alkyl may contain one or two double or triple bonds;

R_2 is C_1-C_{12} alkyl, aryl or (C_1-C_{10} alkylene)aryl wherein said aryl is phenyl, naphthyl, thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidyl, imidazolyl, furanyl, benzofuranyl, benzothiazolyl, isothiazolyl, benzisothiazolyl, thiazolyl, isoxazolyl, benzisoxazolyl, benzimidazolyl, triazolyl, pyrazolyl, pyrrolyl, indolyl, azaindolyl, oxazolyl, or benzoxazolyl; 3- to 8-membered cycloalkyl or (C_1-C_6 alkylene) cycloalkyl, wherein said cycloalkyl may contain one or two of O, S or N-Z wherein Z is hydrogen, C_1-C_4 alkyl, benzyl or C_1-C_4 alkanoyl, wherein R_2 may be substituted independently by from one to three of chloro, fluoro, or C_1-C_4 alkyl, or one of hydroxy, bromo, iodo, C_1-C_6 alkoxy, $S(C_1$-C_6 alkyl), or nitro, and wherein said C_1-C_{12} alkyl or C_1-C_{10} alkylene may contain one to three double or triple bonds; or

R_4 and R_6 are each independently hydrogen, C_1-C_6 alkyl, fluoro, chloro, bromo, iodo, C_1-C_6 alkoxy, amino, $SO_n(C_1$-C_6 alkyl), wherein n is 0, 1 or 2, cyano, wherein said

C_1-C_6 alkyl may be substituted by one C_1-C_3 alkoxy, C_1-C_3 thioalkyl, fluoro, bromo, chloro, iodo, cyano or nitro;

R_5 is phenyl, naphthyl, thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidyl, imidazolyl, furanyl, benzofuranyl, benzothiazolyl, isothiazolyl, benzisothiazolyl, thiazolyl, isoxazolyl, benzisoxazolyl, benzimidazolyl, triazolyl, pyrazolyl, pyrrolyl, indolyl, azaindolyl, benzoxazolyl, oxazolyl, pyrrolidinyl, thiazolidinyl, morpholinyl, piperidinyl, piperazinyl, tetrazolyl, or 3- to 8-membered cycloalkyl or 9- to 12-membered bicycloalkyl, optionally containing one to three of O, S or N-Z wherein Z is hydrogen, C_1-C_4 alkyl, C_1-C_4 alkanoyl, phenyl or phenylmethyl, wherein each one of the above groups may be substituted independently by from one to three of fluoro, chloro, C_1-C_6 alkyl, C_1-C_6 alkoxy or trifluoromethyl, or one of bromo, iodo, cyano, nitro, amino. $NH(C_1$-C_4 alkyl), $N(C_1$-$C_4)(C_1$-C_2 alkyl), $COO(C_1$-C_4 alkyl), $CO(C_1$-C_4 alkyl), $SO_2NH(C_1$-C_4 alkyl), $SO_2N(C_1$-C_4 alkyl)$(C_1$-C_2 alkyl), SO_2NH_2, $NHSO_2(C_1$-C_4 alkyl), $S(C_1$-C_6 alkyl), $SO_2(C_1$-C_6 alkyl), wherein said C_1-C_4 alkyl and C_1-C_6 alkyl may be substituted by one or two of fluoro, chloro, hydroxy, amino, methylamino, dimethylamino or acetyl; with the proviso that R_5 is not unsubstituted phenyl; and R_{16} is hydrogen or $C(O)C_1$-C_6 alkyl; with the proviso that when Q is cyano, R_4 and R_6 are not both methyl.

Whenever reference is made to alkyl, this includes both straight and branched chain alkyl.

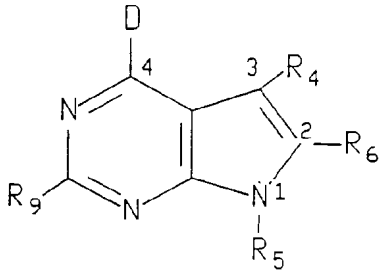
Whenever reference is made herein to 3-to 8-membered cycloalkyl or 9- to 12-membered bicycloalkyl containing one to three of O, S or N-Z, it is understood that the oxygen and sulfur ring atoms are not adjacent to each other. The three membered cycloalkyl has just one O, S or N-Z. An example of a six-membered cycloalkyl having O and N is morpholinyl.

Whenever R_2 or R_5 is a heterocyclic group, the attachment of the group is through a carbon atom.

Whenever reference is made herein to C_1-C_4 alkyl or C_1-C_6 alkyl which "may contain one or two double or triple bonds" in the definitions of R_1, R_2 and R_3, it is understood that at least two carbons are present in the alkyl for one double or triple bond, and at least four carbons for two double and triple bonds.

Whenever an alkoxy group, e.g. in the definitions of R_1 and R_2, may have a double or triple bond, it is understood that such double or triple bond is not directly attached to the oxygen.

The compounds of formula I wherein B is NR_1R_2, $NHCR_1R_2R_{11}$, $OCR_1R_2R_{11}$, $SCR_1R_2R_{11}$, or $NHNR_1R_2$, and R_3 is hydrogen, C_1-C_6 alkyl or chloro (hereafter R_9) may be prepared by reaction of a compound of the formula



II

wherein D is Cl, and R_4, R_5 and R_6 are as defined above with reference to formula I, with a compound of the formula BH wherein B is as defined immediately above. The reaction is carried out in a solvent in the presence of a base at a temperature of between about 0° to about 150°C. Suitable solvents are organic solvents such as acetonitrile, dimethylsulfoxide, acetone, C_2-C_{15} alkyl alcohol, tetrahydrofuran, chloroform, benzene, xylene or toluene, preferably acetontrile or dimethylsulfoxide.

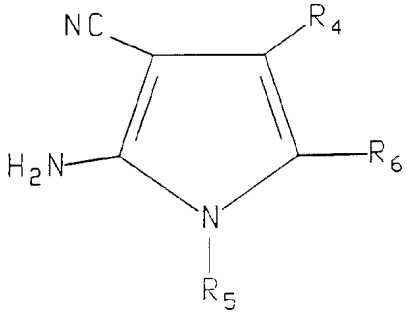
When B is NR_1R_2, $NHNR_1R_2$, or $NHCR_1R_2R_{11}$, an excess of BH is used. Other bases such as potassium carbonate or tri-(C_1-C_6)alkyl amine may be used instead. The reaction is carried out at a temperature of about 75° to 150°C. When the reaction is carried out in the presence of a base, such as sodium hydride or potassium C_1-C_4 alkoxide, a molar equivalent of the amine is used. When B is $OCR_1R_2R_{11}$ or $SCR_1R_2R_{11}$, a base which is capable of deprotonation of BH may be used, such as an alkali metal hydride such as sodium or potassium hydride, or an organometallic base such as sodium diisopropylamide, sodium bis(trimethylsily)amide, lithium diisopropylamide, lithium bis(trimethylsily)amide, sodium C_1-C_4 alkoxide or n-butylithium. The solvent used is dry tetrahydrofuran, dimethylsulfoxide, methylene chloride, or toluene, and the reaction temperature is between about -78°C and the reflux temperature of the reaction mixture, preferably 0°C to 80°C.

The compounds of formula I wherein R_3 is the groups other than R_9 (hereafter R_{10}) may be prepared by reacting a compound of the formula I wherein R_3 is chloro with a nucleophile of the formula $R_{10}H$ with or without an organic or inorganic base. Suitable bases include sodium, sodium hydride, and alkali metal hydroxide such as potassium hydroxide, and weaker bases such as potassium carbonate or triethylamine.

The latter are generally used when $R_{10}H$ is alkanol, C_1-C_6 alkanethiol, an amine, e.g. $NH(C_1$-C_6 alkyl), or tetrahydrobutyl ammonium fluoride. Suitable solvents are dimethylsulfoxide, acetonitrile, C_1-C_5 alkyl alcohol, tetrahydrofuran, benzene, toluene or methylene chloride.

The compounds of formula II wherein D is chloro may be prepared by reacting the corresponding 4-hydroxy compound of formula III (not shown) with an excess of phosphorus oxychloride or thionyl chloride at temperatures between about 60 to 140°C, conveniently at the reflux temperature of the reaction mixture. When the reaction is carried out in a solvent, suitable solvents are halogenated alkanes, such as methylene chloride or chloroform. The reaction may be in the presence of a base such as N,N-diethylaniline, trimethylamine or potassium carbonate.

The compounds of formula III wherein R_9 is hydrogen may be prepared by reaction of a compound of the formula



IV

wherein R_4, R_5, and R_6 are as defined with reference to formula I with formic acid at a temperature between about 60 to 140°C, preferably at the reflux temperature of the reaction mixture.

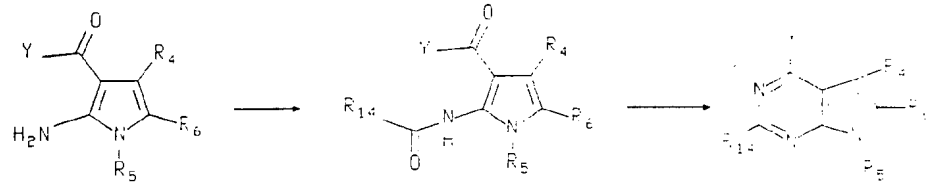
The compounds of formula III wherein R_9 is C_1-C_6 alkyl (hereafter R_{13}) may be prepared by reacting a compound of formula IV with $R_{13}COOCOR_{13}$ in $R_{13}COOH$ or $R_{13}CO(OC_1$-C_2 alkyl)$_3$ in acetic acid or an appropriate organic solvent such as ethyl acetate or toluene, at a temperature between 25° to 120°C, preferably at the reflux temperature of the reaction mixture. The compounds of formula III wherein R_9 is hydroxy may be prepared by reacting a compound of formula IV with chlorosulfonyl isocyanate in an appropriate solvent at temperature between -78°C to 100°C, preferably at -20°C to 60°C, followed by acid hydrolysis. The appropriate solvents include methylene chloride, dimethyl formamide, tetrahydrofuran, and toluene.

preferably dimethyl formamide or methylene chloride. The above formed compounds wherein R_9 is hydrogen, C_1-C_6 alkyl or hydroxy may be heated in aqueous acid to give the compounds of formula III. The appropriate aqueous acids are 85% phosphoric acid, hydrochloric acid, sulfuric acid, or acetic acid, preferably 85% phosphoric acid. The reaction is generally carried out at about 25 to 150°C, preferably 80 to 130°C. Alternatively, the formed compounds may be heated with phosphorous pentoxide and N,N-dimethylcyclohexanamine at about 150 to 200°C.

The compounds of formula IV may be prepared by conventional methods.

The compounds of formula I wherein B is $CR_1R_2R_{11}$ and R_3 is hydrogen, C_1-C_6 alkyl, or hydroxy (hereafter R_{14}) may be prepared, as depicted in Scheme 1, by heating a compound of the formula VI, wherein R_{14} is hydrogen, C_1-C_6 alkyl or amino, R_1, R_2, R_{11}, R_4, R_5, and R_6 are as defined above, and Y is $CR_1R_2R_{11}$, with ammonium chloride and $R_{14}CONH_2$ at reflux temperatures.

Scheme 1



V VI I-A

The compounds of formula I wherein B is $CR_1R_2R_{11}$ as defined above with reference to formula I and R_3 is as defined above with reference to formula I, other than hydrogen, C_1-C_6 alkyl, or hydroxy, may be prepared by reacting the 2-chloro derivatives of formula I wherein R_3 is chloro (formula I-B, not shown) with a nucleophile of formula $R_{15}H$ with or without an organic or inorganic base by the method described previously for the reaction with $R_{10}H$, wherein R_{15} is R_3 other than hydrogen, C_1-C_6 alkyl, hydroxy, and chloro. The compounds of formula I-B may be prepared by a method analogous to that for the conversion of compounds III to compounds II wherein D is chloro.

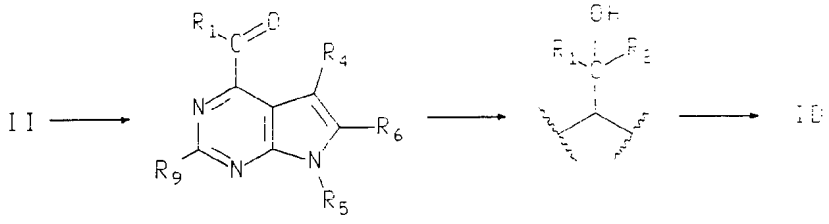
The compounds of formula VI may be prepared, as shown in Scheme I, starting from compounds of the formula V by methods analogous to those for the conversion of compounds IV to compounds III.

The compounds of formula V may be prepared by methods analogous to the conventional methods used for the preparation of compounds of formula IV by using $YCOCH_2CN$ instead of malonitrile, wherein Y is $CR_1R_2R_{11}$.

The compounds of formula I wherein B is $C(O)R_2$ may be prepared by reacting a compound of formula II wherein D is cyano with a Grignard reagent containing group R_2, e.g. R_2MgCl, or R_2MgBr.

The compounds of formula I wherein B is $CR_1R_2R_{11}$, $C(C{=}CR_2R_{12})R_1$, $CR_2R_{11}NHR_1$, $CR_2R_{11}OR_1$, $CR_2R_{11}SR_1$ or $C(O)R_2$, and R_3 is R_9 as defined above with reference to formula II, may be prepared as depicted in Scheme 2.

Scheme 2



VII IC

The compounds of formula II wherein D is cyano and R_4, R_5, R_6 and R_9 are as defined above, prepared by reacting the corresponding compound wherein D is chloro with potassium cyanide in dimethylsulfoxide, are reacted with a Grignard reagent containing group R_1 as defined above to form the compound of formula VII. Further reaction of the compound of formula VII with a Grignard reagent containing group R_2 as defined above provides the compound of formula IC. Corresponding compounds of formula ID wherein B is $CR_1R_2R_{11}$ or $C(=CR_1R_{12})R_1$ may be prepared by conventional methods. Thus, reaction of IC with an acid, such as concentrated sulfuric acid or hydrochloric acid, gives a compound of formula I wherein B is $C(=CR_2R_{12})R_1$. Hydrogenation of a compound wherein B is $C(=CR_2R_{12})R_1$ using Pd/C or platinum oxide catalyst gives a compound I wherein B is CHR_1R_2. Reaction of a compound I wherein B is CR_1R_2OH with diethylamino sulfur trifluoride or triphenylphosphine carbontetrachloride affords a compound I wherein B is CR_1R_2F or CR_1R_2Cl, respectively.

When the compounds of the invention contain one or more chiral centers, it is understood that the invention includes the racemic mixture and the individual diastereomers and enantiomers of such compounds.

The 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 formula I with one chemical equivalent of a pharmaceutically acceptable acid. Conventional concentration or crystallization techniques are employed in isolating the salts. Illustrative of suitable acids are acetic, lactic, succinic, maleic, tartaric, citric, gluconic, ascorbic, benzoic, cinnamic, fumaric, sulfuric, phosphoric, hydrochloric, hydrobromic, hydroiodic, sulfamic, sulfonic acids such as methanesulfonic, benzene sulfonic, p-toluenesulfonic, mandelic, di-p-toluoyl-L-tartaric and related acids.

The novel compound of the invention of formula I may be administered alone or in combination with pharmaceutically acceptable carriers, in either single or multiple, e.g. up to three, doses. Suitable pharmaceutical carriers include inert solid diluents or fillers, sterile aqueous solution and various organic solvents. The pharmaceutical compositions formed by combining the novel compounds of formula I and the pharmaceutically acceptable carriers are then readily administered in a variety of dosage forms such as tablets, powders, lozenges, syrups, injectable solutions and the like. These pharmaceutical compositions can, if desired, contain additional ingredients such as flavorings, binders, excipients and the like. Thus, for purposes of oral administration, tablets containing various excipients such as sodium citrate, calcium carbonate and calcium phosphate may be employed along with various disintegrants such as starch, alginic acid and certain complex silicates, together with binding agents such as polyvinylpyrrolidone, sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often useful for tabletting purposes. Solid compositions of a similar type may also be employed as fillers in soft and hard filled gelatin capsules. Preferred materials for this include lactose or milk sugar and high molecular weight polyethylene glycols. When aqueous suspensions or elixirs are desired for oral administration, the essential active ingredient therein may be combined with various sweetening or flavoring agents, coloring matter or dyes and, if desired, emulsifying or suspending agents, together with diluents such as water, ethanol, propylene glycol, glycerin and combinations thereof.

For parenteral administration, solutions of the novel compound of formula I in sesame or peanut oil, aqueous propylene glycol, or in sterile aqueous solution may be employed. Such aqueous solutions should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular

aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. The sterile aqueous media employed are all readily available by standard techniques known to those skilled in the art.

Additionally, it is possible to administer the compounds of the present invention topically when treating inflammatory conditions of the skin and this may be done by way of creams, jellies, gels, pastes and ointments in accordance with standard pharmaceutical practice.

The effective dosage for the compound of formula I depends on the intended route of administration and other factors such as age and weight of the patient, as generally known to a physician. The dosage also depends on the illness to be treated. The daily dosage will generally range from about 0.1 to 50 mg/kg of the body weight of the patient to be treated. For treatment of inflammatory diseases about 0.1 to about 100 mg/kg will be needed, for Alzheimer's disease, about 0.1 to about 50 mg/kg, as well as for gastrointestinal diseases, anorexia nervosa, hemorrhagic stress, drug and alcohol withdrawal symptoms, etc.

The methods for testing the compounds for formula I for their CRF antagonist activity are according to the procedures of Endocrinology, 116, 1653-1659 (1985) and Peptides, 10, 179-188 (1985) which determine the binding affinity of a test compound for a CRF receptor. The binding affinities for the compounds of formula I. expressed as IC_{50} values, generally range from about 0.2 nanomolar to about 10 micromolar.

The following Examples illustrate the invention. The following abbreviations are used: Ph=phenyl, Me=methyl, Bu=butyl, Et=ethyl, Pr=propyl.

Example 1

A. 2-amino-4-methyl-1-(2,4,6-trimethylphenyl)pyrrole-3-carbonitrile

A mixture 2-(2-bromo-1-methyl-ethylidene)-malononitrile and 2,4,6-trimethylaniline (17.330 g, 91.24 mmol) in 40 mL of isopropanol was stirred at room temperature for 15 hours. The reaction mixture was concentrated to dryness and diluted with chloroform and water. The chloroform layer was neutralized with dilute sodium hydroxide and washed with brine, separated, dried and concentrated to give 33.000 g of brown oily solid. The solid was purified through silica gel column chromatography to give 9.35 g (47.5%) of the title compound as an orange-yellow solid. ^1H NMR ($CDCl_3$) δ 2.0(s.6H). 2.15(s,3H), 2.35(s,3H), 3.75(brs.2H). 5.8(s.1H), 6.95(s,2H) ppm.

B. N-[3-cyano-4-methyl-1-(2,4,6-trimethylphenyl)-1H-pyrrol-2-yl]-acetamide

A mixture of the purified compound of step A (3.000 g, 12.54 mmol) and acetic anhydride (1.410 g, 1.31 ml, 13.82 mmol) in 3 ml of acetic acid was refluxed for 45 minutes, cooled and poured onto crushed ice and extracted with ethyl acetate. The organic layer was neutralized, dried and concentrated to give 3.71 g (105%) of dark-pink glass foam. ^{1}H NMR ($CDCl_3$) δ 1.95(s,6H), 2.2(s,3H), 2.32 (s,3H), 6.2(s,1H), 6.8(brs, 1H, NH), 6.9(s,2H) ppm.

C. 2,5-dimethyl-7-(2,4,6-trimethylphenyl)-3,7-dihydro-pyrrolo[2,3-d]pyrimidin-4-one

A suspension of the compound of step B (3.200 g, 11.38 mmol) in 3 ml of 85% phosphoric acid was immersed in an oil bath preheated to 130°C for 30 minutes. The reaction mixtures was cooled and poured onto crushed ice and stirred until solid formed and ice melted. The solid was filtered, washed with water to give a tannished solid, the title compound, which was purified through silica gel column chromatography to give a tan solid. ^{1}H NMR ($CDCl_3$) δ 1.92(s,6H), 2.32(s,3H), 2.41(s,3H), 2.45(s,3H), 2.46(s,3H), 6.42(d,1H), 6.95(s,2H) ppm.

D. 4-chloro-2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo-[2,3-d]pyrimidine

A mixture of the compound of step C (1.030 g, 3.67 mmol) and $POCl_3$ (3 ml) was heated at reflux for 2.5 hours and cooled. The reaction mixture was poured into ice-water and extracted with ethyl acetate. The organic layer was washed with dilute sodium bicarbonate and brine, dried over anhydrous sodium sulfate, and concentrated to dryness to give the title compound as a tan solid which was purified through silica gel to give an off-white solid. ^{1}H NMR ($CDCl_3$) δ 1.90(s,6H), 2.35(s,3H), 2.50(s,3H), 2.65(s,3H), 6.78(s,1H), 7.00(s,2H) ppm.

EXAMPLE 2

A. 2-amino-4,5-dimethyl-1-(2,4,6-trimethylphenyl)-1H-pyrrole-3-carbonitrile

A mixture of 3-hydroxy-2-butanone (100.000 g, 1.135 mol), 2,4,6-trimethylaniline (153.225 g, 1.135 mol) and p-toluenesulfonic acid (0.670 g) in 500 ml of benzene was refluxed using a Dean-Stark trap to remove water. After 2 hours,malononitrile (75.000 g, 1.135 mol) was added and the mixture was refluxed for an additional 10 hours until all the staring material was consumed. The reaction mixture was cooled and precipitate formed and filtered. The solid was washed with a minimum amount of ethanol. The

solid was diluted with 500 ml of benzene and product was dissolved. Some undesired product was insoluble and was filtered off. The filtrate was concentrated to give a tan solid which was recrystallized from ethanol to give 130.260 g of off-white crystals. ^{1}H NMR ($CDCl_3$) δ 1.68(s,3H), 1.93(s,6H), 2.05(s,3H), 2.31(s,3H), 3.62(brs,2H), 6.95(s,2H) ppm.

B. N-[3-cyano-4,5-dimethyl-1-(2,4,6-trimethyphenyl)-1H-pyrrol-2-yl]-acetamide

The title compound was prepared as a tan solid by the procedure analogous to that of Example 1A starting with the compound of step A and acetic anahydride in acetic acid. The crude material was pure and used directly for the next cyclization step. ^{1}H NMR ($CDCl_3$) δ 1.75(s,3H), 1.80(s,6H), 1.95(s,3H), 2.18(s,3H), 2.30(s,3H), 6.60(brs, 1H), 6.93(s,2H) ppm.

C. 2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-3,7-dihydro-pyrrol[2,3-d]pyrimidin-4-one

A mixture of the compound of step B(157.600 g, 0.53 mol) and 100 ml of 85% phosphoric acid was heated for 0.5 hours in an oil bath at a temperature of 130°C. All the starting material was consumed and the desired product formed. The mixture was cooled, poured into 1200 ml of ice-water, and stirred. Precipitate formed and was filtered. The solid was washed with water, dried overnight to give 113.220 g of the title compound as brick-pink solid. ^{1}H NMR ($CDCl_3$) δ 1.85(s,6H), 1.87(s,3H), 2.34(s,3H), 2,41(s,3H), 2.44(s,3H), 7.00(s,2H) ppm.

EXAMPLE 3

A. 2-amino-4,5-diethyl-1-(2,4,6-trimethylphenyl)-1H-pyrrole-3-carbonitrile

The crude material of the title compound was prepared as an oil by the procedure analogous to that of Example 2A starting with 4-hydroxy-3-hexanone. The crude material was used directly for the next acetylation step without further purification.

B. N-[3-cyano-4,5-diethyl-1-(2,4,6-trimethylphenyl)-1H-pyrrol-2-yl]-acetamide

The title compound was prepared as an oil by the procedure of Example 1A starting with the compound of above step A and acetic anhydride in acetic acid. The crude material was purified through silica gel column chromatography using chloroform as eluent to give the title compound as an oil. ^{1}H NMR ($CDCl_3$) δ 0.85(t,3H), 1.26(t,3H), 1.92(s,6H), 2.19(s,3H), 2.23(q,2H), 2.33(s,3H), 2.59(q,2H), 6.95(s,2H) ppm.

C. 2-methyl-5,6-diethyl-7-(2,4,6-trimethylphenyl)-3,7-dihydro-pyrrolo[2,3-d]pyrimidin-4-one

The title compound was prepared as a brown solid by the procedure of Example 2C starting with the compound of above step B and 85% phosphoric acid. The crude material was used directly for the next chlorination reaction without further purification.

EXAMPLE 4

The following compounds were prepared according to the method of Example 1 starting from the corresponding 2,5,6-trialkyl-7-(2,4,6-trimethylphenyl)-3,7-dihydro-pyrrolo[2,3-d]pyrimidin-4-one.

4-chloro-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine - a tan solid. ^{1}H NMR ($CDCl_3$) δ 1.81(s,6H), 1.99(s,3H), 2.35(s,3H), 2.46(s,3H), 2.59(s,3H), 7.01(s,2H) ppm.

4-chloro-2-methyl-5,6-diethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin - a tan solid. ^{1}H NMR ($CDCl_3$) δ 0.96(t,3H), 1.31(t,3H), 1.85(s,6H), 2.38(s,3H), 2.46(q,2H), 2.62(s,3H), 2.62(s,2H), 2.92(q,2H), 7.02(s,2H) ppm.

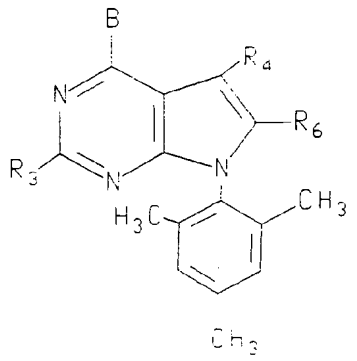
EXAMPLE 5

Butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amine

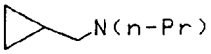
A mixture of 4-chloro-2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine (1.000 g, 3.36 mmol) and N-ethylbutylamine (3.400 g, 33.60 mmol) in 5 ml of dimethylsulfoxide was heated to reflux for 1.5 hours. The mixture was cooled and treated with water and a few drops of 2 N HCl to pH 6.5 and extracted with ethyl acetate. The organic layer was separated, washed with dilute sodium bicarbonate, brine, and dried over sodium sulfate anhydrous and concentrated to dryness. The residue was purified through silica gel column chromatography to give 995 mg (81% yield) of the title compound as an oil. ^{1}H NMR ($CDCl_3$) δ 0.90 (t,3H), 1.23(t,3H), 1.35(m,2H), 1.60-1.70(m,2H), 1.92(s,6H), 2.30(s,3H), 2.40(s,3H), 2.46(s,3H), 3.58(t,2H), 3.66(q,2H), 6.55(s,1H), 6.95(s,2H) ppm. The corresponding hydrogen chloride salt was prepared as a white crystals after recrystallization from ethyl acetate. ^{1}H NMR (D_2O) δ 0.90(t,3H), 1.34(m,5H), 1.75(m,2H), 1.90(s,6H), 2.37(s,3H), 2.48(s,3H), 2.55(s,3H), 3.80-3.94(m,4H), 7.09(s,2H) ppm.

EXAMPLE 6

The following compounds were prepared starting with the appropriate amine and the appropriate 4-chloro-2,5,6-trialkyl-7-(substituted phenyl)-7H-pyrrolo[2,3-d]pyrimidine and employing the general procedure of Example 5.



B	R_3	R_4	R_6	^{1}H NMR ($CDCl_3$) δ (ppm)
NMe_2	Me	Me	Me	1.82(s,6H), 2.00(s,3H) 2.38(s,3H) 2.40(s,3H), 2.90(s,3H), 3.58(s,6H), 7.03(s,2H)
NEt_2	Me	Me	Me	1.22(t,6H), 1.84(s,6H), 1.94(s,3H), 2.35(s,3H), 2.38(s,3H), 2.55(s,3H), 3.60(q,4H), 6.98(s,2H)
$N(n\text{-}Pr)_2$	Me	Me	Me	0.90(t,6H), 1.68(q,4H), 1.85(s,6H), 1.95(s,3H), 2.35(s,3H), 2.39(s,3H), 2.48(s,3H), 3.53(q,4H), 6.99(s,2H)
$N\text{-}(n\text{-}Bu)_2$	Me	Me	Me	0.88(t,6H), 1.30(m,4H), 1.61(m,4H), 1.82(s,6H), 1.92(s,3H), 2.30(s,3H), 2.34(s,3H), 2.47(s,3H), 3.50(t,4H), 6.95(s,2H)
EtN(n-Pr)	Me	Me	Me	0.92(t,3H), 1.20(t,3H), 1.64(m,2H), 1.85(s,6H), 1.94(s,3H), 2.35(s,3H), 2.38(s,3H), 2.47(s,3H), 3.49(t,2H), 3.59(q,2H), 6.99(s,2H)
EtN(n-Bu)	Me	Me	Me	0.90(t,3H), 1.19(t,3H), 1.33(m,2H), 1.60(m,2H), 1.83(s,6H), 1.92(s,3H), 2.33(s,3H), 2.35(s,3H), 2.45(s,3H), 3.51(t,2H), 3.58(q,2H), 6.96(s,2H)

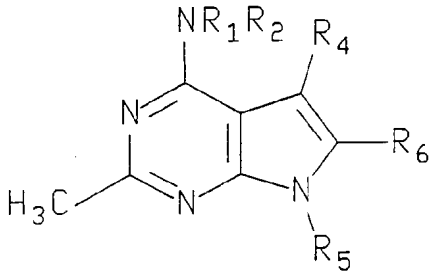
B	R_3	R_4	R_6	^{1}H NMR ($CDCl_3$) δ (ppm)
$EtN(CH_2)_2OH$	Me	Me	Me	1.25(t,3H), 1.78(s,6H), 1.90(s,3H), 2.30(s,3H), 2.36(s,3H), 2.40(s,3H), 3.64(q,2H), 3.75(m,2H), 3.86(t,2H), 6.96(s,2H)
$(n\text{-}Bu)N(CH_2)_2OH$	Me	Me	Me	0.95(t,3H), 1.35(m,2H), 1.71(m,2H), 1.81(s,6H), 1.92(s,3H), 2.31(s,3H), 2.37(s,3H), 2.44(s,3H), 3.55(dd,2H), 3.72(t,2H), 3.87(t,2H), 6.95(s,2H)
$MeN(CH_2CHMe_2)$	Me	Me	Me	0.89(s,3H), 0.91(s,3H), 1.81(s,6H), 1.91(s,3H), 1.96-2.10(m,1H), 2.32(s,3H), 2.35(s,3H), 2.43(s,3H), 3.11(s,3H), 3.32(d,2H), 6.95(s,2H)
	Me	Me	Me	0.35(dd,2H), 0.47(m,2H), 0.90(t,3H), 1.10(m,1H), 1.67(m,2H), 1.83(s,6H), 1.93(s,3H), 2.33(s,3H), 2.37(s,3H), 2.45(s,3H), 3.41(d,2H), 3.62(t,2H), 6.97(s,2H)
$N(CH_2CH{=}CH)_2$	Me	Me	Me	1.85(s,6H), 1.96(s,3H), 2.36(s,3H), 2.39(s,3H), 2.49(s,3H), 4.18(d,4H), 5.20-5.32(m,4H), 5.90-6.10(m,2H), 7.00(s,2H)
MeN-CHMe(Et)	Me	Me	Me	0.87(t,3H), 1.29(d,3H), 1.4.-1.8(m,3H), 1.82(s,3H), 1.86(s,3H), 1.95(s,3H), 2.35(s,3H), 2.37(s,3H), 2.47(s,3H), 3.02(s,3H), 4.34(m,1H), 6.99(s,2H)
$N(CH_2CH_2OH)_2$	Me	Me	Me	1.59(brs,2H), 1.81(s,6H), 1.94(s,3H), 2.34(s,3H), 2.39(s,3H), 3.80-3.95(m,8H), 6.98(s,2H)
$HO(CH_2)_3N(CH_2)_2OH$	Me	Me	Me	1.80(s,6H), 1.93(s,3H), 1.90-2.00(m,2H), 2.33(s,3H), 2.39(s,3H), 2.43(s,3H), 3.65(t,2H), 3.70-3.85(m,2H), 3.89(m,2H), 6.98(s,2H)
$(n\text{-}Bu)N(CH_2CH_2OMe)$	Me	Me	H	0.91(t,3H), 1.31(m,2H), 1.67(m,2H), 1.90(s,6H), 2.32(s,3H), 2.41(s,3H), 2.42(s,3H), 3.36(s,3H), 3.60-3.70(m,4H), 3.82(t,2H), 6.56(s,1H), 6.95(s,2H)

B	R_3	R_4	R_6	^{1}H NMR ($CDCl_3$) δ (ppm)
p-Me-Ph$CH_2N(CH_2)_3OH$	Me	Me	H	1.80(m,2H), 1.90(s,6H), 2.20(s,3H), 2.30(s,3H), 2.34(s,3H), 2.49(s,3H), 3.54(t,2H), 3.82(t,2H), 4.90(s,2H), 6.58(s,1H), 6.95(s,2H), 7.10-7.25(m,4H)
EtN(n-Pr)	Me	Et	Et	0.93(t,6H), 1.1-1.3(m,6H), 1.68(m,2H), 1.88(s,6H), 2.36(s,3H), 2.42(q,2H), 2.49(s,3H), 2.80(q,2H), 3.49(t,2H), 3.58(q,2H), 6.99(s,2H)
EtN(n-Bu)	H	Me	Me	0.91(t,3H), 1.23(t,3H), 1.30(m,2H), 1.62(m,2H), 1.89(s,6H), 2.30(s,3H), 2.44(s,3H), 3.58(t,2H), 3.65(q,2H), 6.67(s,1H), 6.95(s,2H), 8.29(s,1H)
EtN(n-Pr) (HCl salt)	Me	Me	H	0.93(t,3H), 1.25(t,3H), 1.70(m,2H), 1.91(s,6H), 2.33(s,3H), 2.42(s,3H), 3.55(m,2H), 3.69(m,2H), 6.58(s,1H), 6.96(s,2H)
$N(n\text{-}Pr)_2$	Me	Me	H	0.90(t,6H), 1.65(m,4H), 1.90(s,3H), 2.30(s,3H), 2.40(s,3H), 2.45(s,3H), 3.5-3.6(m,4H), 6.55(s,1H), 6.93(s,2H)
$N(CH_2CH{=}CH_2)_2$	Me	Me	H	1.90(s,6H), 2.30(s,3H), 2.40(s,3H), 2.48(s,3H), 4.20(d,4H), 5.15-5.30(m,4H), 5.09-6.10(m,2H), 6.55(s,1H), 6.95(s,2H)
$EtN(CH_2CH(CH_3)_2)$	Me	Me	H	0.95(t,3H), 1.23(t,3H), 1.95(s,6H), 2.11(m,1H), 2.35(s,3H), 2.46(s,3H), 2.50(s,3H), 3.44(d,2H), 3.68(q,2H), 6.59(s,1H), 6.98(s,2H)
$EtN(CH_2C(Me){=}CH_2)$	Me	Me	H	1.21(t,3H), 1.73(d,3H), 1.93(s,6H), 2.34(s,3H), 2.42(s,3H), 2.48(s,3H), 3.63(q,2H), 4.18(s,2H), 4.95(s,1H), 5.05(s,1H), 6.58(s,1H), 6.97(s,2H)
$EtN(CH_2)_2N(CH_3)_2$	Me	Me	H	1.26(t,3H), 1.88(s,6H), 2.31(s,3H), 2.34(s,6H), 2.41(s,3H), 2.43(s,3H), 2.62(m,2H), 3.64(q,2H), 3.74(m,2H), 6.55(s,1H), 6.94(s,2H)
$EtN(CH_2C(Me)_2)$	Me	Me	Me	0.91(d,6H), 1.17(t,3H), 1.84(s,6H), 1.95(s,3H), 2.05(m,1H), 2.35(s,3H), 2.38(s,3H), 2.47(s,3H), 3.36(d,2H), 3.61(q,2H), 6.98(s,2H)

B	R_3	R_4	R_6	^{1}H NMR ($CDCl_3$) δ (ppm)
NH-$CHEt_2$	Me	Me	Me	0.96(t,6H), 1.5-1.8(m,4H), 1.82(s,6H), 1.87(s,3H), 2.3(s,3H), 2.39(s,3H), 2.40(s,3H), 4.30(m,1H), 4.76(d,1H,NH), 6.94(s,2H)
NH-$CHEt_2$	Me	Me	H	0.98(t,6H), 1.5-1.8(m,4H), 1.92(s,6H), 2.32(s,3H), 2.45(s,3H), 2.46(s,3H), 4.32(m,1H), 4.82(d,1H,NH), 6.44(s,1H), 6.95(s,2H)
NHCH(n-$Pr)_2$	Me	Me	Me	0.94(s,6H), 1.3-1.7(m,4H), 1.84(s,6H), 1.89(s,3H), 2.32(s,3H), 2.39(s,3H), 2.41(s,3H), 4.46(s,1H), 4.73(s,1H,NH), 6.96(s,2H)
NHCH(Me)(n-Bu)	Me	Me	Me	0.92(t,3H), 1.27(d,3H), 1.37(m,4H), 1.5-1.7(m,2H), 1.83(s,6H), 1.84(s,3H), 1.89(s,3H), 2.33(s,3H), 2.40(s,3H), 2.43(s,3H), 4.41(m,1H), 4.77(d,1H,NH), 6.96(s,2H)
NH(n-Bu)	Me	Me	H	0.98(t,3H), 1.35-1.45(m,2H), 1.5-1.7(m,2H), 1.90(s,6H), 2.30(s,3H), 2.43(s,3H), 2.44(s,3H), 3.57(q,2H), 4.90(m,t,1H, NH), 6.38(s,1H), 6.93(s,2H)
NHEt	Me	Me	H	1.30(t,3H), 1.90(s,6H), 2.30(s,3H), 2.44(s,3H), 2.46(s,3H), 3.62(m,2H), 4.90(t,1H,NH), 6.40(s,1H), 6.93(s,2H)
NH-cyclopropyl	Me	Me	Me	0.57(m,2H), 0,85(m,2H), 1.81(s,6H), 1.87(s,3H), 2.31(s,3H), 2.34(s,3H), 2.48(s,3H), 3.00(m,1H), 5.17(s,1H), 6.95(s,2H)
NH-(R)-CH(Et)(CH_2OH)	Me	Me	Me	1.05(t,3H), 1.5-1.8(m,2H), 1.80(s,6H), 1.89(s,3H), 2.31(s,3H), 2.40(s,6H), 3.84(2sets of ABq. 2H), 3.96(m,1H), 5.14(d,1H,NH), 6.95(s,2H), 7.04(s,1H)
NHCH(Me)(Et)	Me	Me	Me	0.99(t,3H), 1.25(d,3H), 1.57(m,2H), 1.82(s,6H), 1.88(s,3H), 2.31(s,3H), 2.39(s,3H), 2.41(s,3H), 4.35(m,1H), 4.78(d,1H,NH), 6.94(s,2H)

B	R_3	R_4	R_6	^{1}H NMR ($CDCl_3$) δ (ppm)
NH-(S)-CH(Et)(CH_2OH)	Me	Me	Me	1.05(t,3H), 1.5-1.8(m,2H), 1.80(s,6H), 1.89(s,3H), 2.31(s,3H), 2.40(s,6H), 3.84(2sets of ABq, 2H), 3.96(m,1H), 5.14(d,1H,NH), 6.95(s,2H), 7.04(s,1H)
NH-cyclopentyl	Me	Me	Me	1.49(m,2H), 1.67(m,2H), 1.81(s,6H), 1.87(s,3H), 2.13(m,2H), 2.31(s,3H), 2.37(s,3H), 2.42(s,3H), 4.58(m,1H), 4.93(d,1H,NH), 6.94(S,2H)
NH-(S)-CH(Et)(CH_2OH)	Me	Me	H	1.08(t,3H), 1.5-1.8(m,2H), 1.89(s,6H), 2.30(s,3H), 2.43(s,3H), 2.448(s,3H), 2.453(s,3H), 3.86(2sts of ABq,2H), 3.98(m,1H), 5.17(d,1H,NH), 6.48(s,1H), 6.81(s,1H), 6.94(s,1H)
NH-(S)-CH(Et)(CH_2OMe)	Me	Me	H	0.98(t,3H), 1.6-1.8(m,2H), 1.90(s,3H), 1.91(s,3H), 2.30(s,3H), 2.42(s,3H), 2.44(s,3H), 3.39(s,3H), 3.53(2 sets of ABq,2H), 4.46(m,1H), 5.24(d,1H,NH), 6.42(s,1H), 6.92(s,2H)
NHCH(Me)(Et)	Me	Me	H	0.99(t,3H), 1.26(d,3H), 1.5-1.7(m,2H), 1.91(s,6H), 2.30(s,3H), 2.44(s,6H), 4.34(m,1H), 4.79(d,1H,NH), 6.42(s,1H), 6.93(s,2H)
NH-(R)-CH(Et)(CH_2OMe)	Me	Me	Me	1.00(t,3H), 1.55-1.8(m,2H), 1.82(s,6H), 1.87(s,3H), 2.31(s,3H), 2.38(s,3H), 2.39(s,3H), 3.39(s,3H), 3.54(m,2H), 4.45(m,1H), 5.25(d,1H,NH), 6.94(s,2H)
NH-(S)-CH(Et)(CH_2OMe)	Me	Me	Me	1.00(t,3H), 1.55-1.8(m,2H), 1.82(s,6H), 1.87(s,3H), 2.31(s,3H), 2.38(s,3H), 2.39(s,3H), 3.39(s,3H), 3.54(m,2H), 4.45(m,1H), 5.25(d,1H,NH), 6.94(s,2H)

B	R_3	R_4	R_6	^{1}H NMR ($CDCl_3$) δ (ppm)
NH-CH_2CH(Me)(Et)	Me	Me	Me	0.96(t,3H), 1.00(d,3H), 1.1-1.3(m,2H), 1.4-1.6(m,2H), 1.6-1.8(m,1H), 1.82(s,6H), 1.88(s,3H), 2.31(s,3H), 2.39(s,3H), 2.42(s,3H), 3.40(m,1H), 3.54(m,1H), 5.00(t,1H,NH), 6.94(s,2H)
NH-(S)-CH(CH_2Ph)(CH_2OH)	Me	Me	Me	1.77(s,3H), 1.78(s,3H), 1.82(s,3H), 1.99(s,3H), 2.30(s,3H), 2.41(s,3H), 2.84(m,1H), 3.12(m,1H), 3.75(m,1H), 3.93(m,1H), 4.27(m,1H), 5.15(d,1H,NH), 6.94(s,2H), 7.2-7.4(m,5H)
NH-(R)-CH(CH_2Ph)(CH_2OH)	Me	Me	Me	1.77(s,3H), 1.78(s,3H), 1.82(s,3H), 1.99(s,3H), 2.30(s,3H), 2.41(s,3H), 2.84(m,1H), 3.12(m,1H), 3.75(m,1H), 3.93(m,1H), 4.27(m,1H), 5.15(d,1H,NH), 6.94(s,2H), 7.2-7.4(m,5H)
NH-(S)-CH(CH_2Ph)(CH_2OMe)	Me	Me	Me	1.80(s,3H), 1.83(s,3H), 1.88(s,3H), 2.31(s,3H), 2.33(s,3H), 2.44(s,3H), 2.90(m,1H), 3.13(m,1H), 3.40(s,3H), 3.44(m,2H), 4.70(m,1H), 5.35(d,1H,NH), 6.95(s,2H), 7.2-7.4(m,5H)
NH-(R)-CH(CH_2Ph)(CH_2OMe)	Me	Me	Me	1.80(s,3H), 1.83(s,3H), 1.88(s,3H), 2.31(s,3H), 2.33(s,3H), 2.44(s,3H), 2.90(m,1H), 3.13(m,1H), 3.40(s,3H), 3.44(m,2H), 4.70(m,1H), 5.35(d,1H,NH), 6.95(s,2H), 7.2-7.4(m,5H)
NH-(S)-CH(Et)(CH_2OEt)	Me	Me	H	1.00(t,3H), 1.20(t,3H), 1.6-1.8(m,2H0, 1.90(s,3H), 1.91(s,3H), 2.30(s,3H), 2.42(s,3H), 2.43(s,3H), 3.4-3.6(m,2H), 4.41(m,1H), 5.34(d,1H,NH), 6.42(s,1H), 6.93(s,2H)
NHCH_2CH(n-Bu)(Et)	Me	Me	Me	0.89(t,3H), 0.95(t.3H), 1.2-1.4(m,7H), 1.54-1.62(m.1H), 1.82(s,6H), 1.88(s.3H), 2.31(s,3H), 2.39(s,3H), 2.42(s.3H), 3.53(m.2H), 4.90(m.1H), 6.95(s.2H)



NR_1R_2	R_4	R_6	R_5
	^{1}H-NMR($CDCl_3$) δ (ppm)		
EtN(n-Bu)	Me	Me	2,4-dimethylphenyl
	0.89(t,3H), 1.15(t,3H), 1.30(m,2H), 1.2-1.4(m,2H), 1.87(s,3H), 1.97(s,3H), 2.33(s,3H), 2.37(s,3H), 2.44(s,3H), 3.49(t,2H), 3.55(q,2H), 6.9-7.2(m,3H)		
$N(n\text{-}Pr)_2$	Me	Me	2,4-dimethylphenyl
	0.86(t,6H), 1.62(m,4H), 1.87(s,3H), 1.97(s,3H), 2.34(s,3H), 2.37(s,3H), 3.48(m,4H), 6.95-7.20(m,3H)		
EtN(n-Bu)	Me	Me	2,6-dimethylphenyl
	0.89(t,3H), 1.31(t,3H), 1.31(m,2H), 1.62(m,2H), 1.86(s,3H), 1.90(s,3H), 2.35(s,3H), 2.43(s,3H), 3.50(t,2H), 3.56(q,2H), 7.1-7.2(m,3H)		
EtN(n-Pr)	Me	Me	2,4-dimethylphenyl
	0.89(t,3H), 1.18(t,3H), 1.66(m,2H), 1.86(s,6H), 1.91(s,3H), 2.35(s,3H), 2.43(s,3H), 3.43(m,2H), 3.56(m,2H), 7.0-7.2(m,3H)		
EtN(n-Bu)	Me	H	2,5-dimethylphenyl
	0.93(t,3H),1.22(t,3H), 1.25-1.45(m,2H), 1.6-1.8(m,2H), 2.04(s,6H), 2.33(s,3H), 2.41(s,3H), 2.42(s,3H), 3.58(t,2H), 3.64(q,2H), 6.70(s,1H), 7.06(s,1H), 7.1-7.25(m,2H)		
EtN(n-Bu)	Me	H	3-methyl-4-chlorophenyl
	0.94(t,3H), 1.23(t,3H), 1.23-1.45(m,2H), 1.4-1.6(m,2H), 2.43(s,3H), 2.44(s,3H), 2.58(s,3H), 3.4-3.75(m,4H), 6.94(s,1H), 7.4-7.65(m,3H)		
EtN(n-Bu)	Me	H	2.6-dimethyl-4-bromophenyl

NR_1R_2	R_4	R_5	R_5
	^{1}H-NMR($CDCl_3$) δ (ppm)		
	0.92(t,3H), 1.22(t,3H), 1.25-1.40(m,2H), 1.55-1.60(m,2H), 1.91(s,6H), 2.40(s,3H), 2.43(s,3H), 3.57(m,2H), 3.64(q,2H), 6.50(s,1H), 7.27(s,2H)		
EtN(n-Bu)	Me	H	2,4-dimethyl-6-bromophenyl
	0.98(t,3H), 1.28(T,3H), 1.3-1.5(m,2H), 1.6-1.8(M,2H), 2.00(s,3H), 2.36(s,3H), 2.48(s,3H), 2.53(s,3H), 3.64(t,2H), 3.71(q,2H), 6.63(s,1H), 7.09(d,1H), 7.38(d,1H)		
$NHCH(Et)_2$	Me	H	2,6-dimethyl-4-bromophenyl
	0.98(t,6H), 1.5-1.8(m,4H), 1.94(s,6H), 2.44(s,3H), 2.45(s,3H), 4.30(M,1H), 4.80(d,1H,NH), 6.39(S,1H), 7.28(s,2H)		
NHCH(Et)(CH_2OH) (S)-isomer	Me	H	2,6-dimethyl-4-bromophenyl
	1.07(t,3H), 1.5-1.8(m,2H), 1.90(s,6H), 2.42(s,3H), 2.44(s,3H), 3.5-3.8(m,2H), 3.87(m,1H), 5.18(d,1H,NH), 6.45(s,1H), 6.56(s,1H), 7.27(s,2H)		
NHCH(Et)(CH_2OMe)	Me	H	2,6-dimethyl-4-bromophenyl
	1.00(t,3H), 1.55-1.75(m,2H), 1.92(s,6H), 2.42(s,3H), 2.43(s,3H), 3.39(s,3H), 3.55(2 sets of ABq,2H), 4.46(m,1H), 5.28(d,1H,NH), 6.38(s,1H), 7.26(s,2H)		
NHCH(Et)(CH_2OH)	Me	H	2,6-dimethyl-4-chlorophenyl
	1.07(t,3H), 1.55-1.80(m,2H), 1.91(s,6H), 2.42(s,3H), 2.45(s,3H), 3.74(2 sets of ABq,2H), 4.00(m,1H), 5.18(d,1H,NH), 6.46(s,1H), 6.60(brs,1H), 7.11(s,2H)		
NH-CH(Et)(CH_2OH) (R)-isomer	Me	H	2,6-dimethyl-4-bromophenyl
	1.07(t,3H), 1.55-1.75(m,2H), 1.90(s,6H), 2.42(s,3H), 2.44(s,3H), 3.75(2 sets of ABq,2H), 4.00(m,1H), 5.14(d,1H,NH), 6.45(s,1H), 6.50(brs,1H), 7.27(s,2H)		

EXAMPLE 7

A. 1-[2-Amino-4,5-dimethyl-1-(2,4,6-trimethylphenyl)-1H-pyrrol-3-yl]-2-ethyl-butan-1-one

A mixture of 3-hydroxy-2-butanone (0.637 g, 7.23 mmol), 2,4,6-trimethylaniline (0.973 g, 7.19 mmol) and p-toluenesulfonic acid (0.012 g, 0.06 mmol) in 15 ml of

benzene was refluxed using a Dean-Stark trap to remove water. After 3 hours, 4-ethyl-3-oxo-hexanenitrile (1.008 g, 0.724 mmol) was added and the mixture was refluxed for an additional 15 hours until all the starting material was consumed. The mixture was cooled to room temperature, diluted with water, and extracted with ethyl acetate. The organic layer was dried and concentrated to give 1.679 g of brown oil which was purified by silica gel column chromatography to give 368 mg of the title compound as a brown oil and 732 mg of undesired 2-(2-ethyl-butyl)-4,5-dimethyl-1-(2,4,6-trimethylphenyl)-1H-pyrrole-3-carbonitrile as a yellow solid. ^{1}H-NMR ($CDCl_3$) (the title compound) δ 0.94(t,6H), 1.4-1.8(m,4H), 1.73(s,3H), 1.98(s,6H), 2.25(s,3H). 2.34(s,3H), 3.00(m,1H), 5.80(brs,2H), 6.99(s,2H) ppm. ^{1}H-NMR ($CDCl_3$) (2-(2-ethyl-butyl)-4,5-dimethyl-1-(2,4,6-trimethylphenyl)-1H-pyrrole-3-carbonitrile)δ0.85(t,6H),1.5-1.85(m,4H), 1.71(s,3H), 1.88(s,6H), 1.95-2.10(m,1H), 2.14(s,3H), 2.34(s,3H), 6.96(s,2H) ppm.

B. N-[3-(2-ethyl-butyryl)-4,5-dimethyl-1-(2,4,6-trimethylphenyl)-1H-pyrrol-2-yl]-acetamide

A mixture of 1-[2-amino-4,5-dimethyl-1-(2,4,6-trimethylphenyl)-1H-pyrrol-3-yl]-2-ethyl-butan-1-one (326 mg, 1 mmol) and acetic anhydride (108 mg, 1.05 mmol) in 3 ml of acetic acid was heated to reflux for 2 hours. The mixture was cooled, quenched with water, neutralized with saturated potassium carbonate, and extracted with ethyl acetate. The organic layer was washed with brine, dried, and concentrated to give the title compound as a dark oil. The oil was purified through silica gel column chromatography to give 107 mg of the title compound as a brown oil. ^{1}H-NMR ($CDCl_3$) δ 0.88(t,6H), 1.4-1.8(m,4H), 1.76(s,3H), 1.88(s,3H), 1.93(s,6H), 2.25(s,3H). 2.28(s,3H), 2.98(m,1H), 6.89(s,2H) ppm.

C. 4-(1-Ethyl-propyl)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine

A mixture of N-[3-(2-ethyl-butyl)-4,5-dimethyl-1-(2,4,6-trimethylphenyl)-1H-pyrrol-2-yl]-acetamide (100 mg, 0.27 mmol), ammonium chloride (290 mg. 5.42 mmol). and acetamide (1.635 g) was heated to reflux for 2 hours. The mixture was cooled. quenched with water and extracted with ethyl acetate. The organic layer was dried and concentrated to give 56 mg of the title compound as a dark oil. The oil was purified through silica gel column chromatography to give the title compound as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ 0.85(t,6H), 1.70-2.0(m,4H), 1.83(s,6H), 1.99(s,3H 2.36(s,3H) 2.44(s,3H). 2.61(s,3H), 3.26(m,1H). 7.00(s,2H) ppm.

EXAMPLE 8

Butyl-ethyl-[2,5-dimethyl-7-(2,6-dimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine-4-yl]amine and 4-[4-(butyl-ethyl-amino)-2,5-dimethyl-pyrrolo[2,3-d]pyrimidin-7-yl]-3,5-dimethyl-benzoic acid

A solution of 7-(4-bromo-2,6-dimethyl-phenyl)-2,5-dimethyl-7H-pyrrolo[2,3-d]pyrimidine-4-yl]-butyl-ethyl-amine (0.700 g, 1.63 mmol) in 5 ml of dry tetrahydrofuran (THF) was added to a cooled solution of n-butyl lithium (n-BuLi) (2.5 M in hexane, 1.79 mmol) in 5 ml of dry THF at -78°C and stirred at that temperature for 20 minutes.

A part (1 mL) was taken from the reaction mixture and was quenched with an excess of water and extracted with ethyl acetate, dried and concentrated to give butyl-ethyl-[2,5-dimethyl-7-(2,6-dimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine-4-yl]amine as a clear oil. The oil was treated with 1 N HCl in methanol and concentrated to dryness. The residue was recrystallized from ethyl acetate to give the corresponding HCl salt as white crystals, mp 148-150°C.

The rest of the reaction mixture was quenched with an excess of dry ice at -78°C and the -78°C bath was removed. After 30 minutes, tlc showed that no starting material was left, and the mixture was quenched with water and extracted with ethyl acetate. The organic layer was washed with brine, dried and concentrated to give an off-white solid (0.550 g). The solid was recrystallized from 2-propanol to give the second title compound as white crystals, mp 228-230°C.

EXAMPLE 9

4-[4-(Butyl-ethyl-amino)-2,5-dimethyl-pyrrolo[2,3-d]pyrimidin-7-yl]-3,5-dimethyl-benzoic acid methyl ester

A mixture of 4-[4-(butyl-ethyl-amino)-2,5-dimethyl-pyrrolo[2,3-d]pyrimidin-7-yl]-3,5-dimethyl-benzoic acid (0.230g, 0.583 mmol) in 40 ml of 1 N HCl and methanol was heated at reflux for 3 hours (tlc showed that all starting materials were consumed). The mixture was concentrated to dryness. The residue was diluted with water and extracted with ethyl acetate. The organic layer was washed with saturated sodium bicarbonate, brine, dried and concentrated to give the title compound as a light brown oil. The oil was purified through silica gel column chromatography using 5% ethyl acetate in hexane as an eluent to give a golden oil. The corresponding HCl salt was prepared as an off-white solid, mp 58-60°C.

EXAMPLE 10

[4-(Butyl-ethyl-amino)-2,5-dimethyl-pyrrolo[2,3-d]pyrimidin-7-yl]-3,5-dimethylphenyl}-methanol

A solution of 4-[4-(butyl-ethyl-amino)-2,5-dimethyl-pyrrolo[2,3-d]pyrimidin-7-yl]-3,5-dimethyl-benzaldehyde (0.100 g, 0.264 mmol) in 1 ml MeOH was treated with sodium borohydride (0.030 g, 0.793 mmol) and stirred at room temperature for 20 minutes. The mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried and concentrated to dryness to give a clear oil. The oil was purified through silica gel column chromatography to give the title compound (0.092 g, 92% yield) as a white solid, mp 93-95°C.

EXAMPLE 11

Butyl-ethyl-[7-(4-fluoromethyl-2,6-dimethyl-phenyl)-2,5-dimethyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-amine

A solution of {4-[4-(butyl-ethyl-amino)-2,5-dimethyl-pyrrolo[2,3-d]pyrimidin-7-yl]-3,5-dimethylphenyl}-methanol (0.071 g, 0.186 mmol) in 2 ml anhydrous methylene chloride was cooled to -78°C and treated with dimethylaminosulfur trifluoride (0.063g, 0.390 mmol) and stirred at room temperature for 1 hour. The mixture was quenched with water and extracted with chloroform. The organic layer was washed with brine, dried, and concentrated to give an oil which was purified through silica gel using 2% methanol/chloroform as eluent to give the title compound as an off-white solid, mp 163-165°C.

EXAMPLE 12

Butyl-ethyl-[7-(4-methoxymethyl-2,6-dimethyl-phenyl)-2,5-dimethyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-amine

A solution of {4-[4-(butyl-ethyl-amino)-2,5-dimethyl-pyrrolo[2,3-d]pyrimidin-7-yl]-3,5-dimethylphenyl}-methanol (0.100 g, 0.263 mmol) in 1 ml of dry tetrahydrofuran was treated with sodium hydride (0.0116 g, 0.289 mmol, 60% in oil). After stirring for 10 minutes, an excess of methyl iodide was added. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was washed with brine, dried and concentrated to give an oil. The oil was purified through silica gel column using 10% ethyl acetate in hexane as eluent to give 0.081 g (78%) of the title compound as a white glass form.

EXAMPLE 13

[7-(4-aminomethyl-2,6-dimethyl-phenyl)-2,5-dimethyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-butyl-ethyl-amine

A solution of 4-[4-(butyl-ethyl-amino)-2,5-dimethyl-pyrrolo[2,3-d]pyrimidin-7-yl]-3,5-dimethyl-benzaldehyde (0.200 g, 0.528 mmol) in 2 ml of methanol was treated with sodium cyanoborohydride (0.023 g, 0.37 mmol), ammonium acetate (0.407 g, 5.28 mmol) and sodium sulfate. After stirring for 1 hour, the mixture was concentrated to remove methanol and the residue was dissolved in water, saturated sodium bicarbonate and extracted with ethyl acetate. The organic layer was washed with brine, dried and concentrated to give an oil. The oil was purified through silica gel column using 10% methanol in chloroform as eluent to give the title compound as a clear oil. The corresponding di-HCl salt was prepared as a white solid, mp 158-160°C.

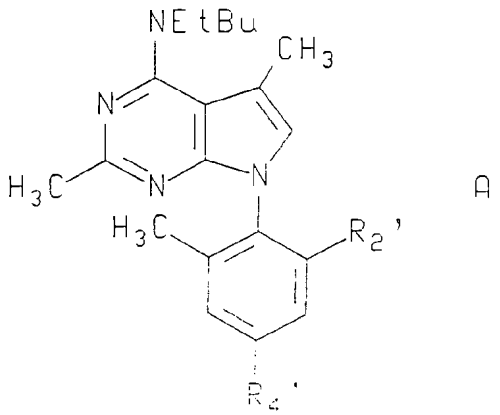
EXAMPLE 14

Butyl-ethyl-[7-(4-methoxyethyl-2,6-dimethyl-phenyl)-2,5-dimethyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-amine

The title compound was prepared starting from the 1-{4-[4-(butyl-ethyl-amino)-2,5-dimethyl-pyrrolo[2,3-d]pyrimidin-7-yl]-3,5-dimethyl-phenyl}-ethanol,sodiumhydride and methyl iodide and employing the procedure of Example 12.

The ^{1}H NMR data of the compounds prepared by the methods of Examples 8 to 15 as well as other compounds prepared by these methods are listed in the following Table.

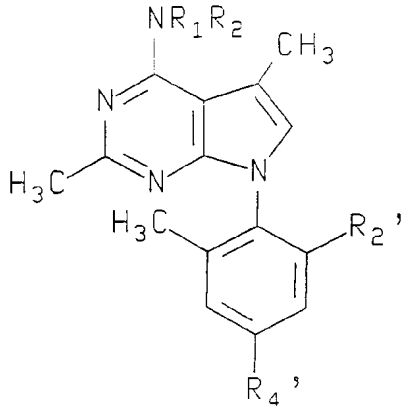
Table I



A

Example	R2'	R4'	^{1}H NMR ($CDCl_3$) δ (ppm)
8	Me	H	0.93(t,3H), 1.23(t,3H), 1.25-1.40(m,2H), 1.55-1.60(m,2H), 1.95(s,6H), 2.42(s,3H), 2.45(s,3H), 3.58(m,2H), 3.64(q,2H), 6.56(s,1H), 7.05-7.20(M,3H)
8	Me	COOH	0.95(t,3H), 1.27(t,3H), 1.3-1.45(m,2H), 1.6-1.8(m,2H), 1.96(s,6H), 2.43(s,3H), 2.56(s,3H), 3.65(t,2H), 3.72(q,2H), 6.61(s,1H), 7.52(s,2H)
	Me	CHO	0.92(t,3H), 1.23(t,3H), 1.25-1.40(m,2H), 1.55-1.70(m,2H), 2.03(s,6H), 2.42(s,3H), 2.43(s,3H), 3.58(m,2H), 3.64(q,2H), 6.54(s,1H), 7.65(s,2H), 9.99(s,1H)
10	Me	CH_2OH	0.92(t,3H), 1.22(t,3H), 1.25-1.40(m,2H), 1.55-1.70(m,2H), 1.94(s,6H), 2.41(s,3H), 2.45(s,3H), 3.58(t,2H), 3.65(q,2H), 4.55(s,2H), 6.54(s,1H), 7.09(s,2H)
	Me	CH(Me)(OH)	0.91(t,3H), 1.21(t,3H), 1.2-1.4(m,2H), 1.44(d,3H), 1.5-1.7(m,2H), 1.91(s,6H), 2.39(s,3H), 2.42(s,3H), 3.57(t,2H), 3.64(q,2H), 4.75(q,1H), 6.53(s,1H), 7.11(s,1H), 7.13(s,1H)
9	Me	COOMe	0.92(t,3H), 1.23(t,3H), 1.25-1.30(m,2H), 1.5-1.7(m,2H), 1.99(s,6H), 2.41(s,3H), 2.43(s,3H), 3.58(t,2H), 3.64(q,2H), 3.91(s,3H), 6.53(s,1H), 7.81(s,2H)
11	Me	CH_2F	0.90(t,3H), 1.20(t,3H), 1.24-1.40(m,2H), 1.5-1.7(m,2H), 1.95(s,6H), 2.38(s,3H), 2.42(s,3H), 3.54(t,2H), 3.62(q,2H). 4.30(d, 2H), 6.50(s,1H), 7.10(s,2H)
13	Me	CH_2NH_2	0.90(t,3H), 1.20(t,3H), 1.2-1.4(m,2H), 1.5-1.7(m,2H), 1.93(s,6H), 2.40(s,3H), 2.42(s,3H), 3.54(t,2H), 3.62(q,2H), 3.82(s,2H), 6.52(s,1H), 7.10(s,2H)
	Me	CONHMe	0.94(t,3H), 1.2-1.4(m,5H), 1.4-1.6(m,2H), 1.96(s,6H), 2.43(s,3H), 2.75(s,1.5H), 2.82(s,1.5H), 3.24(s,1H), 3.5-3.8(m.4H), 6.53(s,1H), 7.22(s,1H), 7.48(s,1H)
	Me	OH	0.89(t,3H), 1.20(t,3H), 1.2-1.4(m,2H), 1.5-1.7(m,2H), 1.76(s,6H), 2.379s,3H), 2.52(s,3H), 3.58(t,2H), 3.65(q,2H), 6.26(s,2H), 6.50(s,1H)

Example	R2'	R4'	^{1}H NMR ($CDCl_3$) δ (ppm)
	Me	I	0.92(t,3H), 1.22(t,3H), 1.2-1.35(m,2H), 1.5-1.7(m,2H), 1.89(s,6H), 2.40(s,3H), 2.44(s,3H), 3.57(t,2H), 3.64(q,2H), 6.50(s,1H), 7.48(s,2H)
	Me	Et	0.93(t,3H), 1.25(m,6H), 1.2-1.4(m,2H), 1.55-1.60(m,2H), 1.92(s,6H), 2.41(s,3H), 2.46(s,3H), 2.63(q,2H), 3.57(t,2H), 3.64(q,2H), 6.55(s,1H), 6.96(S,2H)
14	Me	CH(Me)(OMe)	0.88(t,3H), 1.18(t,3H), 1.2-1.4(m,2H), 1.38(d,3H), 1.5-1.7(m,2H), 1.90(s,6H), 2.36(s,3H), 2.40(s,3H), 3.24(s,3H), 3.4-3.65(m,4H), 4.20(q,1H), 6.50(s,1H), 7.00(s,2H)
12	Me	CH_2OMe	0.92(t,3H), 1.22(t,3H), 1.2-1.4(m,2H), 1.5-1.65(m,2H), 1.94(s6H), 2.41(s,3H), 2.44(s,3H), 3.42(s,3H), 3.45-3.52(m,4H), 4.42(s,2H), 6.53(s,1H), 7.10(s,2H)
	Me	$C(Me)_2(OH)$	0.92(t,3H), 1.22(t,3H), 1.25-1.40(m,2H), 1.5-1.7(m,2H), 1.58(s,6H), 1.95(s,6H), 2.40(s,3H), 2.45(s,3H), 3.5-3.7(m,4H), 6.54(s,1H), 7.23(s,2H)



NR_1R2	R2'	$R_{4'}$	^{1}H NMR ($CDCl_3$) δ (ppm)
$NHCH(Et)_2$	Me	H	0.98(t,6H), 1.5-1.8(m,4H), 1.97(s,6H), 2.44(s,3H), 2.46(s,3H), 4.34(m,1H), 4.81(d,1H), 6.44(s,1H), 7.1-7.2(m,3H)

$NHCH(Et)_2$	Me	CHO	0.98(t,6H), 1.5-1.8(m,4H), 2.06(s,6H), 2.43(s,3H), 2.46(s,3H), 4.31(m,1H), 4.83(d,1H), 6.43(s,1H), 7.66(s,1H), 9.99(s,1H)
$NHCH(Et)(CH_2OMe)$	Me	H	1.01(t,3H), 1.4-1.6(m,2H), 1.95(s,6H), 2.42(s,3H), 2.44(s,3H), 3.40(s,3H), 3.55(2 sets of ABq,2H), 4.48(m,1H), 5.26(d,1H,NH), 6.43(s,1H), 7.0-7.2(m,3H)

EXAMPLE 15

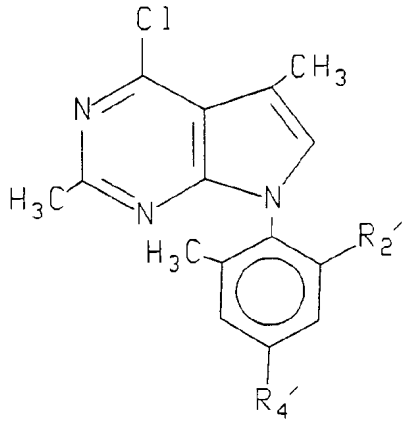
The following compounds of above formula A (Example 14) were prepared by procedures analogous to those in Examples 8 to 13.

R2'	R4'	^1H NMR ($CDCl_3$) δ (ppm)
H	Me	0.95(t,3H), 1.23(t,3H), 1.2-1.4(m,2H), 1.55-1.77(m,2H), 2.08(s,3H), 2.38(s,3H), 2.44(s,3H), 2.50(s,3H)3.59(t,2H), 3.66(q,2H), 6.71(ws,1H), 7.0-7.2(m,3H)
CHO	Me	0.95(t,3H), 1.26(t,3H), 1.25-1.45(m,2H), 1.6-1.8(m,2H), 2.05(s,3H), 2.438(s,3H), 2.443(s,3H), 2.448(s,3H), 3.5-3.8(m,4H), 6.7(s,1H), 7.39(d,1H), 7.68((d,1H), 9.33(s,1H)
CH_2OH	Me	0.96(t,3H), 1.27(t,3H), 1.25-1.45(m,2H), 1.6-1.75(m,2H), 1.95(s,3H), 2.41(s,3H), 2.43(s,3H), 2.44(s,3H), 3.5-3.8(m,4H), 4.15(m,2H), 6.6(s,1H), 7.11(s,1H), 7.26(s,1H)
CH_2F	Me	0.96(t,3H), 1.28(t,3H), 1.25-1.45(m,2H), 1.6-1.8(m,2H), 1.95(s,3H), 2.41(s,3H), 2.43(s,3H), 2.46(s,3H), 3.5-3.8(m,4H), 5.01(2 sets of ABq,2H), 6.63(s,1H), 7.15(s,1H), 7.25(s,1H)
CH(Me)(OH)	Me	0.96(t,3H), 1.27(t,3H), 1.25-1.409m,2H), 1.43(d,3H), 1.6-1.8(m,2H), 1.93(s,3H), 2.42(s,6H), 2.45(s,3H), 3.4-3.8(m,4H), 4.37(q,2H), 5.10(s,1H), 6.62(s,1H), 7.09(s,1H), 7.35(s,1H)

R2'	R4'	^{1}H NMR ($CDCl_3$) δ (ppm)
I	Me	0.96(t,3H), 1.27(t,3H), 1.25-1.45(m,2H), 1.6-1.8(m,2H), 1.97(s,3H), 2.34(s,3H), 2.46(s,3H), 2.50(s,3H), 3.5-3.8(m,4H), 6.58(s,1H), 7.10(S,1H), 7.62(s,1H)
Cl	Me	0.95(t,3H), 1.25(t,3H), 1.25-1.45(m,2H), 1.6-1.8(m,2H), 1.97(s,3H), 2.36(s,3H), 2.44(s,3H), 2.48(s,3H), 3.5-3.8(m,4H), 6.61(s,1H), 7.04(s,1H), 7.19(s,1H)
$C(Me)_2(OH)$	Me	0.94(t,3H), 1.18(s,3H), 1.25(t,3H), 1.25-1.5(m,2H), 1.55(s,3H), 1.6-1.8(m,2H), 1.69(s,3H), 2.38(s,3H), 2.42(s,3H), 2.43(s,3H), 3.5-3.8(m,4H), 4.13(brs,1H), 6.57(s,1H), 7.04(s,1H), 7.39(s,1H)
CH_2NH_2	Me	0.96(t,3H), 1.26(t,3H), 1.3-1.5(m,2H), 1.6-1.8(M,2H), 1.85(s,3H), 2.28(S<3H), 2.38(s,6H), 3.28(q,2H), 3.5-3.8(m,4H), 6.58(s,1H), 6.93(s,1H), 6.99(s,1H)
CH_2OMe	Me	0.96(t,3H), 1.26(t,3H), 1.25-1.45(m,2H), 1.6-1.8(m,2H), 1.92(s,3H), 2.38(s,3H), 2.44(s,3H), 2.46(s,3H), 3.25(s,3H), 3.61(t,2H), 3.68(q,2H), 4.04(ABq,2H), 6.62(s,1H), 7.06(s,1H), 7.22(s,1H)
Et	Me	0.95(t,3H), 1.04(t,3H), 1.26(t,3H), 1.25-1.45(m,2H), 1.90(s,3H), 2.15-2.35(m,2H), 2.37(s,3H), 2.44(s,3H), 2.47(s,3H), 3.63(m,2H), 3.67(q,2H), 6.57(s,1H), 6.98(s,1H), 7.01(s,1H)
CH(Me)(OMe)	Me	0.96(t,3H), 1.2-1.4(m,6H), 1.25-1.45(m,2H), 1.6-1.8(m,2H), 1.91(s,3H), 2.41(s,3H), 2.43(s,3H), 2.44(s,3H), 3.14(s,3H), 3.5-3.75(m,4H), 3.81(q,1H), 6.54(s,1H), 7.06(s,1H), 7.25(s,1H)

Example 16

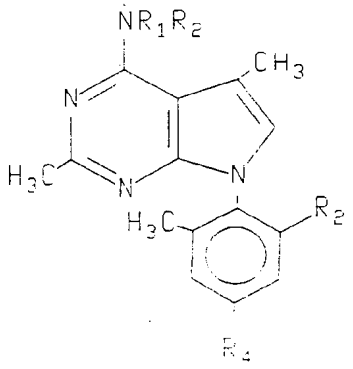
A. The following compounds were prepared by the procedures analogous to those in Examples 8 to 13 starting from n-BuLi with 4-chloro-2,5-dimethyl-7-(2,6-dimethyl-4-bromo- or 2,4-dimethyl-6-bromo-phenyl)-7H-pyrrolo-[2,3-d]pyrimidine, followed by quenching with an appropriate electrophile compound.



$R^{2'}$	$R^{4'}$	^1H NMR ($CDCl_3$) δ (ppm)
Me	Et	1.25(t,3H), 1.89(s,6H), 2.50(s,3H), 2.63(s,3H), 2.62(m,2H), 6.78(s,1H), 6.99(s,2H)
Me	OH	1.79(s,6H), 2.50(s,3H), 2.74(s,3H), 6.36(s,2H), 6.80(s,1H), 8.60(s,1H)
Me	$C(Me_2)(OH)$	1.60(s,6H), 1.93(s,6H), 2.50(s,3H), 2.63(s,3H), 6.78(s,1H), 7.23(s,1H)
CH_2F	Me	1.92(s,3H), 2.43(s,3H), 2.52(s,3H), 2.53(s,3H), 4.87(ABq,1H), 5.11(ABq,1H), 6.87(s,1H), 7.26(s,1H), 7.27(s,1H)
Et	Me	1.01(t,3H), 1.87(s,3H), 2.20(q,2H), 2.38(s,3H), 2.53(s,3H), 2.65(s,3H), 6.81(s,1H), 7.01(s,1H), 7.04(s,1H)
$C(Me_2)(OH)$	Me	1.26(s,3H), 1.43(s,3H), 1.66(s,3H), 2.40(s,3H), 2.52(s,3H), 2.62(s,3H), 6.83(s,1H), 7.07(s,1H), 7.45(s,1H)
H	Me	2.04(s,3H), 2.40(s,3H), 2.51(s,3H), 2.67(s,3H), 6.92(s,1H), 7.13(s,2H), 7.18(s,1H)

$R^{2'}$	$R^{4'}$	^1H NMR ($CDCl_3$) δ (ppm)
CHO	Me	2.00(s,3H), 2.47(s,3H), 2.54(s,3H), 2.63(s,3H), 6.92(s,1H), 7.45(m,1H), 7.70(m,1H), 9.39(s,1H)
CH_2OH	Me	1.88(s,3H), 2.36(s,3H), 2.50(s,3H), 2.57(s,3H), 3.56(brs,1H), 4.05-4.25(m,2H), 6.84(s,1H), 7.08(s,1H), 7.25(s,1H)

B. The following compounds were prepared starting with the appropriate amine and the appropriate 4-chloro-2,5-dimethyl-7-(substituted phenyl)-7H-pyrrolo[2,3-d]pyrimidine (compounds listed in the table above under 16A or other related compounds) in DMSO and employing the general procedure of Example 5.



NR_1R_2	R_2'	R_4'	^1H NMR ($CDCl_3$) δ (ppm)
NH-(S)-CH(Et)(CH_2OH)	Me	Et	1.07(t,3H), 1.23(t,3H), 1.45-1.56(m,2H), 1.90(s,3H), 1.91(s,3H), 2.43(s,3H), 2.45(s,3H), 2.60(q,2H), 3.65(m,1H), 3.83(m,1H), 4.00(m,1H), 5.17(d,1H), 6.49(s,1H), 6.95(s,2H)
NH-(S)-CH(Et)(CH_2OH)	Et	Me	1.00(t,3H), 1.07(t,3H), 1.6-1.85(m,2H), 1.88(s,3H), 2.15-2.30(m,2H), 2.32(s,3H), 2.42(s,3H), 2.48(s,3H), 3.6-3.9(m,2H), 3.92-4.10(m,1H),5.25(d,1H), 6.5(s,1H), 6.94(s,1H), 7.0(s,1H)

NR_1R_2	R_2'	R_4'	^1H NMR ($CDCl_3$) δ (ppm)
NH-(S)-CH(Et)(CH_2OH)	Me	CMe_2OH	1.08(t,3H), 1.58(s,6H), 1.6-1.9(m,2H), 1.94(s,3H), 1.95(s,3H), 2.46(s,3H), 2.50(s,3H), 3.5-3.95(m,2H), 4.2(brs ,1H), 5.28(brs,1H), 6.53(s,1H), 7.24(s,2H)
NH-(S)-CH(Et)(CH_2OH)	C(OH)(Me_2)	Me	1.11(t,3H), 1.24(s,3H), 1.5-1.9(m,2H), 1.50(s,0.5x3H), 1.53(0.5x3H), 1.71(s,3H), 2.39(s,3H), 2.43(s,3H), 2.48(s,3H), 3.16(brs,0.5H), 3.29(brs,0.5H), 3.6-3.95(m,2H), 3.95-4.10(m,1H), 5.2-5.3(m,1H), 6.54(s,1H), 7.05(s,1H), 7.40(s,0.5H), 7.43(s,0.5H)
NH-(S)-CH(Et)(CH_2OH)	H	Me	1.10(t,3H), 1.5-1.9(m,2H), 2.05(s,3H), 2.37(s,3H), 2.48(s,6H), 3.6-3.9(m,2H), 3.9-4.1(m,1H), 5.23(d,1H), 6.64(s,1H), 7.0-7.2(m,2H)
NH-(S)-CH(Et)(CH_2OH)	CHO	Me	1.09(t,3H), 1.55-1.90(m,2H), 2.02(s,3H), 2.42(s,6H), 2.47(s,3H), 3.6-3.9(m,2H), 4.0-4.15(m,1H), 5.27(t,1H), 6.62(s,1H), 7.39(s,1H), 7.65(s,1H), 9.36(s,1H)
NH-(S)-CH(Et)(CH_2OH)	CH_2OH	Me	both diastereoisomers were separated by column chromatography and showed identical spectra. 1.11(t,3H), 1.55-1.90(m,2H), 1.95(s,3H), 2.40(s,3H), 2.43(s,3H), 2.49(s,3H), 3.6-3.95(m,2H), 4.0-4.3(m,2H), 4.4(brs,1H), 5.30(d,1H), 6.57(s,1H), 7.11(s,1H), 7.27(s,1H)
NH-(S)-CH(Et)(CH_2OH)	CH_2F	Me	1.11(t,3H), 1.5-1.85(m,2H), 1.94(s,3H), 2.41(s,3H), 2.45(s,3H), 2.47(s,3H), 3.6-3.9(m,2H), 4.0-4.2(m,1H), 4.75-5.25(m,2H), 5.24(m,1H), 6.58(s,1H), 7.16(s,1H), 7.24(s,1H)

Example 17

The following compounds were prepared by the procedures analogous to those in Examples 8 to 13 starting from an excess of n-BuLi with 4-substituted amino-2,5-dimethyl-7-(2,4,6-tri-substituted-phenyl)-7H-pyrrolo-[2.3-d]pyrimidine, followed by quenching with an appropriate electrophile.

NR_1R_2	R_2'	R_4'	^1H NMR ($CDCl_3$) δ
NH-(S)-CH(Et)(CH_2OH)	CH_2CH_2OH	Me	1.11(t,3H), 1.5-1.9(m,2H), 1.84(s,3H), 2.37(s,3H), 2.45(s,3H), 2.48(s,3H), 2.45-2.65(m,2H), 3.6-3.95(m,4H), 4.15(m,1H), 5.28(d,1H), 6.54(s,1H), 7.02(s,1H), 7.09(s,1H)
NH-(S)-CH(Et)(CH_2OH)	Me	CHO	1.08(t,3H), 1.5-1.8(m,2H), 2.02(s,6H), 2.42(s,3H), 2.46(s,3H), 3.6-3.9(m,2H), 4.0(brs,1H), 5.20(d,1H), 6.49(s,1H), 7.65(s,2H), 9.98(s,1H)
NH-(S)-CH(Et)(CH_2OH)	Me	I	1.06(t,3H), 1.6-1.9(m,2H), 1.88(s,6H), 2.42(s,3H), 2.44(s,3H), 3.6-3.9(m,2H), 4.08(brs,1H), 5.20(d,1H), 6.45(s,1H), 7.48(s,2H)
NH-(S)-CH(Et)(CH_2OH)	Me	CH_2OH	1.08(t,3H), 1.6-1.85(m,2H), 1.93(s,6H), 2.45(s,6H), 3.6-3.95(m,2H), 4.10(brs,1H), 4.60(s,2H), 5.24(brs,1H), 6.50(s,1H), 7.11(s,2H)
NH-(S)-CH(Et)(CH_2OH)	Me	C(Me)-($C=CH_2$)	1.08(t,3H), 1.5-1.8(m,2H), 1.94(s,6H), 2.13(s,3H), 2.44(s,3H), 2.45(s,3H), 3.55-3.90(m,2H), 4.00(brs,1H), 5.07(s,1H), 5.20(d,1H), 5.35(s,1H), 6.50(s,1H), 7.21(s,2H)

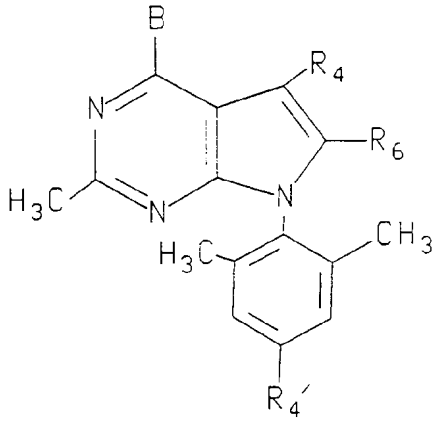
EXAMPLE 18

4-sec-Butoxy-1-(2,5,6-trimethylphenyl-7-(2,4,6-trimethyl-phenyl)-7H-pyrrolo[2,3-d]pyrimidine

Sodium hydride (0.114g, 4.77mmol, 60% in oil) was washed with hexane, then treated with 2-butanol (1.18g, 15.90 mmol). After 20 minutes, a mixture of 4-chloro-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine(0.500g,1.59mmol) in 5 ml of anhydrous tetrahydrofuran was added to the reaction mixture and stirred for 2 hours. The mixture was concentrated to dryness, dissolved in ethyl acetate and water. The organic layer was separated, washed with brine, dried, and concentrated to give a clear oil. The oil residue was purified through silica gel column chromatography using 20% ethyl acetate in hexane as eluent to give a clear oil which crystallized under high vacuo to give 0.450 g (80.5%) of an off-white solid. The solid was recrystallized from i-propanol to give gold crystals, mp 178-180°C.

EXAMPLE 19

The following compounds were prepared starting with the appropriate alcohol or thiol and 4-chloro-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine or 4-chloro-2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine and employing the general procedure of Example 18.



B	R_4'	R_4	R_6	^{1}H NMR ($CDCl_3$) δ (ppm)
$OCHEt_2$	Me	Me	Me	0.99(t,6H), 1.74(m,4H), 1.84(s,6H), 1.92(s,3H), 2.34(s,3H), 2.37(s,3H), 2.48(s,3H), 5.34(m,1H), 6.98(s,2H)
$OCHMe_2$	Me	Me	Me	1.41(d,6H), 1.82(s,6H), 1.91(s,3H), 2.33(s,3H), 2.36(s,3H), 2.48(s,3H), 5.55(m,1H), 6.98(s,2H)
OCH(Me)(Et)	Me	Me	Me	1.02(t,3H), 1.28(d,3H), 1.65-1.80(m,2H), 1.83(s,6H), 1.92(s,3H), 2.33(s,3H), 2.37(s,3H), 2.48(s,3H), 5.38(m,1H), 6.98(s,2H)
OCH(Et)(n-Pr)	Me	Me	Me	0.94(t,3H), 0.97(t,3H), 1.38-1.60(m,2H), 1.6-1.8(m,4H), 1.82(s,6H), 1.90(s,3H), 2.32(s,3H), 2.35(S,3H), 2.46(s,3H), 6.96(s,2H)
OCH(Et)(n-Bu)	Me	Me	Me	0.90(t,3H), 0.99(t,3H), 1.3-1.5(m,4H), 1.6-1.8(m,4H), 1.832(s,3H), 1.837(s,3H), 1.92(s,3H), 2.34(s,3H), 2.36(s,3H), 2.48(s,3H), 5.39(m,1H), 6.98(s,2H)

B	R_4'	R_4	R_6	^{1}H NMR ($CDCl_3$) δ (ppm)
OCH(Et)(n-pentyl)	Me	Me	Me	0.88(t,3H), 0.98(t,3H), 1.4-1.6(m,6H), 1.6-1.8(m,4H), 1.82(s,6H), 1.90(s,3H), 2.32(s,3H), 2.36(s,3H), 2.47(s,3H), 5.40(m,1H), 6.96(s,2H)
OCH(Et)(n-hexyl)	Me	Me	Me	0.85(t,3H), 0.97(t,3H), 1.20-1.50(m,8H), 1.6-1.8(m,4H), 1.82(s,6H), 1.90(s,3H), 2.32(s,3H), 2.35(s,3H), 2.46(s,3H), 5.37(m,1H), 6.96(s,2H)
$OCH(n\text{-}Pr)_3$	Me	Me	Me	0.94(t,3H), 1.4-1.6(m,4H), 1.6-1.8(m,4H), 1.83(s,6H), 1.91(s,3H), 2.33(s,3H), 2.36(S,3H), 2.48(s,3H), 5.50(m,1H), 6.97(s,2H)
$OCH(Et)(CH_2OMe)$	Me	Me	Me	1.03(t,3H), 1.82(s,3H), 1.83(s,3H), 1.91(s,3H), 2.33(s,3H), 2.37(s,3H), 2.47(s,3H), 3.43(s,3H), 3.68(m,2H), 5.55(m,1H), 6.97(s,2H)
$OCHEt_2$	Me	Me	H	0.99(t,6H), 1.63(m,4H), 1.92(s,6H), 2.32(s,3H), 2.41(s,3H), 2.50(s,3H), 5.35(m,1H), 6.52(s,1H), 6.96(s,2H)
$OCH(Et)(CH_2OMe)$	Me	Me	H	1.00(t,3H), 1.6-1.8(m,2H), 1.86(s,3H), 1.87(s,3H), 2.28(s,3H), 2.40(s,3H), 2.489s,3H), 3.40(s,3H), 3.62(m,2H), 5.51(m,1H), 6.48(s,1H), 6.92(s,2H)
OCH_2-(S)-$CH(NH_2)(Et)$	Br	Me	H	1.03(t,3H), 1.3-1.5(m,2H), 1.91(s,6H), 2.42(s,3H), 2.51(s,3H), 4.13(m,1H), 4.26(m,1H), 4.44(m,1H), 6.52(s,1H), 7.29(s,2H)
S-CH(Me)-CH(OH)(Me)	Br	Me	H	1.25(d,3H), 1.41(d,3H), 1.87(s,3H), 1.89(s,3H), 2.50(s,3H), 2.55(s,3H), 4.1-4.3(m,2H), 6.63(s,1H), 6.65(brs,1H), 7.30(s,2H)

EXAMPLE 20

A. 2,5,6-Trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine-4-carbonitrile

A mixture of 4-chloro-2,5,6-trimethyl-7-(2,4,6-trimethyl-phenyl)-7H-pyrrolo[2,3-d]pyrimidine (10.000 g, 31.90 mmol) and potassium cyanide (20.75 g, 319 mmol) in 100

ml dimethylsulfoxide was heated at 130°C oil bath over weekend. The mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated to give 9.61 g (99%) of brown soild. The solid was recrystallized from i-propanol to give 6.34 g (65%) of the title compound as light golden crystals, mp 188-190°C. ^{1}H NMR ($CDCl_3$) δ 1.8(s,6H), 2.07(s,3H), 2.36(s,3H), 2.50(s,3H), 2.65(s,3H), 7.00(s,2H).

B. 2-Methyl-1-[2,5,6-trimethyl-7-(2,4,6-trimethyl-phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-butan-1-one

To a solution of sec-butyl magnesium chloride (1.5 ml, 3.0 mmol, 2 M in diethyl ether) in 24 ml of dry tetrahydrofuran was added 2,5,6-trimethyl-7-(2,4,6-trimethyl-phenyl)-7H-pyrrolo[2,3-d]pyrimidine-4-carbonitrile (0.814 g, 2.67 mmol) at room temperature and stirred for 5 hours. The mixture was quenched with 5 ml of 2N HCl, neutralized with saturated sodium bicarbonate, extracted with ethyl acetate. The organic layer was dried and concentrated to give a yellow solid. The solid was purified through silica gel column chromatography using chloroform as eluent to give 0.884 g (90%) of the title compound as yellow crystals, mp 133-135°C.

EXAMPLE 21

[2,5,6-Trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-propan-1-one and 1-[2,5,6-Trimethyl-7-(2,4,6-trimethyl-phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-pentan-1-one were prepared starting from 2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine-4-carbonitrile, and ethyl magnesium chloride and n-BuLi. respectively, employing the general procedure of Example 20B.

EXAMPLE 22

[2,5,6-Trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-propan-1-ol

A solution of 2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-propan-1-one (0.300 g, 0.89 mmol) in 10 ml of methanol was treated with sodium borohydride ($NaBH_4$) (0.169 g, 4.47 mmol) at room temperature and stirred for 15 minutes. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried, and concentrated to give 0.291 g (96%) of the title compound as light yellow crystals. The crystals were recrystallized from i-propanol to give light yellow crystals, mp 143-144°C.

EXAMPLE 23

The following compounds were prepared by reduction of the corresponding ketone derivative with $NaBH_4$ by the procedure described in the Example 22:

1-[2,5,6-Trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-pentan1-ol; and

2-Methyl-1-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-butan-ol.

EXAMPLE 24

The following compounds were prepared by reaction of the corresponding alcohol derivative with NaH, followed by reacting with alkyl iodide using the procedure analogous to that described in Example 12:

4-(1-Methoxy-propyl)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine;

4-(1-Ethoxy-propyl)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine; and

4-(1-Methoxy-2-methyl-butyl)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine.

EXAMPLE 25

[2,5,6-Trimethyl-7-(2,4,6-trimethyl-phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-pentan-3-ol

A solution of 1-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-propan-1-one (0.220 g, 0.656 mmol) in 10 ml of dry THF was treated with ethyl magnesium bromide (0.787 mmol, 0.39 ml, 2.0 m in THF) at 0°C and stirred at room temperature for 1 hour. The mixture was quenched with diluted HCl, neutralized with aqueous NaOH and extracted with ethyl acetate. The organic layer was dried and concentrated to give a yellow solid. The solid was redcrystallized from ethyl ether/ethyl acetate to give off-white crystals, mp 164-166.5°C.

EXAMPLE 26

[2,5,6-Trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-hexan-3-ol

The title compound was prepared by reacting 1-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-propan-1-one with n-propyl magnesium chloride using the procedure described in Example 25.

EXAMPLE 27

(1-Ethyl-1-fluoro-propyl)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine

The title compound was prepared by reacting of 3-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-pentan-3-ol with dimethylaminosulfur trifluoride using the procedure described in Example 11.

EXAMPLE 28

(1-Ethyl-propenyl)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine

A mixture of 3-[2,5,6-trimethyl-7-(2,4,6-trimethyl-phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-pentan-3-ol (0.041 g, 0.122 mmol), concentrated sulfuric acid (0.055 g, 0.56 mmol) and acetic acid (0.136 g, 2.27 mmol) was heated to reflux for 1 hour, cooled, diluted with water, basified to pH 10 with 2 N NaOH and extracted with ethyl acetate. The organic layer was washed with brine, dried and concentrated to dryness to give 43 mg of the title compound as a clear oil. The oil was purified through silica gel column chromatography to give 40 mg of the title compound as a white solid, mp 59-61°C.

EXAMPLE 29

Compounds listed in the following Table II in which B is CH(OAc)(CHMeEt) and a mixture of two isomers 4-(1-ethyl-butenyl)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine and 4-(1-n-propyl-propenyl)-2,5,6-trimethyl-7-(2,4,6-trimethyl-phenyl)-7H-pyrrolo[2,3-d]pyrimidine [see Table II in which B is C(=CHEt)(Et) and C(=CHMe)(n-Pr)] were prepared by a procedure analogous to that described in Example 28.

EXAMPLE 30

(1-Ethyl-butyl)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine

A mixture of two isomers, 4-(1-ethyl-butenyl)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine and 4-(1-n-propyl-propenyl)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine (67 mg, 0.185 mmol) in ethyl acetate (18 ml) and 10% Pd/C (38 mg) was hydrogenated at 50 psi for 15 hours. The mixture was filtered through celite. The filtrated was washed with brine, dried and concentrated to give 119 mg of oil. The oil was purified through silica gel column

chromatography using 7% ethyl acetate in hexane as eluent to give 31 mg (46%) of the title compound as off-white crystals, mp 100-102°C.

EXAMPLE 31

[-2,5,6-Trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-propan-1-one oxime

A mixture of 1-[-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-propan-1-one (0.598 g, 1.783 mmol), hydroxylamino hydrochloride (0.370 g, 5.35 mmol), sodium acetate (0.439 g, 5.35 mmol) in MeOH (30 ml) was stirred at room temperature for 24 hours. The mixture was concentrated to dryness. The residue was diluted with water and extracted with ethyl acetate. The organic layer was dried and concentrated to give 0.657 g of a white glass form. The glass form was purified through silica gel column chromatography to separated both E (white crystals, mp 162-164°C, confirmed by X-ray structural analysis) and Z (white crystals, mp 84-87°C) isomers and a mixture of E and Z isomers (mp-150-190°C).

EXAMPLE 32

1-[2,5,6-Trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-propylamine

Hydrogenation of 1-[-2,5,6-Trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-propan-1-one oxime with 10% Pd/C in MeOH using the general procedure described in Example 28 resulted in the title compound.

EXAMPLE 33

[2,5,6-Trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-ylmethyl]-formamide

A mixture of 2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine-4-carbonitrile (1.000 g, 3.29 mmol), 1:1 Al/Ni alloy (1.0 g) in 70% aqueous formic acid (10 ml) was stirred at room temperature for 1 hour. The mixture was filtered through Celite, washed with 100 ml of water and 100 ml of ethyl acetate. The organic layer was separated, dried and concentrated to give a light green oil. The oil was purified through silica gel column chromatography using 2% methanol in chloroform as eluent to give 0.960 g (86.5%) of the title compound as an off-white soild. The solid was recrystallized from ethyl acetate to give a light yellow crystals, mp 202-204°C.

EXAMPLE 34

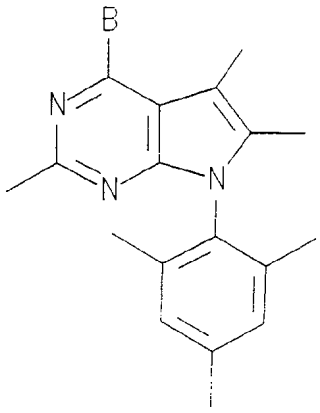
N-[2,5,6-Trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-ylmethyl]-acetamide

A mixture of 2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine-4-carbonitrile (0.500 g, 1.64 mmol) and 10% Pd/C (0.500 g) in ethanol was hydrogenated at 55 psi for 5 hours. The mixture was filtered through celite and the filtrate was concentrated to give 0.500g (98.8%) of N-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-ylmethyl]-amine.

A mixture of N-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-ylmethyl]-amine (0.200 g, 0.648 mmol), acetic anhydride (0.132 g, 1.30 mmol), triethylamine (0.132 g, 1.30 mmol) in anhydrous methylene chloride (1 ml) was stirred at room temperature for 1 hour. The mixture was quenched with water and extracted with methylene chloride. The organic layer was separated, dried and concentrated to give 0.217 g (95.6%) of the title compound as a light tan solid. The solid was purified through silica gel column chromatography using 5% methanol in chloroform as eluent to give 0.200 g (88.1%) of the title compound as golden crystals, mp 140-143°C.

The ^{1}H NMR data of the compounds which are described in the Examples 20 to 34 are listed in the following Table.

Table II



B	^{1}H NMR ($CDCl_3$) δ (ppm)
CO-(n-Bu)	1.00(t,3H), 1.4-1.6(m,2H), 1.7-1.9(m,2H), 1.83(s,6H), 2.06(s,3H), 2.34(s,3H), 2.35(s,3H), 2.38(s,3H), 3.27(t,2H), 7.03(s,2H)
COEt	1.26(t,3H), 1.81(s,6H), 2.04(s,3H), 2.32(s,3H), 2.36(s,3H), 2.68(s,3H), 3.27(q,2H), 7.00(s,2H)
CO-CH(Me)(Et)	0.99(t,3H), 1.24(d,3H), 1.45-1.65(m,1H), 1.7-1.9(m,1H), 1.83(s,6H), 2.05(s,3H), 2.30(s,3H), 2.37(s,3H), 2.68(s,3H), 3.91(m,1H0, 7.03(s,2H)
CH(OH)(n-Bu)	0.95(t,3H), 1.2-1.8(m,6H), 1.77(s,3H), 1.87(s,3H), 2.00(s,3H), 2.37(s,3H), 2.39(s,3H), 2.63(s,3H), 5.20(dd,1H), 7.02(s,2H)
CH(OH)(Et)	1.12(t,3H), 1.6-2.0(m,2H), 1.77(s,3H), 1.87(s,3H), 2.00(s,3H), 2.37(s,3H), 2.39(s,3H), 2.63(s,3H), 4.97(d,1H), 5.15(m,1H), 7.02(s,2H)
CH(OMe)(Et)	1.02(t,3H), 1.82(s,3H), 1.83(s,3H), 2.01(s,3H), 1.8-2.1(m,2H), 2.36(s,3H), 2.45(s,3H), 2.669s,3H), 3.359s,3H0, 4.68(t,1H), 7.01(s,2H)
CH(OEt)(Et)	1.02(t,3H), 1.22(t,3H), 1.82(s,3H), 1.83(s,3H), 1.7-2.1(m,2H), 2.36(s,3H), 2.46(s,3H), 2.65(s,3H), 3.49(m,2H), 4.75(t,1H), 7.01(s,2H)
CH(OMe)(CHMeEt)	0.68(d,1.8H), 0.83(t,1.2H), 0.95(t,1.8H), 1.10(d,1.2H), 1.1-1.5(m,2H), 1.9-2.2(m,1H), 1.8(3 sets of s,6H), 2.0(s,3H), 2.359s,3H0, 2.53(s,3H0, 2.65(s,3H), 3.25(s,1.8H), 3.30(s,1.2H), 4.42(d,0.6H), 4.5(d,0.4H), 7.0(s,2H)
CH(OAc)(CHMeEt)	0.7(d,1.5H), 0.85(t,1.5H), 0.94(t,1.5H), 1.1(d,1.5H), 1.1-1.5(m,2H), 1.81(s,1.5H), 1.83(s,3H), 1.869s,1.5H), 2.0(s,3H), 2.22(s,1.5H), 2.24(s,1.5H), 2.2-2.4(m,0.5H), 2.32(s,3H), 2.49(s,1.5H), 2.51(s,1.5H), 2.60(s,3H), 3.0-3.2(m,0.5H), 6.12(m,1H), 7.0(s,2H)
$CFEt_2$	0.90(t,6H), 1.83(s,6H), 2.03(s,3H), 2.0-2.4(m,4H), 2.38(s,6H), 2.59(s,3H), 7.02(s,2H)
$CEt_2(OH)$	071(t,6H), 1.79(s,6H), 2.02(s,3H), 2.0-2.4(m,4H), 2.36(s,3H), 2.47(s,3H), 2.61(s,3H), 7.01(s,2H)

B	^{1}H NMR ($CDCl_3$) δ (ppm)
C(Et)(n-Pr)(OH)	0.71(t,3H), 0.84(t,3H), 1.4-1.6(m,2H), 1.80(s,3H), 1.81(s,3H), 2.04(s,3H), 1.9-2.2(m,4H), 2.38(s,3H), 2.49(s,3H), 2.63(s,3H), 6.83(s,1H), 7.03(s,2H)
CH(Et)(NH-n-Pr)	0.87(t,3H), 0.90(t,3H), 1.5-1.7(m,2H), 1.80(s,3H), 1.83(s,3H), 2.00(s,3H), 1.9-2.2(m,2H), 2.36(s,3H), 2.41(s,3H), 2.42(s,3H), 2.3-2.5(m,1H), 2.7-2.9(m,1H), 4.48(m,1H), 7.019s,2H), 7.15(s,1H),
=NOH)(Et)	1.0-1.2(m,3H), 1.79(s,1.5H), 1.80(s,1.5H), 1.99(s,1,.5H), 2.00(s,1.5H), 2.22(s,3H0, 2.35(s,3H), 2.65(s,1.5H), 2.68(s,1.5H), 2.7(q,1H), 2.99(q,1H), 6.93(s,2H), 9.05(brs,1H)
CH(Et)(NH2)	1.04(t,3H), 1.79(s,3H), 1.85(s,3H), 1.7-2.0(m,2H), 1.99(s,3H), 2.36(s,3H), 2.42(s,3H), 2.62(s,3H), 4.52(m,1H), 7.01(s,2H)
=CHMe)(Et)	1.00(t,2.1H), 1.1(t,0.9H), 1.47(d,0.9H), 1.82(s,6H), 1.9(d,2.1H), 2.02(s,3H), 2.25(s,3H), 2.4-2.8(m,5H), 5.6-5.8(m,1H), 7.0(s,2H)
=CHEt)(Et) + C(=CHMe)(n-Pr)	(m,5.4H), 1.82(s,6H), 1.869d,1.8H), 2.0(s,3H), 2.20(s,.1.2H), 2.21(s,1.8H), 2.359s,3H), 2.60(s,1.8H), 2.61(s,1.2H), 2.3-2.8(m,2.8H),5.4-5.8(m,1H), 6.959s,2H)
CH(n-Bu)(Et)	0.83(t,3H), 0.88(t,3H), 1.1-1.49(m,2H), 1.6-2.2(m,4H), 1.82(s,3H), 1.83(s,3H), 1.98(s,3H), 2.35(s,3H), 2.43(s,3H), 2.61(s,3H), 3.33(m,1H), 7.00(s,2H)
CH2NHCHO	1.79(s,6H), 2.00(s,3H), 2.35(s,3H), 2.48(s,3H), 2.62(s,3H), 4.98(d,2H), 7.01(s,2H), 8.05(brs,1H), 8.38(s,1H)
CH2NHCOCH3	1.79(s,6H), 1.97(s,3H), 2.12(s,3H), 2.34(s,3H), 2.43(s,3H), 2.61(s,3H), 4.90(d,2H), 6.99(s,2H), 7.46(brs,1H)

Example 35

A. 1-[2-Amino-4,5-dimethyl-1-(2,4,6-trimethylphenyl)-1H-pyrrol-3-yl]-2-ethyl-butan-1-one

A mixture of 3-hydroxy-2-butanone (0.637 g, 7.23 mmol), 2,4,6-trimethylaniline (0.973 g, 0.719 mmol) and p-toluene sulfonic acid (0.012 g) in 15 ml of benzene was heated at reflux under Dean-Stark trap for 3 hours. A solution of $(Et)_2CHCOCH_2CN$ (1.008 g, 7.24 mmol) was added to the reaction mixture and heated at reflux overnight. The mixture was cooled and diluted with ethyl acetate and water. The organic layer was separated and washed with water, aqueous sodium carbonate, and then brine; dried and concentrated to give a brown oil which contains the desired compound. 0.368 g of the desired compound was isolated after silica gel column chromatography using chloroform as eluent. ^1H NMR ($CDCl_3$) δ 0.94 (t,6H), 1.5-1.8 (m,4H), 1.73 (s,3H), 1.98 (s,6H), 2.26 (s,3H), 2.34 (s,3H), 3.00 (m,1H), 5.78 (brs,2H), 6.99 (s,2H) ppm.

B. N-[3-(2-Ethyl-butyryl)-4,5-dimethyl-1-(2,4,6-trimethylphenyl)-1H-pyrrol-2-yl]-acetamide

A mixture of the title compound from Example 35A (0.326 g, 1 mmol) and acetic anhydride (0.108 g, 1.05 mmol) in acetic acid (3 ml) was heated at reflux for 2 hours. The mixture was concentrated to dryness, diluted with water and extracted with ethyl acetate. The organic layer was washed with aqueous sodium carbonate and brine, dried and concentrated to give a dark oil. The oil was purified by silica gel column chromatography to give 107 mg of the title compound as a brown oil. ^1H NMR ($CDCl_3$) δ 0.88 (t,6H), 1.6-1.8 (m,4H), 1.76 (s,3H), 1.88 (s,3H), 1.93 (s,6H), 2.25 (s,3H), 2.28 (s,3H), 2.90-3.00 (m,1H), 6.89 (s,2H) ppm.

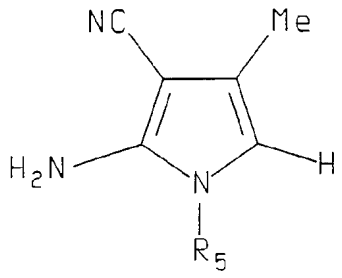
C. 4-(1-Ethylpropyl)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine

A mixture of the title compound of Example 35B (100 mg, 0.27 mmol) and ammonium chloride in 1.6 g of acetamide was heated at reflux for 2 hours. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried and concentrated to give the desired product which was purified by silica gel column chromatography to give the title compound as a yellow oil. ^1H NMR ($CDCl_3$) δ 0.85 (t,6H), 1.7-2.0 (m,4H), 1.83 (s,6H), 1.99 (s,3H), 2.35 (s,3H), 2.44 (s,3H), 2.61 (s,3H), 3.25-3.35 (m,1H), 7.00 (s,2H) ppm.

The following Preparations illustrate the synthesis of intermediates.

Preparation 1

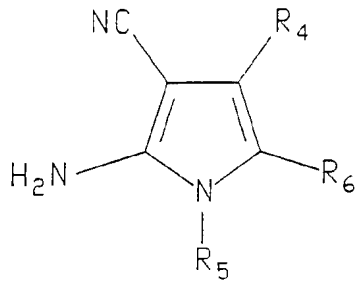
The following compounds were prepared starting from the appropriate aniline and employing the general procedure of Example 1A.



R_5	^{1}H-NMR($CDCl_3$) δ (ppm)
3,5-ditrifluoromethylphenyl	2.2(s,3H), 4.0(s,2H), 6.15(s,1H), 7.9(s,2H)
2,5-dimethylphenyl	2.04(s,3H), 2.12(s,3H), 2.35(s,3H), 3.85(s,2H), 5.90(s,1H), 7.0(s,1H), 7.10-7.25(m,2H)
2-methyl-4-iodophenyl	2.05(s,3H), 2.10(s,3H), 3.80(s,2H), 5.85(s,1H), 6.92(d,1H), 7.60(dd,1H), 7.70(d,1H)
3-methyl-4-chlorophenyl	2.10(s,3H), 2.40(s,3H), 4.03(s,2H), 6.03(s,1H), 7.10(dd,1H), 7.21(d,1H), 7.45(d,1H)
4-bromo-2,6-dimethylphenyl	2.01(s,6H), 2.10(s,3H), 3.70(brs,2H), 5.72(s,1H), 7.30(s,2H)
2-bromo-4,6-dimethylphenyl	2.06(s,3H), 2.13(s,3H), 2.35(s,3H), 3.83(brs,2H), 5.81(s,1H), 7.08(s,1H), 7.35(s,3H)
4-chloro-2,6-dimethylphenyl	2.01(s,6H), 2.10(s,3H), 3.75(brs,2H), 5.75(s,1H), 7.14(s,2H)

Preparation 2

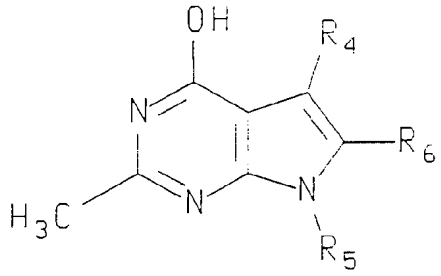
The following compounds were prepared starting from 3-hydroxy-2-butanone or 4-hydroxy-3-hexanone and the appropriate aniline and employing the general procedure of Example 2A.



R_4 and R_6	R_5	^{1}H-NMR($CDCl_3$) δ (ppm)
Me	2,4-dimethylphenyl	1.70(s,3H), 1.95(s,3H), 2.05(s,3H), 2.38(s,3H), 3.7(s,2H), 6.95-7.20(m,3H)
Me	2,6-dimethylphenyl	1.67(s,3H), 1.98(s,6H), 2.05(s,3H), 2.90(brs,2H), 7.05-7.21(m,3H)
Et	2,4,6-trimethylphenyl	No purification, the material was used directly for the next reaction step

Preparation 3

The following compounds were prepared starting from the corresponding compounds of preparations 1 and 2 and employing the general procedures of Examples 1B and 1C.

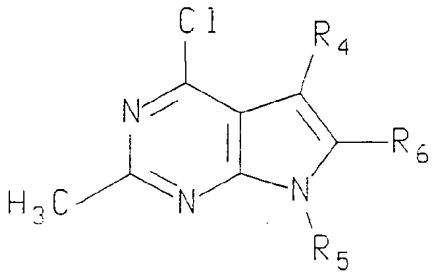


R_4=Me, R_6=H	^{1}H-NMR (solvent) δ (ppm)
R_5=3,5-ditrifluoromethylphenyl	(DMSO-d6) 2.32(s,3H), 7.50(s,1H), 8.05(s,1H), 8.55(s,1H), 12.10(s,1H)
R_5=2,5-dimethylphenyl	($CDCl_3$) 2.04(s,3H), 2.35(s,3H), 2.467(s,3H), 2.470(s,3H), 6.57(s,1H), 7.0-7.3(m,3H), 12.08(s,3H)

R_5=3-methyl-4-chlorophenyl	(DMSO-d_6) 2.29(s,3H), 2.31(s.3H), 2.38(s,3H), 7.12(s,1H), 7.55(m.2H), 7.67(d,1H), 11.90(s,1H)
R_5=4-bromo-2,6-dimethylphenyl	($CDCl_3$) 1.94(s,6H), 2.40(s,3H), 2.45(s,3H), 6.39(s,1H), 7.29(s,2H)
R_5=2-bromo-4,6-dimethylphenyl	(DMSO-d_6) 1.91(s,3H), 2.20(s.3H), 2.32(s,3H), 2.34(s,3H), 6.68(s,1H), 7.21(s,1H), 7.44(s,1H),11.80(s,1H)
R_5 = 4-chloro-2,6-dimethylphenyl	($CDCl_3$) 1.91(s,6H), 2.38(s,3H), 2.40(s,3H), 6.34(s,1H), 7.08(s.2H)
R_4 & R_6 = Me	^{1}H-NMR (solvent) δ (ppm)
R_5=2,4,6-trimethylphenyl	($CDCl_3$) 1.85(s,6H), 1.87(s,3H), 2.34(s,3H), 2.41(s,3H), 2.44(s.3H), 7.00(s,2H), 12.2(s,1H)
R_5=2,4-dimethylphenyl	($CDCl_3$) 1.90(s,3H), 1.93(s,3H), 2.38(s,3H), 2.42(s,6H), 7.0-7.2(m,3H), 12.25(s,1H)
R_5=2,6-dimethylphenyl	($CDCl_3$) 1.80-1.90(m,9H), 2.39(s,3H), 2.49(s,3H), 7.04-7.20(m,3H), 12.2(s,1H)

Preparation 4

The following compounds were prepared starting from the corresponding compounds of Preparation 3 and employing the general procedure in Example 1D.

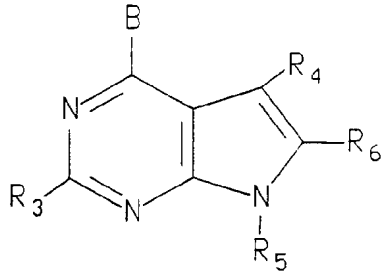


R_4=Me, R_6=H	^{1}H-NMR ($CDCl_3$) δ (ppm)
R_5=3,5-ditrifluoromethylphenyl	2.53(s,3H), 2.74(s,3H), 7.27(s.1H), 7.82(s,1H), 8.29(s,2H)

R_5=2,5-dimethylphenyl	2.01(s,3H), 2.35(s,3H), 2.50(s,3H), 2.66(s,3H), 6.91(s,1H), 7.05(s,1H), 7.10-7.30(m2H)
R_5=3-methyl-4-chlorophenyl	2.46(s,3H), 2.51(s,3H), 2.74(s,3H), 7.15(s,1H), 7.47(s,2H), 7.55(s,1H)
R_5=4-bromo-2,6-dimethylphenyl	1.89(s,6H), 2.49(s,3H), 2.62(s,3H), 6.75(s,1H), 7.32(s,2H)
R_5 = 2-bromo-4,6-dimethylphenyl	1.96(s,3H), 2.37(s,3H), 2.52(s,3H), 2.65(s,3H), 6.82(s,1H), 7.11(s,1H), 7.38(s,1H)
R_4 and R_6 = Me	^{1}H-NMR ($CDCl_3$) δ (ppm)
R_5=2,4,6-trimethylphenyl	1.81(s,6H), 1.99(s,3H), 2.35(s,3H), 2.46(s,3H), 2.59(s,3H), 7.01(s,2H)
R_5=2,4-dimethylphenyl	1.84(s,3H), 2.03(s,3H), 2.39(s,3H), 2.44(s,3H), 2.59(s,3H), 6.90-7.15(m,3H)
R_5=2,6-dimethylphenyl	1.83(s,6H), 1.98(s,3H), 2.45(s,3H), 2.58(s,3H), 7.10-7.30(m,3H)
R_5=4-chloro-2,6-dimethylphenyl	1.91(s,6H), 2.51(s,3H), 2.64(s,3H), 6.77(s,1H), 7.17(s,2H)
R_4 and R_6 = Et	^{1}H-NMR ($CDCl_3$) δ (ppm)
R_5=2,4,6-trimethylphenyl	0.96(t,3H), 1.31(t,3H), 1.85(s,6H), 2.38(s,6H), 2.46(q,2H), 2.62(s,3H), 2.92(q,2H), 7.02(s,2H)

CLAIMS

1. A compound of the formula



I

and the pharmaceutically acceptable acid addition salts thereof, wherein

B is NR_1R_2, $CR_1R_2R_{11}$, $C(=CR_2R_{12})R_1$, $NHCR_1R_2R_{11}$, $OCR_1R_2R_{11}$, $SCR_1R_2R_{11}$, $NHNR_1R_2$, $CR_2R_{11}NHR_1$, $CR_2R_{11}OR_1$, $CR_2R_{11}SR_1$, or $C(O)R_2$;

R_1 is hydrogen, or C_1-C_6 alkyl which may be substituted by one or two substituents R_7 independently selected from the group consisting of hydroxy, fluoro, chloro, bromo, iodo, C_1-C_8 alkoxy, O-C -(C_1-C_6 alkyl), O-C NH(C_1-C_4 alkyl), O-C -N(C_1-C_4 alkyl)(C_1-C_2 alkyl), amino, NH(C_1-C_4 alkyl), N(C_1-C_2 alkyl)(C_1-C_4 alkyl), S(C_1-C_6 alkyl), N(C_1-C_4alkyl)C (C_1-C_4 alkyl), NHC (C_1-C_4 alkyl), COOH, C O(C_1-C_4 alkyl), C NH(C_1-C_4 alkyl), C N(C_1-C_4 alkyl)(C_1-C_2 alkyl), SH, CN, NO_2, SO(C_1-C_4 alkyl), SO_2(C_1-C_4 alkyl), SO_2NH(C_1-C_4 alkyl), SO_2N(C_1-C_4 alkyl)(C_1-C_2 alkyl), and said C_1-C_5 alkyl may contain one or two double or triple bonds;

R_2 is C_1-C_{12} alkyl, aryl or (C_1-C_{10} alkylene)aryl wherein said aryl is phenyl, naphthyl, thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidyl, imidazolyl, furanyl, benzofuranyl, benzothiazolyl, isothiazolyl, benzisothiazolyl, thiazolyl, isoxazolyl, benzisoxazolyl, benzimidazolyl, triazolyl, pyrazolyl, pyrrolyl, indolyl, pyrrolopyridyl, oxazolyl, or benzoxazolyl; 3- to 8-membered cycloalkyl or (C_1-C_5 alkylene) cycloalkyl, wherein said cycloalkyl may contain one or two of O, S or N-Z wherein Z is hydrogen, C_1-C_4 alkyl, benzyl or C_1-C_4 alkanoyl, wherein R_2 may be substituted independently by from one to three of chloro, fluoro, or C_1-C_4 alkyl, or one of hydroxy, bromo, iodo, C-

C_6 alkoxy, O-C-(C_1-C_6 alkyl), O-C-N(C_1-C_4 alkyl)(C_1-C_2 alkyl), S(C_1-C_6 alkyl), NH_2, NH(C_1-C_2 alkyl), N(C_1-C_2 alkyl) (C_1-C_4 alkyl), N(C_1-C_4 alkyl)-C (C_1-C_4 alkyl), NHC (C_1-C_4 alkyl), COOH, C O(C_1-C_4 alkyl), C NH(C_1-C_4 alkyl), C N(C_1-C_4 alkyl)(C_1-C_2 alkyl), SH, CN, NO_2, SO(C_1-C_4 alkyl), SO_2(C_1-C_4 alkyl), SO_2NH(C_1-C_4 alkyl), SO_2N(C_1-C_4 alkyl)(C_1-C_2 alkyl), and wherein said C_1-C_{12} alkyl or C_1-C_{10} alkylene may contain one to three double or triple bonds; or

NR_1R_2 or $CR_1R_2R_{11}$ may form a saturated 3- to 8-membered carbocyclic ring of which the 5- to 8-membered ring may contain one or two double bonds or one or two of O, S or N-Z wherein Z is hydrogen, C_1-C_4 alkyl, benzyl or C_1-C_4 alkanoyl;

R_3 is hydrogen, C_1-C_6 alkyl, fluoro, chloro, bromo, iodo, hydroxy, amino, O(C_1-C_6 alkyl), NH(C_1-C_6 alkyl), N(C_1-C_4 alkyl)(C_1-C_2 alkyl), SH, S(C_1-C_4 alkyl), SO(C_1-C_4 alkyl), or SO_2(C_1-C_4 alkyl), wherein said C_1-C_4 alkyl and C_1-C_6 alkyl may contain one double or triple bond and may be substituted by from 1 to 3 substituents R_5 independently selected from the group consisting of hydroxy, C_1-C_3 alkoxy, fluoro, chloro or C_1-C_3 thioalkyl;

R_4 is hydrogen, C_1-C_6 alkyl, fluoro, chloro, bromo, iodo, C_1-C_6 alkoxy, amino, NH(C_1-C_6 alkyl), N(C_1-C_6 alkyl)(C_1-C_2 alkyl), SO_n(C_1-C_6 alkyl), wherein n is 0, 1 or 2, cyano, hydroxy, carboxy, or amido, wherein said C_1-C_6 alkyls may be substituted by one hydroxy, trifluoromethyl, amino, carboxy, amido, NHC (C_1-C_4 alkyl), NH(C_1-C_4 alkyl), N(C_1-C_4 alkyl)(C_1-C_2 alkyl), C O(C_1-C_4 alkyl), C_1-C_3 alkoxy, C_1-C_3 thioalkyl, fluoro, bromo, chloro, iodo, cyano or nitro;

R_5 is phenyl, naphthyl, thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidyl, imidazolyl, furanyl, benzofuranyl, benzothiazolyl, isothiazolyl, benzisothiazolyl, thiazolyl, isoxazolyl, benzisoxazolyl, benzimidazolyl, triazolyl, pyrazolyl, pyrrolyl, indolyl, pyrrolopyridyl, benzoxazolyl, oxazolyl, pyrrolidinyl, thiazolidinyl, morpholinyl, piperidinyl,

piperazinyl, tetrazolyl, or 3- to 8-membered cycloalkyl or 9- to 12-membered bicycloalkyl, optionally containing one or two of O, S or N-Z wherein Z is hydrogen, C_1-C_4 alkyl, C_1-C_4 alkanoyl, phenyl or phenylmethyl, wherein each one of the above groups may be substituted independently by from one to three of fluoro, chloro, bromo, formyl, C_1-C_6 alkyl, C_1-C_6 alkoxy or trifluoromethyl, or one of hydroxy, iodo, cyano, nitro, amino, $NH(C_1$-C_4 alkyl), $N(C_1$-$C_4)(C_1$-C_2 alkyl), $COO(C_1$-C_4 alkyl), $CO(C_1$-C_4 alkyl), $SO_2NH(C_1$-C_4 alkyl), $SO_2N(C_1$-C_4 alkyl)$(C_1$-C_2 alkyl), SO_2NH_2, $NHSO_2(C_1$-C_4 alkyl), $S(C_1$-C_6 alkyl), $SO_2(C_1$-C_6 alkyl), wherein said C_1-C_4 alkyl and C_1-C_6 alkyl may be substituted by one or two of fluoro, chloro, hydroxy, C_1-C_4 alkoxy, amino, methylamino, dimethylamino or acetyl wherein said C_1-C_4 alkyl and C_1-C_6 alkyl may contain one double or triple bond; with the proviso that R_5 is not unsubstituted phenyl;

R_6 is hydrogen, C_1-C_6 alkyl, fluoro, chloro, bromo, iodo, C_1-C_6 alkoxy, formyl, amino, $NH(C_1$-C_6 alkyl), $N(C_1$-C_6 alkyl$)(C_1$-C_2 alkyl), $SO_n(C_1$-C_6 alkyl), wherein n is 0, 1 or 2, cyano, carboxy, or amido, wherein said C_1-C_6 alkyls may be substituted by one hydroxy, trifluoromethyl, amino, carboxy, amido, $NHC(=O)(C_1$-C_4 alkyl), $NH(C_1$-C_4 alkyl), $N(C_1$-C_4 alkyl$)(C_1$-C_2 alkyl), $C(=O)O(C_1$-C_4 alkyl), C_1-C_3 alkoxy, C_1-C_3 thioalkyl, fluoro, bromo, chloro, iodo, cyano or nitro;

R_{11} is hydrogen, hydroxy, fluoro, chloro, $COO(C_1$-C_2 alkyl), cyano, or $CO(C_1$-C_2 alkyl); and

R_{12} is hydrogen or C_1-C_4 alkyl; with the proviso that (1) B is not straight chain C_1-C_{12} alkyl, (2) when R_5 is unsubstituted cycloakyl, R_3 and R_4 are hydrogen, and R_6 is hydrogen or methyl, then B is not NHR_2 wherein R_2 is benzyl or thienylmethyl, and (3) when R_5 is p-bromophenyl, and R_3, R_4 and R_6 are methyl, then B not methylamino or hydroxyethylamino.

2. A compound according to claim 1 wherein B is NR_1R_2, $NHCHR_1R_2$, or $OCHR_1R_2$, wherein R_1 is C_1-C_6 alkyl, which may be substituted by one of hydroxy, fluoro or C_1-C_2 alkoxy, and may contain one double or triple bond, and R_2 is benzyl or C_1-C_5 alkyl which may contain one double or triple bond, wherein said C_1-C_5 alkyl or the phenyl in said benzyl may be substituted by fluoro, C_1-C_5 alkyl, or C_1-C_5 alkoxy

3. A compound according to claim 1 wherein B is $CR_1R_2R_{11}$, wherein R_1 is C_1-C_6 alkyl which may be substituted by one C_1-C_6 alkoxy or hydroxy, R_2 is benzyl or C_1-C_6 alkyl wherein said C_1-C_6 alkyl or the phenyl in said benzyl may be substituted by one C_1-C_6 alkyl, C_1-C_6 alkoxy, fluoro, chloro or bromo, and R_{11} is hydrogen or fluoro.

4. A compound according to claim 1 wherein B is as defined in claim 1 and R_2 is C_1-C_6 alkyl which may be substituted by fluoro, C_1-C_6 alkyl or C_1-C_6 alkoxy and may contain one double or triple bond.

5. A compound according to claim 1 wherein B is as defined in claim 1 and R_2 is benzyl or methylthienyl, the phenyl or thienyl of which may be substituted by fluoro, chloro, C_1-C_4 alkyl or C_1-C_4 alkoxy.

6. A compound according to any one of claims 1 to 5 wherein R_3 is methyl, ethyl, fluoro, chloro, or methoxy.

7. A compound according to any one of claims 1 to 6 wherein R_4 and R_6 are hydrogen, methyl or ethyl.

8. A compound according to any one of claims 1 to 7 wherein R_5 is phenyl substituted by two or three substituents.

9. A compound according to claim 8 wherein said substituent is independently fluoro, chloro, bromo, iodo, C_1-C_4 alkoxy, trifluoromethyl, C_1-C_6 alkyl which may be substituted by one of hydroxy, C_1-C_4 alkoxy or fluoro and may have one double or triple bond, -(C_1-C_4 alkylene)O(C_1-C_2 alkyl), C_1-C_3 hydroxyalkyl, hydroxy, formyl, COO(C_1-C_2 alkyl), -(C_1-C_2 alkylene)amino, or -C(O)(C_1-C_4 alkyl).

10. A compound according to claim 1 wherein said compound is

n-butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amine;

di-n-propyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amine;

ethyl-n-propyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amine;

diethyl-2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amine;

n-butyl-ethyl-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amine;

2-{N-n-butyl-N-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amino}-ethanol;

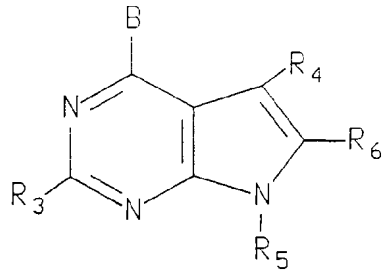
4-(1-ethyl-propyl)-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidine;

n-butyl-ethyl-[2,5-dimethyl-7-(2,4-dimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amine;

2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidyl-4-yl]-(1-ethyl-propyl)amine; or

2-[7-(4-bromo-2,6-dimethylphenyl)-2,5-dimethyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamino]-butan-1-ol.

11. A pharmaceutical composition for the treatment of (a) illnesses induced or facilitated by corticotropin releasing factor or (b) inflammatory disorders such as arthritis, asthma and allergies; anxiety; depression; fatigue syndrome; headache; pain; cancer; irritable bowel syndrome, including Crohn's disease, spastic colon and irritable colon; immune dysfunction; human immunodeficiency virus (HIV) infections; neurogenerative diseases such as Alzheimer's disease; gastrointestinal diseases; eating disorders such as anorexia nervosa; hemorrhagic stress; drug and alcohol withdrawal symptoms; drug addiction; stress-induced psychotic episodes; and fertility problems, which comprises a compound of the formula



I

and the pharmaceutically acceptable acid addition salts thereof, wherein

B is NR_1R_2, $CR_1R_2R_{11}$, $C(=CR_2R_{12})R_1$, $NHCR_1R_2R_{11}$, OCR_1R_2R... SCR_1R_2R..., $NHNR_1R_2$, $CR_2R_{11}NHR_1$, $CR_2R_{11}OR_1$, $CR_2R_{11}SR_1$, or $C(O)R_2$;

R_1 is hydrogen, or C_1-C_6 alkyl which may be substituted by one or two substituents R_7 independently selected from the group consisting of hydroxy, fluoro, chloro, bromo, iodo, C_1-C_8 alkoxy, O-C -(C_1-C_6 alkyl), O-C NH(C_1-C_4 alkyl), O-C -N(C_1-C_4

O O O

alkyl)(C_1-C_2 alkyl), amino, NH(C_1-C_4 alkyl), N(C_1-C_2 alkyl)(C_1-C_4 alkyl), S(C_1-C_6 alkyl), N(C_1-C_4alkyl)C (C_1-C_4 alkyl), NHC (C_1-C_4 alkyl), COOH, C O(C_1-C_4 alkyl), C NH(C_1-C_4 alkyl), C N(C_1-C_4 alkyl)(C_1-C_2 alkyl), SH, CN, NO_2, SO(C_1-C_4 alkyl), SO_2(C_1-C_4 alkyl), SO_2NH(C_1-C_4 alkyl), SO_2N(C_1-C_4 alkyl)(C_1-C_2 alkyl), and said C_1-C_6 alkyl may contain one or two double or triple bonds;

R_2 is C_1-C_{12} alkyl, aryl or (C_1-C_{10} alkylene)aryl wherein said aryl is phenyl, naphthyl, thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidyl, imidazolyl, furanyl, benzofuranyl, benzothiazolyl, isothiazolyl, benzisothiazolyl, thiazolyl, isoxazolyl, benzisoxazolyl, benzimidazolyl, triazolyl, pyrazolyl, pyrrolyl, indolyl, pyrrolopyridyl, oxazolyl, or benzoxazolyl; 3- to 8-membered cycloalkyl or (C_1-C_6 alkylene) cycloalkyl, wherein said cycloalkyl may contain one or two of O, S or N-Z wherein Z is hydrogen, C_1-C_4 alkyl, benzyl or C_1-C_4 alkanoyl, wherein R_2 may be substituted independently by from one to three of chloro, fluoro, or C_1-C_4 alkyl, or one of hydroxy, bromo, iodo, C_1-C_6 alkoxy, O-C -(C_1-C_6 alkyl), O-C -N(C_1-C_4 alkyl)(C_1-C_2 alkyl), S(C_1-C_6 alkyl), NH_2, NH(C_1-C_2 alkyl), N(C_1-C_2 alkyl) (C_1-C_4 alkyl), N(C_1-C_4 alkyl)-C (C_1-C_4 alkyl), NHC (C_1-C_4 alkyl), COOH, C O(C_1-C_4 alkyl), C NH(C_1-C_4 alkyl), C N(C_1-C_4 alkyl)(C_1-C_2 alkyl), SH, CN, NO_2, SO(C_1-C_4 alkyl), SO_2(C_1-C_4 alkyl), SO_2NH(C_1-C_4 alkyl), SO_2N(C_1-C_4 alkyl)(C_1-C_2 alkyl), and wherein said C_1-C_{12} alkyl or C_1-C_{10} alkylene may contain one to three double or triple bonds; or

NR_1R_2 or $CR_1R_2R_{11}$ may form a saturated 3- to 8-membered carbocyclic ring, the 5- to 8-membered rings of which may contain one or two double bonds or one or two of O, S or N-Z wherein Z is hydrogen, C_1-C_4 alkyl, benzyl or C_1-C_4 alkanoyl;

R_3 is hydrogen, C_1-C_6 alkyl, fluoro, chloro, bromo, iodo, hydroxy, amino, O(C_1-C_6 alkyl), NH(C_1-C_6 alkyl), N(C_1-C_4 alkyl)(C_1-C_2 alkyl), SH, S(C_1-C_4 alkyl), SO(C_1-C_4 alkyl), or SO_2(C_1-C_4 alkyl), wherein said C_1-C_4 alkyl and C_1-C_6 alkyl may contain one

double or triple bond and may be substituted by from 1 to 3 substituents R_6 independently selected from the group consisting of hydroxy, C_1-C_3 alkoxy, fluoro, chloro or C_1-C_3 thioalkyl;

R_4 is hydrogen, C_1-C_6 alkyl, fluoro, chloro, bromo, iodo, C_1-C_6 alkoxy, formyl, $NH(C_1$-C_6 alkyl), $N(C_1$-C_6 alkyl)$(C_1$-C_2 alkyl), $SO_n(C_1$-C_6 alkyl), wherein n is 0, 1 or 2, cyano, hydroxy, carboxy, or amido, wherein said C_1-C_6 alkyls may be substituted by one hydroxy, trifluoromethyl, amino, carboxy, amido, NHC(C_1-C_4 alkyl), $NH(C_1$-C_4 alkyl), $N(C_1$-C_4 alkyl)$(C_1$-C_2 alkyl), C O(C_1-C_4 alkyl), C_1-C_3 alkoxy, C_1-C_3 thioalkyl, fluoro, bromo, chloro, iodo, cyano or nitro;

R_5 is phenyl, naphthyl, thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidyl, imidazolyl, furanyl, benzofuranyl, benzothiazolyl, isothiazolyl, benzisothiazolyl, thiazolyl, isoxazolyl, benzisoxazolyl, benzimidazolyl, triazolyl, pyrazolyl, pyrrolyl, indolyl, pyrrolopyridyl, benzoxazolyl, oxazolyl, pyrrolidinyl, thiazolidinyl, morpholinyl, piperidinyl, piperazinyl, tetrazolyl, or 3- to 8-membered cycloalkyl or 9- to 12-membered bicycloalkyl, optionally containing one or two of O, S or N-Z wherein Z is hydrogen, C_1-C_4 alkyl, C_1-C_4 alkanoyl, phenyl or phenylmethyl, wherein each one of the above groups may be substituted independently by from one to three of fluoro, chloro, bromo, formyl, C_1-C_6 alkyl, C_1-C_6 alkoxy or trifluoromethyl, or one of hydroxy, iodo, cyano, nitro, amino, $NH(C_1$-C_4 alkyl), $N(C_1$-$C_4)(C_1$-C_2 alkyl), $COO(C_1$-C_4 alkyl), $CO(C_1$-C_4 alkyl), $SO_2NH(C_1$-C_4 alkyl), $SO_2N(C_1$-C_4 alkyl)$(C_1$-C_2 alkyl), SO_2NH_2, $NHSO_2(C_1$-C_4 alkyl), $S(C_1$-C_6 alkyl), $SO_2(C_1$-C_6 alkyl), wherein said C_1-C_4 alkyl and C_1-C_6 alkyl may be substituted by one or two of fluoro, chloro, hydroxy, C_1-C_4 alkoxy, amino, methylamino, dimethylamino or acetyl wherein said C_1-C_4 alkyl and said C_1-C_6 alkyl may contain one double or triple bond; with the proviso that R_5 is not unsubtituted phenyl;

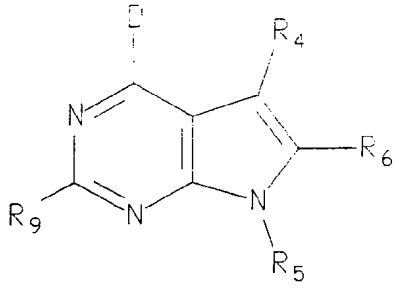
R_6 is hydrogen, C_1-C_6 alkyl, fluoro, chloro, bromo, iodo, C_1-C_6 alkoxy, formyl, amino, $NH(C_1$-C_6 alkyl), $N(C_1$-C_6 alkyl)$(C_1$-C_2 alkyl), $SO_n(C_1$-C_6 alkyl), wherein n is 0, 1 or 2, cyano, carboxy, or amido, wherein said C_1-C_6 alkyls may be substituted by one hydroxy, trifluoromethyl, amino, carboxy, amido, NHC(C_1-C_4 alkyl), $NH(C_1$-C_4 alkyl).

$N(C_1\text{-}C_4$ alkyl)$(C_1\text{-}C_2$ alkyl), C O$(C_1\text{-}C_4$ alkyl), $C_1\text{-}C_3$ alkoxy, $C_1\text{-}C_3$ thioalkyl, fluoro, bromo, chloro, iodo, cyano or nitro;

R_{11} is hydrogen, hydroxy, fluoro, chloro, $COO(C_1\text{-}C_2$ alkyl), cyano, or $CO(C_1\text{-}C_2$ alkyl); and

R_{12} is hydrogen or $C_1\text{-}C_4$ alkyl, in an amount effective in the treatment of said illnesses, and a pharmaceutically acceptable carrier.

12. A compound of the formula



II

wherein

D is hydroxy, chloro, or cyano;

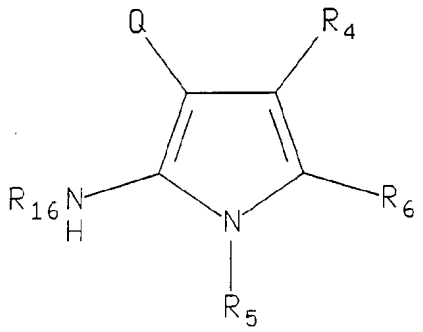
R_4 and R_6 are each independently hydrogen, $C_1\text{-}C_6$ alkyl, fluoro, chloro, bromo, iodo, $C_1\text{-}C_6$ alkoxy, $SO_n(C_1\text{-}C_6$ alkyl), wherein n is 0, 1 or 2, or cyano, wherein said $C_1\text{-}C_6$ alkyls may be substituted by one to three of hydroxy, amino, carboxy, amido, $NHC(O)(C_1\text{-}C_4$ alkyl), $NH(C_1\text{-}C_4$ alkyl), $N(C_1\text{-}C_4$ alkyl)$(C_1\text{-}C_2$ alkyl), $C(O)O(C_1\text{-}C_4$ alkyl), $C_1\text{-}C_3$ alkoxy, $C_1\text{-}C_3$ thioalkyl, fluoro, bromo, chloro, iodo, cyano or nitro;

R_5 is phenyl, naphthyl, thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidyl, imidazolyl, furanyl, benzofuranyl, benzothiazolyl, isothiazolyl, benzoisothiazolyl, thiazolyl, isoxazolyl, benzisoxazolyl, benzimidazolyl, triazolyl, pyrazolyl, pyrrolyl, indolyl, azaindolyl, benzoxazolyl, oxazolyl, pyrrolidinyl, thiazolidinyl, morpholinyl, piperdinyl, piperazinyl, tetrazolyl, or 3- to 8-membered cycloalkyl or 9- to 12-membered bicycloalkyl, optionally containing one to three of O, S or N-Z wherein Z is hydrogen, $C_1\text{-}C_4$ alkyl, $C_1\text{-}C_4$ alkanoyl, phenyl or benzyl, wherein each of the above groups may be substituted independently by from one to three of fluoro, chloro, $C_1\text{-}C_6$ alkyl, $C_1\text{-}C_6$ alkoxy or trifluoromethyl, or one of hydroxy, bromo, iodo, cyano, nitro, amino, $NH(C_1\text{-}C_4$ alkyl), $N(C_1\text{-}C_4)(C_1\text{-}C_2$ alkyl), $COO(C_1\text{-}C_4$ alkyl), $CO(C_1\text{-}C_4$ alkyl),

$SO_2NH(C_1\text{-}C_4$ alkyl), $SO_2N(C_1\text{-}C_4$ alkyl)$(C_1\text{-}C_2$ alkyl), SO_2NH_2, $NHSO_2(C_1\text{-}C_4)$, $S(C_1\text{-}C_6$ alkyl), $SO_2(C_1\text{-}C_6$ alkyl), wherein said $C_1\text{-}C_4$ alkyl and $C_1\text{-}C_6$ alkyl may be substituted by one or two of fluoro, chloro, hydroxy, amino, methylamino, dimethylamino or acetyl; with the proviso that R_5 is not unsubstituted phenyl; and

R_9 is hydrogen, $C_1\text{-}C_6$ alkyl or chloro; with the proviso that when (a) R_4 and R_6 are methyl, R_9 is hydrogen and D is hydroxy, then R_5 is not phenyl (1) substituted by one of halogen, nitro, $C_1\text{-}C_6$ alkyl, $C_1\text{-}C_6$ alkoxy, or trifluoromethyl, and optionally in addition substituted by one or two of halogen, $C_1\text{-}C_6$ alkyl or $C_1\text{-}C_6$ alkoxy, or (2) di-or trisubstituted by one of nitro or trifluoromethyl and one or two of halogen, $C_1\text{-}C_6$ alkyl or $C_1\text{-}C_6$ alkoxy, and (b) when D is chloro, R_4 and R_9 are hydrogen, and R_6 is $C_1\text{-}C_6$ alkyl, then R_5 is not unsubstituted cyclohexyl.

13. A compound of the formula



VII

wherein

Q is $C(O)CHR_1R_2$ or cyano;

R_1 is hydrogen, or $C_1\text{-}C_6$ alkyl which may be substituted by one or two substituents R_7 independently selected from the group consisting of hydroxy, fluoro, chloro, bromo, iodo, $C_1\text{-}C_8$ alkoxy, or nitro, and said $C_1\text{-}C_6$ alkyl may contain one or two double or triple bonds;

R_2 is $C_1\text{-}C_{12}$ alkyl, aryl or ($C_1\text{-}C_{10}$ alkylene)aryl wherein said aryl is phenyl, naphthyl, thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidyl, imidazolyl, furanyl, benzofuranyl, benzothiazolyl, isothiazolyl, benzisothiazolyl, thiazolyl, isoxazolyl, benzisoxazolyl, benzimidazolyl, triazolyl, pyrazolyl, pyrrolyl, indolyl, pyrrolopyridyl, oxazolyl, or benzoxazolyl; 3- to 8-membered cycloalkyl or ($C_1\text{-}C_6$ alkylene) cycloalkyl, wherein said cycloalkyl may contain one or two of O, S or N-Z wherein Z is hydrogen, $C_1\text{-}C_4$ alkyl, benzyl or $C_1\text{-}C_4$ alkanoyl, wherein R_2 may be substituted independently by

from one to three of chloro, fluoro, or C_1-C_4 alkyl, or one of hydroxy, bromo, iodo, C_1-C_6 alkoxy, nitro, SO(C_1-C_4 alkyl), SO_2(C_1-C_4 alkyl), SO_2NH(C_1-C_4 alkyl), SO_2N(C_1-C_4 alkyl)(C_1-C_2 alkyl), and wherein said C_1-C_{12} alkyl or C_1-C_{10} alkylene may contain one to three double or triple bonds;

R_4 and R_6 are each independently hydrogen, C_1-C_6 alkyl, fluoro, chloro, bromo, iodo, C_1-C_6 alkoxy, amino, or SO_n(C_1-C_6 alkyl), wherein n is 0, 1 or 2, or cyano, wherein said C_1-C_6 alkyl may be substituted by one C_1-C_3 alkoxy, C_1-C_3 thioalkyl, fluoro, bromo, chloro, iodo, cyano or nitro;

R_5 is phenyl, naphthyl, thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidyl, imidazolyl, furanyl, benzofuranyl, benzothiazolyl, isothiazolyl, benzisothiazolyl, thiazolyl, isoxazolyl, benzisoxazolyl, benzimidazolyl, triazolyl, pyrazolyl, pyrrolyl, indolyl, pyrrolopyridyl, benzoxazolyl, oxazolyl, pyrrolidinyl, thiazolidinyl, morpholinyl, piperidinyl, piperazinyl, tetrazolyl, or 3- to 8-membered cycloalkyl or 9- to 12-membered bicycloalkyl, optionally containing one or two O, S or N-Z wherein Z is hydrogen, C_1-C_4 alkyl, C_1-C_4 alkanoyl, phenyl or benzyl, wherein each one of the above groups may be substituted independently by from one to three of fluoro, chloro, C_1-C_6 alkyl, C_1-C_6 alkoxy or trifluoromethyl, or one of hydroxy, bromo, iodo, cyano, nitro, amino, NH(C_1-C_4 alkyl), N(C_1-C_4)(C_1-C_2 alkyl), COO(C_1-C_4 alkyl), CO(C_1-C_4 alkyl), SO_2NH(C_1-C_4 alkyl), SO_2N(C_1-C_4 alkyl)(C_1-C_2 alkyl), SO_2NH_2, $NHSO_2$(C_1-C_4 alkyl), S(C_1-C_6 alkyl), SO_2(C_1-C_6 alkyl), wherein said C_1-C_4 alkyl and C_1-C_6 alkyl may be substituted by one or two of fluoro, chloro, hydroxy, amino, methylamino, dimethylamino or acetyl; with the proviso that R_5 is not unsubstituted phenyl; and

R_{16} is hydrogen or C(O)C_1-C_6 alkyl; with the proviso that when Q is cyano, R_4 and R_6 are not both methyl.

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/US 93/10715

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07D487/04 A61K31/505 C07D207/34 //(C07D487/04,239:00, 209:00)

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 5 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	DE,A,31 45 287 (TROPONWERKE) 19 May 1983 cited in the application see page 4, line 1 - line 5; claim 1 ---	1,11
X	EP,A,0 475 411 (MARION MERRELL DOW) 18 March 1992 see claim 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
9 March 1994	23. 03. 94

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	Alfaro Faus, I

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

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/US 93/10715

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
DE-A-3145287	19-05-83	NONE		
EP-A-0475411	18-03-92	AU-A-	8372991	19-03-92
		CA-A-	2051012	15-03-92
		CN-A-	1059909	01-04-92
		JP-A-	4270282	25-09-92
		NZ-A-	239750	25-02-94
		US-A-	5244896	14-09-93

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07D 487/04, 471/04, A61K 31/505, 31/435 // (C07D 487/04, 239:00, 231:00) (C07D 471/04, 249:00, 221:00) (C07D 487/04, 239:00, 209:00)

A1

(11) International Publication Number: **WO 98/08847**

(43) International Publication Date: 5 March 1998 (05.03.98)

(21) International Application Number: PCT/IB97/00922

(22) International Filing Date: 25 July 1997 (25.07.97)

(30) Priority Data:
60/025,039 28 August 1996 (28.08.96) US

(71) Applicant *(for all designated States except US)*: PFIZER INC. [US/US]; 235 East 42nd Street, New York, NY 10017 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: CHEN, Yuhpyng, Liang [US/US]; 8 Waterview Drive, Waterford, CT 06385 (US).

(74) Agents: SPIEGEL, Allen, J. et al.; Pfizer Inc., 235 East 42nd Street, New York, NY 10017 (US).

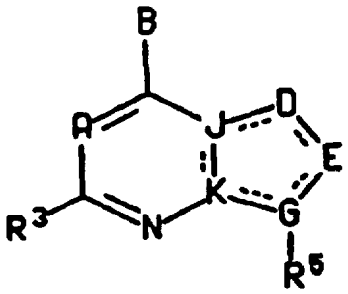
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, 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.

(54) Title: SUBSTITUTED 6,5-HETERO-BICYCLIC DERIVATIVES

(57) Abstract

This invention relates to compounds of formula (I), wherein the dashed lines represent optional double bonds; A is nitrogen or CR^7; B is $-NR^1R^2$, $-CR^1R^2R^{10}-C(=CR^2R^{11})R^1$, $-NHCR^1R^2R^{10}$, $-OCR^1R^2R^{10}$, $-SCR^1R^2R^{10}$, $-CR^2R^{10}NHR^1$, $-CR^2R^{10}OR^1$, $-CR^2R^{10}SR^1$ or $-COR^2$; J and K are each independently nitrogen or carbon and both J and K are not nitrogens; D and E are each selected, independently, from nitrogen, CR^4, C=O, C=S, sulfur, oxygen, CR^4R^6 and NR^8; G is nitrogen or carbon; and their use for the prevention or inhibition of a disorder that can be treated by antagonizing CRF.



(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.

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		

SUBSTITUTED 6,5- HETERO-BICYCLIC DERIVATIVES

Background of the invention

This invention relates to certain pharmaceutically active substituted 6,5-hetero-bicyclic derivatives, pharmaceutical compositions containing them and methods of administering them to subjects in need of their corticotropin releasing factor antagonist activity.

The substituted heterocyclic derivatives claimed in this case exhibit activity as corticotropin releasing factor (hormone) CRF (CRH) antagonists.

CRF antagonists are mentioned in U.S. Patents 4,605,642 and 5,063,245 referring to peptides and pyrazolinones, respectively. They are also referred to in the following: PCT Patent Application PCT/IB95/00439, which designates the United States and was filed on June 6, 1995 and published on December 14, 1995; PCT Patent Application PCT/IB95/00373, which designates the United States and was filed on May 18, 1995 and published on December 21, 1995; U.S. Patent Application 08/448,539, which was filed in the PCT on Nov. 12, 1993 and entered the U.S. national phase on June 14, 1995; PCT Patent Application WO 95/10506, which was filed on October 12, 1993 and published on April 20, 1995, and U.S. Patent Application 08/481,413, which was filed in the PCT on November 26, 1993 and entered the U.S. national phase on July 24, 1995; U.S. Patent Application 08/254,820, which was filed on April 19, 1995; Provisional U.S. Patent Application 60/008,396, which was filed on December 8, 1995; and Provisional U.S. Patent Application 60/006,333, which was filed on November 8, 1995. All the foregoing patent applications are incorporated herein by reference in their entireties.

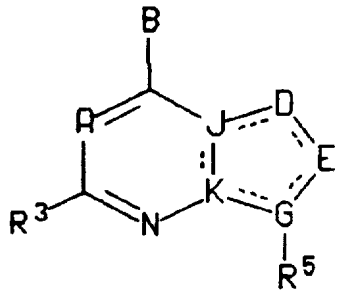
The importance of CRF antagonists is set out in the literature, e.g., P. Black, Scientific American SCIENCE & MEDICINE, 1995, p. 16-25; T. Lovenberg et al., Current Pharmaceutical Design, 1995, 1, 305-316; and United States Patent 5,063,245, which is referred to above. A recent outline of the different activities possessed by CRF antagonists is found in M. J. Owens et al., Pharm. Rev., Vol. 43, pages 425 to 473 (1991), also incorporated herein by reference. Based on the research described in these two and other references, CRF antagonists are effective in the treatment of a wide range of stress-related illnesses, mood disorders such as depression, major depressive disorder, single episode depression, recurrent depression, child abuse induced depression, postpartum depression, dysthemia, bipolar disorders and cyclothymia;

chronic fatigue syndrome; eating disorders such as anorexia and bulimia nervosa; generalized anxiety disorder; panic disorder; phobias; obsessive-compulsive disorder, post-traumatic stress disorder, pain perception such as fibromyalgia; headache; gastrointestinal diseases; hemorrhagic stress; ulcers; stress-induced psychotic episodes; fever; diarrhea; post-operative ileus, colonic hypersensitivity; irritable bowel syndrome; Crohn's disease; spastic colon; inflammatory disorders such as rheumatoid arthritis and osteoarthritis; pain; asthma; psoriasis; allergies; osteoporosis; premature birth; hypertension, congestive heart failure; sleep disorders; neurodegenerative diseases such as Alzheimer's disease, senile dementia of the Alzheimer's type, multiinfarct dementia, Parkinson's disease, and Huntington's disease; head trauma; ischemic neuronal damage; excitotoxic neuronal damage; epilepsy; stroke; spinal cord trauma; psychosocial dwarfism; euthyroid sick syndrome; syndrome of inappropriate antidiarrhetic hormone; obesity; chemical dependencies and addictions; drug and alcohol withdrawal symptoms; infertility, cancer; infertility; muscular spasms; urinary incontinence; hypoglycemia and immune dysfunctions including stress induced immune dysfunctions, immune suppression and human immunodeficiency virus infections; and stress-induced infections in humans and animals.

The compounds of this invention are also believed to be inhibitors of CRH binding protein and therefore useful in the treatment of disorders the treatment of which can be effected or facilitated by inhibiting such protein. Examples of such disorders are Alzheimer's disease and obesity.

Summary of the Invention

The present invention relates to compounds of the formula



I

or a pharmaceutically acceptable salt thereof, wherein

the dashed lines represent optional double bonds;

A is nitrogen or CR^7;

B is $-NR^1R^2$, $-CR^1R^2R^{10}$, $-C(=CR^2R^{11})R^1$, $-NHCR^1R^2R^{10}$, $-OCR^1R^2R^{10}$, $-SCR^1R^2R^{10}$, $-CR^2R^{10}NHR^1$, $-CR^2R^{10}OR^1$, $-CR^2R^{10}SR^1$ or $-COR^2$;

J and K are each independently nitrogen or carbon and both J and K are not nitrogens ;

D and E are each selected, independently, from nitrogen, CR^4, C=O, C=S, sulfur, oxygen, CR^4R^6 and NR^8 ;

G is nitrogen or carbon;

the ring containing D, E, G, K, and J in formula I may be a saturated or unsaturated 5-membered ring and may optionally contain one or two double bonds and may optionally contain from one to three heteroatoms in the ring and may optionally have one or two C=O or C=S groups;

R^1 is C_1-C_6 alkyl optionally substituted with one or two substituents independently selected from hydroxy, fluoro, chloro, bromo, iodo, -O-(C_1-C_4 alkyl), CF_3, -C(=O)O-(C_1-C_4alkyl), -OC(=O)(C_1-C_4 alkyl), -OC(=O)N(C_1-C_4 alkyl)(C_1-C_2 alkyl), -NHCO(C_1-C_4 alkyl), -COOH, -COO(C_1-C_4 alkyl), -CONH(C_1-C_4 alkyl), -CON(C_1-C_4 alkyl)(C_1-C_2 alkyl), -S(C_1-C_4 alkyl), -CN, $-NO_2$, -SO(C_1-C_4 alkyl), $-SO_2$(C_1-C_4 alkyl), $-SO_2$NH(C_1-C_4 alkyl) and $-SO_2$N(C_1-C_4 alkyl)(C_1-C_2 alkyl), wherein each of the C_1-C_4 alkyl groups in the foregoing R^1 groups may optionally contain one or two double or triple bonds;

R^2 is C_1-C_{12} alkyl which may optionally contain from one to three double or triple bonds, aryl or (C_1-C_4 alkylene)aryl, wherein said aryl and the aryl moiety of said (C_1-C_4 alkylene)aryl is selected from phenyl, naphthyl, thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidinyl, imidazolyl, furanyl, benzofuranyl, benzothiazolyl, isothiazolyl, pyrazolyl, pyrrolyl, indolyl, pyrrolopyridyl, oxazolyl and benzoxazolyl; C_3-C_8 cycloalkyl or (C_1-C_6 alkylene)(C_3-C_8 cycloalkyl), wherein one or two of the carbon atoms of said cycloalkyl and the 5 to 8 membered cycloalkyl moieties of said (C_1-C_6 alkylene)(C_3-C_8 cycloalkyl) may optionally and independently be replaced by an oxygen or sulfur atom or by NZ^2 wherein Z^2 is selected from hydrogen, C_1-C_4 alkyl, benzyl and C_1-C_4 alkanoyl, and wherein each of the foregoing R^2 groups may optionally be substituted with from one to three substituents independently selected from chloro, fluoro, hydroxy and C_1-C_4 alkyl, or with one substituent selected from bromo, iodo, C_1-C_6 alkoxy, -OC(=O)(C_1-C_6 alkyl), -OC(=O)N(C_1-C_4 alkyl)(C_1-C_2 alkyl), -S(C_1-C_6 alkyl), amino, -NH(C_1-C_2 alkyl), -N(C_1-C_2 alkyl)(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)-CO-(C_1-C_4 alkyl), -NHCO(C_1-C_4 alkyl),

-COOH, -COO(C_1-C_4 alkyl), -CONH(C_1-C_4 alkyl), -CON(C_1-C_4 alkyl)(C_1-C_2 alkyl), -SH, -CN, -NO_2, -SO(C_1-C_4 alkyl), -SO_2(C_1-C_4 alkyl), -SO_2NH(C_1-C_4 alkyl) and -SO_2N(C_1-C_4 alkyl)(C_1-C_2 alkyl);

-NR^1R^2 or -$CR^1R^2R^{10}$ may form a saturated 3 to 8 membered carbocyclic ring which may optionally contain from one to three double bonds and wherein one or two of the ring carbon atoms of such 5 to 8 membered rings may optionally and independently be replaced by an oxygen or sulfur atom or by NZ^3 wherein Z^3 is hydrogen, C_1-C_4 alkyl, benzyl or C_1-C_4 alkanoyl;

R^3 is hydrogen, C_1-C_4 alkyl, -O(C_1-C_4 alkyl), chloro, fluoro, bromo, iodo, (C_1-C_2 alkylene)-O-(C_1-C_2 alkyl), (C_1-C_2 alkylene)-OH, or -S(C_1-C_4 alkyl);

each R^4 is, independently, hydrogen, (C_1-C_6 alkyl), fluoro, chloro, bromo, iodo, hydroxy, cyano, amino, (C_1-C_2 alkylene)-OH, CF_3, CH_2SCH_3, nitro, -O(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)(C_1-C_2 alkyl), -S(C_1-C_4 alkyl), -CO(C_1-C_4 alkyl), -C(=O)H or -C(=O)O(C_1-C_4alkyl);

R^6 is hydrogen, methyl or ethyl;

R^8 is hydrogen or C_1-C_4 alkyl;

R^5 is phenyl, pyridyl, pyrazinyl, pyrimidyl, pyridazinyl and wherein each of the foregoing R^5 groups is substituted with from one to four substituents R^{13} wherein one to three of said substituents may be selected, independently, from fluoro, chloro, C_1-C_6 alkyl and -O(C_1-C_6 alkyl) and one of said substituents may be selected from bromo, iodo, formyl, OH, (C_1-C_4 alkylene)-OH, (C_1-C_4alkylene)-O-(C_1-C_2 alkyl), -CN, -CF_3, -NO_2, -NH_2, -NH(C_1-C_4 alkyl), -N(C_1-C_2 alkyl)(C_1-C_6 alkyl), -OCO(C_1-C_4 alkyl), (C_1-C_4 alkylene)-O-(C_1-C_4 alkyl), -S(C_1-C_6 alkyl), (C_1-C_4 alkylene)-S-(C_1-C_4 alkyl), -C(=O)O(C_1-C_4 alkyl), -C(=O)(C_1-C_4 alkyl), -COOH, -SO_2NH(C_1-C_4 alkyl), -SO_2N(C_1-C_2 alkyl)(C_1-C_4 alkyl), -SO_2NH_2, -$NHSO_2$(C_1-C_4 alkyl), -S(C_1-C_6 alkyl) and -SO_2(C_1-C_6 alkyl), and wherein each of the C_1-C_4 alkyl and C_1-C_6 alkyl moieties in the foregoing R^5 groups may optionally have one or two double bonds;

R^7 is hydrogen, C_1-C_4 alkyl, halo (e.g., chloro, fluoro, iodo or bromo), hydroxy, -O(C_1-C_4 alkyl), -C(=O)(C_1-C_4 alkyl), -C(=O)O(C_1-C_4 alkyl), -OCF_3, -CF_3, -CH_2OH or -CH_2O(C_1-C_2 alkyl);

R^{10} is hydrogen, hydroxy, methoxy or fluoro;

R^{11} is hydrogen or C_1-C_4 alkyl; and

with the proviso that: a) when both J and K are carbon and D is CR^4 and E is nitrogen, then G can not be nitrogen; (b) when both J and K are carbons and D and G are nitrogens, then E can not be CR^4 or C=O or C=S; (c) when both J and K are carbons and D and E are carbons, then G can not be nitrogen; (d) when G is carbon, it must be double bonded to E; and (e) in the ring containing J, K, D, E and G, there can not be two double bonds adjacent to each other.

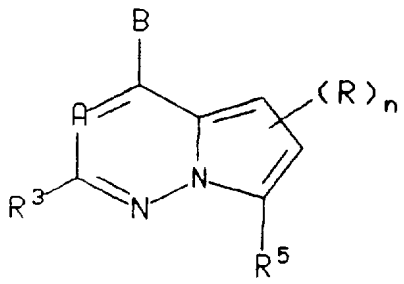
and the pharmaceutically acceptable salts of such compounds.

When the ring containing D, E, G, K and J is a 5-membered heteroaromatic ring, it may be, e.g., pyrazolo, imidazolo, thieno, furano, thiazolo, oxazolo, triazolo or thiadiazolo.

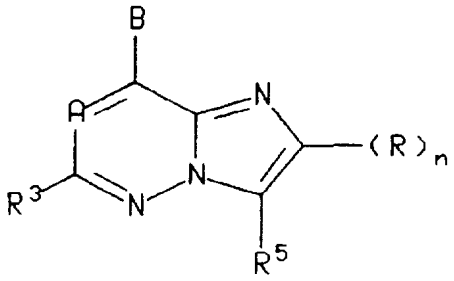
The term "alkyl", as used herein, unless otherwise indicated, includes saturated monovalent hydrocarbon radicals having straight branched or cyclic moieties or combinations thereof.

The term "alkoxy", as used herein, unless otherwise indicated, means -O-alkyl, where "alkyl" is defined as above.

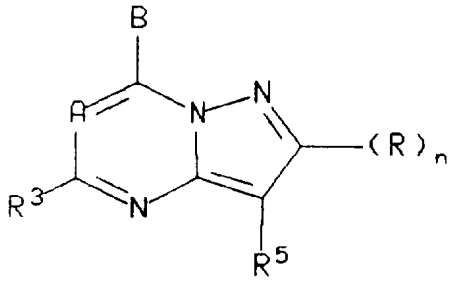
Examples of more specific embodiments of formula I are the following, wherein $(R)_n$ represents from zero to two substituents, wherein such substitutents are as defined above in the definition of formula I.



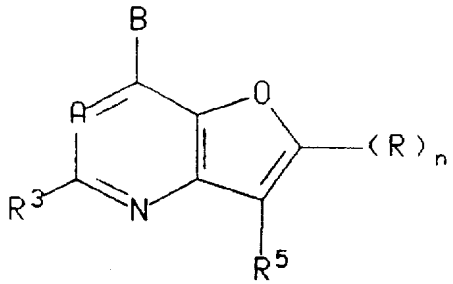
I-A



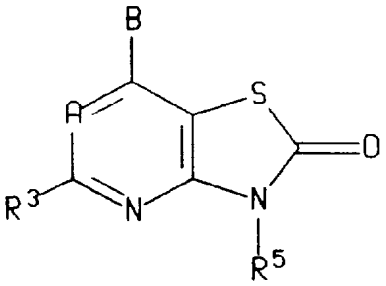
I-B



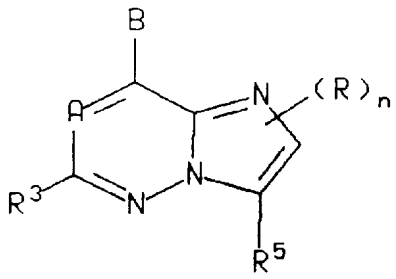
I-E



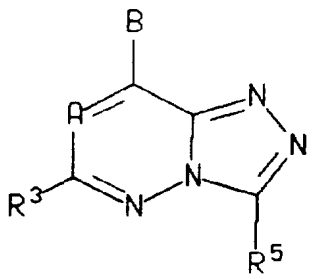
I-J



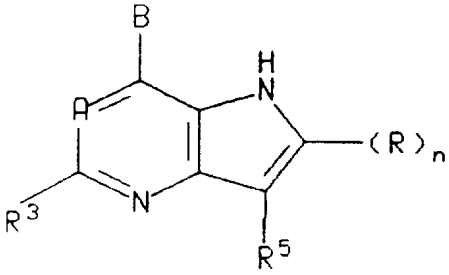
I-K



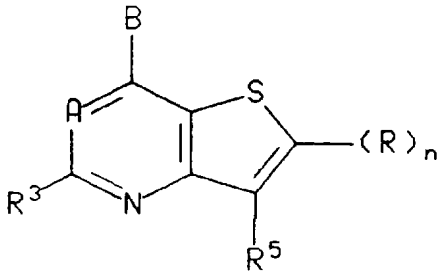
I-C



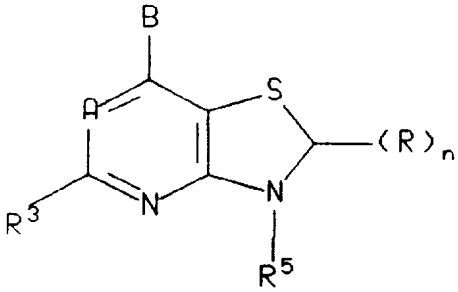
I-D



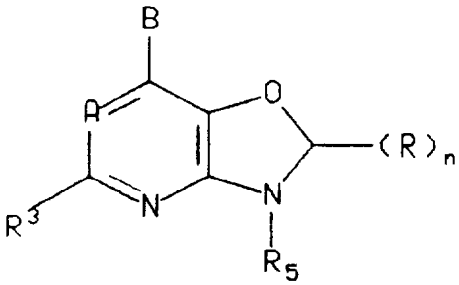
I-G



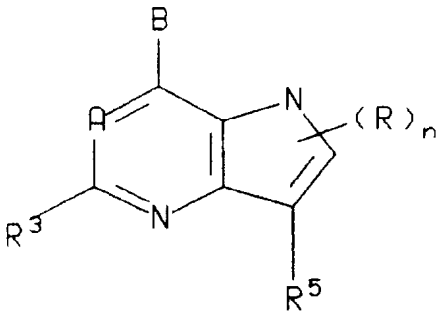
I-H



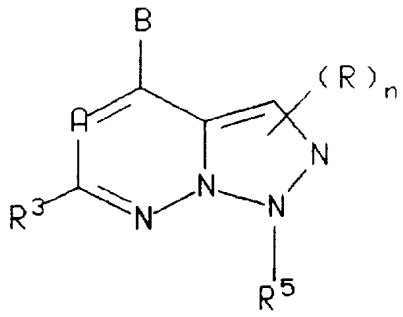
I-L



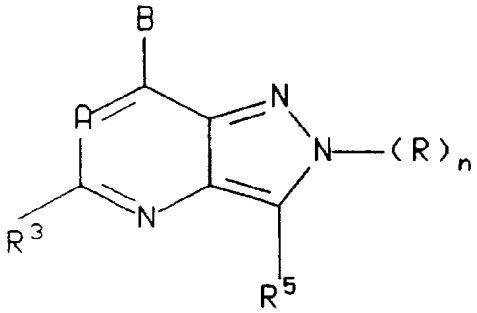
I-M



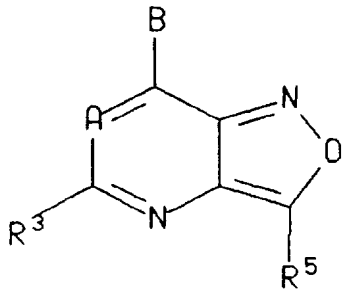
I-N



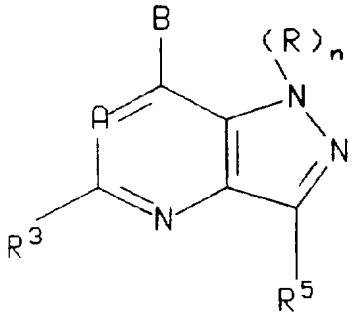
I-O



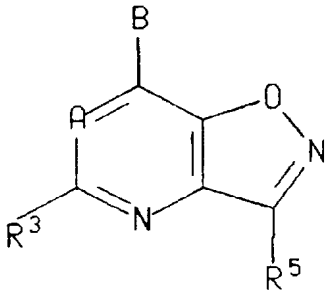
I-R



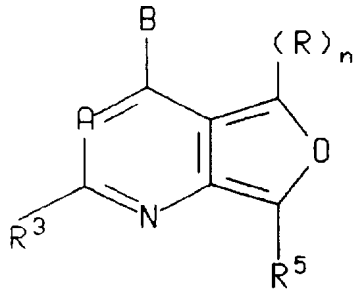
I-S



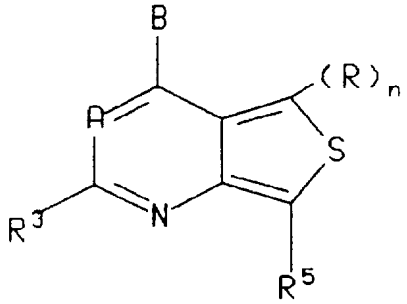
I-V



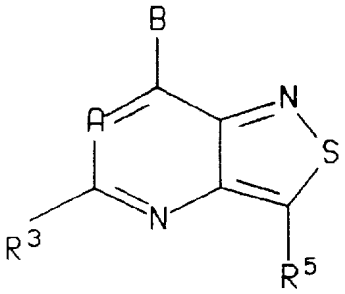
I-W



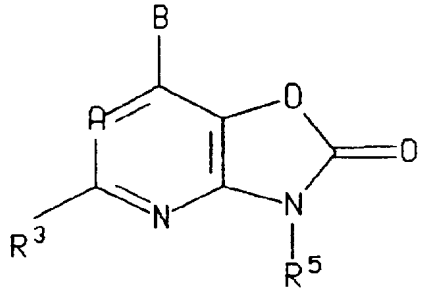
I-P



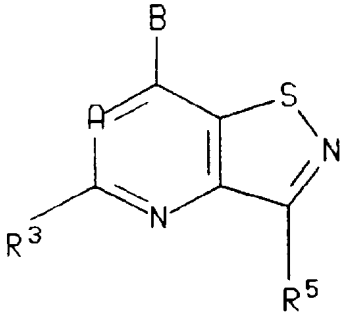
I-Q



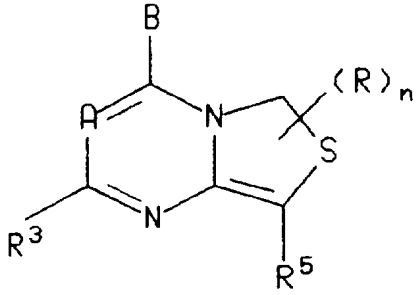
I-T



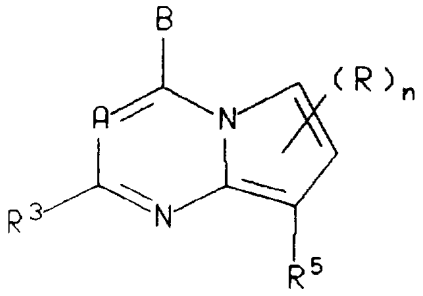
I-U



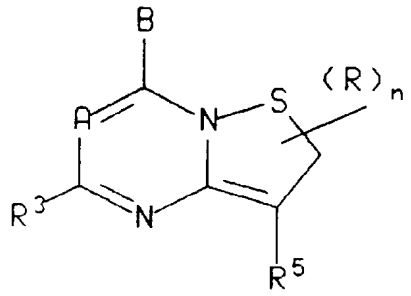
I-X



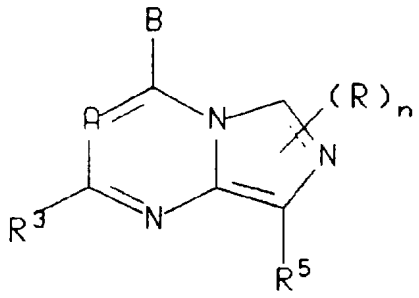
I-B′



I-Y



I-Z



I-A'

More specific embodiments of this invention include compounds of the above formula I wherein B is $-CHR^1R^2$, $-NR^1R^2$, $-NHCHR^1R^2$, $-OCHR^1R^2$ or $-SCHR^1R^2$, and R^1 is C_1-C_6 alkyl, which may optionally be substituted with one hydroxy, fluoro, CF_3 or C_1-C_4 alkoxy group and which may optionally contain one double or triple bond; and R^2 is benzyl or C_1-C_6 alkyl, which may optionally contain one double or triple bond, wherein said C_1-C_6 alkyl and the phenyl moiety of said benzyl may optionally be substituted with one fluoro, CF_3, C_1-C_2 alkyl, C_1-C_2 alkoxy or chloro group.

Other more specific embodiments of this invention include compounds of formula I wherein R^3 is methyl, ethyl, chloro or methoxy; R^4 and R^6 are selected from hydrogen, methyl and ethyl; R^5 is di- or tri-substituted phenyl, pyridyl, or pyrimidyl, in which the two or three substitutents on said phenyl, pyridyl or pyrimidyl are selected, independently, from C_1-C_4 alkyl, -O-(C_1-C_4 alkyl), (C_1-C_2 alkyl)-O-(C_1-C_4 alkyl), $-CF_3$, $-OCF_3$, -CHO, -(C_1-C_4 alkyl)-OH, cyano, chloro, fluoro, bromo and iodo, wherein each of the forgoing C_1-C_4 alkyl groups may optionally contain one double or triple bond;

Other more specific embodiments of this invention include compounds of the formula I wherein B is or contains an NR^1R^2 or $CR^1R^2R^{10}$ moiety which forms a saturated or unsaturated 5-membered carbocyclic ring wherein one of the ring carbons may optionally be replaced by a sulfur or oxygen atom.

Other more specific embodiments of this invention include compounds of the formula I wherein A is nitrogen or CR^7 and R^7 is hydrogen or methyl.

Other more specific embodiments of this invention include compounds of the formula I wherein B is $-CR^1R^2R^{10}$, $-C(=CR^2R^{11})R^1$, $-OCR^1R^2R^{10}$, $-SCR^1R^2R^{10}$, $-CR^2R^{10}NHR^1$, $CR^2R^{10}OR^1$, $-CR^2R^{10}SR^1$ or $-COR^2$.

Other more specific embodiments of this invention include compounds of the formula I wherein A is CH, J and K are carbon and D, E, and G are nitrogen;

Other more specific embodiments of this invention include compounds of the formula I wherein J and D are nitrogen, K and G are carbon, and E is CH, CCH_3, or CC_2H_5.

Other more specific embodiments of this invention include compounds of the formula I wherein B contains a $-NR^1R^2$ or $-CR^1R^2R^{10}$ moiety that is a saturated or unsaturated three to five membered ring.

Other more specific embodiments of this invention include compounds of the formula I wherein J and K are carbon, and D--E--G is O-$C(CH_3)$=C, O-CH=C,

S-C(CH_3)=C, S-CH=C, N(CH_3)-C(CH_3)=C, N(CH_3)-CH=C, O-N=C, S-N=C, N(CH_3)-N=C, O-CH_2N, S-CH_2N, or NH-C(C_1-C_2alkyl)=C.

Examples of preferred compounds of the formula I:

7-(1-ethyl-propoxy)-5-methyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidine;

[2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-(1-ethyl-propyl)-amine;

(1-ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-amine;

7-(1-ethyl-propoxy)-2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidine;

[2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-ethyl-propyl-amine;

[6-bromo-5-bromomethyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b]-pyridin-7-yl]-(1-ethyl-propyl)-amine;

(1-ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b]-pyridin-7-yl]-amine;

[6-bromo-5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b]pyridin-7-yl]-(1-ethyl-propyl)-methyl-amine; and

7-(1-ethyl-propoxy)-5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b]-pyridine.

Other compounds of formula I include the following:

4-(1-ethyl-propoxy)-2,7-dimethyl-8-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-a]-pyrimidine;

(1-ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-amine;

[3-(4-bromo-2,6-dimethyl-phenyl)-5-methyl-pyrazolo[1,5-a]pyrimidin-7-yl]-(1-ethyl-propyl)-amine;

butyl-ethyl-[5-methyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-amine;

3-(4-bromo-2,6-dimethyl-phenyl)-7-(1-ethyl-propoxy)-5-methyl-pyrazolo[1,5-a]-pyrimidine;

[2,7-dimethyl-8-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-a]pyrimidin-4-yl]-diethyl-amine;

[2,7-dimethyl-8-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-a]pyrimidin-4-yl]-ethyl-propyl-

amine;

[8-(4-chloro-2,6-dimethyl-phenyl)-2,7-dimethyl-pyrrolo[1,2-a]pyrimidin-4-yl]-ethyl-propyl-amine;

[8-(4-chloro-2,6-dimethyl-phenyl)-2,7-dimethyl-pyrrolo[1,2-a]pyrimidin-4-yl]-diethyl-amine;

[8-(4-chloro-2,6-dimethyl-phenyl)-2,6-dimethyl-pyrrolo[1,2-a]pyrimidin-4-yl]-diethyl-amine;

[2,6-dimethyl-8-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-a]pyrimidin-4-yl]-diethyl-amine;

[2,6-dimethyl-8-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-a]pyrimidin-4-yl]-ethyl-propyl-amine;

butyl-[2,6-dimethyl-8-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-a]pyrimidin-4-yl]-ethyl-amine;

[2,6-dimethyl-8-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-a]pyrimidin-4-yl]-(1-ethyl-propyl)-amine;

[8-(4-chloro-2,6-dimethyl-phenyl)-2,6-dimethyl-pyrrolo[1,2-a]pyrimidin-4-yl]-(1-ethyl-propyl)-amine;

8-(4-chloro-2,6-dimethyl-phenyl)-4-(1-ethyl-propoxy)-2,6-dimethyl-pyrrolo[1,2-a]-pyrimidine;

4-(1-ethyl-propoxy)-2,6-dimethyl-8-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-a]-pyrimidine;

4-(1-ethyl-propoxy)-2-methyl-8-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-a]pyrimidine;

(1-ethyl-propyl)-[2-methyl-8-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-a]pyrimidin-4-yl]-amine;

butyl-ethyl-[2-methyl-8-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-a]pyrimidin-4-yl]-amine;

butyl-ethyl-[5-methyl-3-(2,4,6-trimethyl-phenyl)-2H-isothiazolo[2,3-a]-pyrimidin-7-yl]-amine;

(1-ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-2H-isothiazolo[2,3-a]-pyrimidin-7-yl]-amine;

7-(1-ethyl-propoxy)-5-methyl-3-(2,4,6-trimethyl-phenyl)-2H-isothiazolo[2,3-a]-pyrimidine;

4-(1-ethyl-propoxy)-2-methyl-8-(2,4,6-trimethyl-phenyl)-imidazo[1,5-a]-

pyrimidine;

(1-ethyl-propyl)-[2-methyl-8-(2,4,6-trimethyl-phenyl)-imidazo[1,5-a]pyrimidin-4-yl]-amine;

[2,6-dimethyl-8-(2,4,6-trimethyl-phenyl)-imidazo[1,5-a]pyrimidin-4-yl]-(1-ethyl-propyl)-amine;

4-(1-ethyl-propoxy)-2,6-dimethyl-8-(2,4,6-trimethyl-phenyl)-imidazo[1,5-a]-pyrimidine;

4-(1-ethyl-propoxy)-2,6-dimethyl-8-(2,4,6-trimethyl-phenyl)-6,7-dihydro-imidazo [1,5-a]pyrimidine;

4-(1-ethyl-propoxy)-2,6-dimethyl-8-(2,4,6-trimethyl-phenyl)-thiazolo[3,4-a]-pyrimidine;

4-(1-ethyl-propoxy)-2-methyl-7-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-b]pyridazine;

4-(1-ethyl-propoxy)-2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-b]-pyridazine;

4-(1-ethyl-propoxy)-2,6-dimethyl-7-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-b]; pyridazine;

4-(1-ethyl-propoxy)-2,5,6-trimethyl-7-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-b]-pyridazine;

[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-b]pyridazin-4-yl]-(1-ethyl-propyl)-amine;

(1-ethyl-propyl)-[2-methyl-7-(2,4,6-trimethyl-phenyl)-pyrrolo[1,2-b]pyridazin-4-yl]-amine;

[2,6-dimethyl-3-(2,4,6-trimethyl-phenyl)-imidazo[1,2-b]pyridazin-8-yl]-(1-ethyl-propyl)-amine;

8-(1-ethyl-propoxy)-2,6-dimethyl-3-(2,4,6-trimethyl-phenyl)-imidazo[1,2-b]-pyridazine;

8-(1-ethyl-propoxy)-6-methyl-3-(2,4,6-trimethyl-phenyl)-imidazo[1,2-b]pyridazine;

(1-ethyl-propyl)-[6-methyl-3-(2,4,6-trimethyl-phenyl)-imidazo[1,2-b]pyridazin-8-yl]-amine;

(1-ethyl-propyl)-[2-methyl-7-(2,4,6-trimethyl-phenyl)-imidazo[1,5-b]pyridazin-4-yl]-amine;

[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-imidazo[1,5-b]pyridazin-4-yl]-(1-ethyl-propyl)-amine;

4-(1-ethyl-propoxy)-2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-imidazo[1,5-b]-pyridazine-b]pyridazine;

butyl-[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-imidazo[1,5-b]pyridazin-4-yl]-ethyl-amine;

[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-imidazo[1,5-b]pyridazin-4-yl]-ethyl-propyl-amine;

[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-imidazo[1,5-b]pyridazin-4-yl]-diethyl-amine;

diethyl-[6-methyl-3-(2,4,6-trimethyl-phenyl)-[1,2,4]triazolo[4,3-b]pyridazin-8-yl]-amine;

ethyl-[6-methyl-3-(2,4,6-trimethyl-phenyl)-[1,2,4]triazolo[4,3-b]pyridazin-8-yl]-propyl-amine;

butyl-ethyl-[6-methyl-3-(2,4,6-trimethyl-phenyl)-[1,2,4]triazolo[4,3-b]pyridazin-8-yl]-amine;

(1-ethyl-propyl)-[6-methyl-3-(2,4,6-trimethyl-phenyl)-[1,2,4]triazolo[4,3-b]-pyridazin-8-yl]-amine;

8-(1-ethyl-propoxy)-6-methyl-3-(2,4,6-trimethyl-phenyl)-[1,2,4]triazolo[4,3-b]-pyridazine;

7-(1-ethyl-propoxy)-1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]-pyridine;

7-(1-ethyl-propoxy)-1,2,5-trimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]-pyridine;

(1-ethyl-propyl)-[1,2,5-trimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]-pyridin-7-yl]-amine;

[1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridin-7-yl]-(1-ethyl-propyl)-amine;

butyl-[1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridin-7-yl]-ethyl-amine;

[1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridin-7-yl]-ethyl-propyl-amine;

[1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridin-7-yl]-diethyl-amine;

ethyl-[5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b]pyridin-7-yl]-

propyl-amine;

butyl-ethyl-[5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b]pyridin-7-yl]-amine;

diethyl-[5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b]pyridin-7-yl]-amine;

[3-(4-bromo-2,6-dimethyl-phenyl)-5-methyl-3H-[1,2,3]triazolo[4,5-b]pyridin-7-yl]-(1-ethyl-propyl)-amine;

[3-(4-chloro-2,6-dimethyl-phenyl)-5-methyl-3H-[1,2,3]triazolo[4,5-b]pyridin-7-yl]-(1-ethyl-propyl)-amine;

3-(4-chloro-2,6-dimethyl-phenyl)-7-(1-ethyl-propoxy)-5-methyl-3H-[1,2,3]triazolo[4,5-b]pyridine;

3-(4-bromo-2,6-dimethyl-phenyl)-7-(1-ethyl-propoxy)-5-methyl-3H-[1,2,3]triazolo[4,5-b]pyridine;

7-(1-ethyl-propoxy)-5-methyl-3-(2,4,6-trimethyl-phenyl)-isothiazolo[4,5-b]pyridine;

(1-ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-isothiazolo[4,5-b]pyridin-7-yl]-amine;

(1-ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-isoxazolo[4,5-b]pyridin-7-yl]-amine;

7-(1-ethyl-propoxy)-5-methyl-3-(2,4,6-trimethyl-phenyl)-isoxazolo[4,5-b]pyridine;

7-(1-ethyl-propoxy)-5-methyl-3-(2,4,6-trimethyl-phenyl)-thieno[3,2-b]pyridine;

7-(1-ethyl-propoxy)-2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-thieno[3,2-b]pyridine;

[2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-thieno[3,2-b]pyridin-7-yl]-(1-ethyl-propyl)-amine;

(1-ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-thieno[3,2-b]pyridin-7-yl]-amine;

(1-ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-furo[3,2-b]pyridin-7-yl]-amine;

[2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-furo[3,2-b]pyridin-7-yl]-(1-ethyl-propyl)-amine;

7-(1-ethyl-propoxy)-2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-furo[3,2-b]pyridine;

7-(1-ethyl-propoxy)- 5-methyl-3-(2,4,6-trimethyl-phenyl)-furo[3,2-b]pyridine;

4-(1-ethyl-propoxy)-2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-6H-pyrrolo[3,4-b]-pyridine;

4-(1-ethyl-propoxy)-2,5,6-trimethyl-7-(2,4,6-trimethyl-phenyl)-6H-pyrrolo[3,4-b]-

pyridine;

(1-ethyl-propyl)-[2,5,6-trimethyl-7-(2,4,6-trimethyl-phenyl)-6H-pyrrolo[3,4-b]pyridin-4-yl]-amine;

[1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-2-thia-4-aza-inden-7-yl]-(1-ethyl-propyl)-amine;

7-(1-ethyl-propoxy)-1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-2-thia-4-aza-indene;

4-(1-ethyl-propoxy)-2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-furo[3,4-b]pyridine;

[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-furo[3,4-b]pyridin-4-yl]-(1-ethyl-propyl)-amine;

[2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-2H-pyrazolo[4,3-b]pyridin-7-yl]-(1-ethyl-propyl)-amine;

7-(1-ethyl-propoxy)-2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-2H-pyrazolo[4,3-b]-pyridine;

7-(1-ethyl-propoxy)-5-methyl-3-(2,4,6-trimethyl-phenyl)-isothiazolo[4,3-b]pyridine;

(1-ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-isothiazolo[4,3-b]pyridin-7-yl]-amine;

(1-ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-d]-pyrimidin-7-yl]-amine;

diethyl-[5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl]-amine;

ethyl-[5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl]-propyl-amine;

butyl-ethyl-[5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-d]-pyrimidin-7-yl]-amine;

[2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-diethyl-amine;

butyl-[2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-ethyl-amine;

butyl-ethyl-[5-methyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-amine;

ethyl-[5-methyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-propyl-amine;

diethyl-[5-methyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-amine;

[3-(4-chloro-2,6-dimethyl-phenyl)-5-methyl-pyrazolo[1,5-a]pyrimidin-7-yl]-(1-ethyl-propyl)-amine;

[3-(4-chloro-2,6-dimethyl-phenyl)-5-methyl-pyrazolo[1,5-a]pyrimidin-7-yl]-(1-ethyl-propyl)-amine;

3-(4-chloro-2,6-dimethyl-phenyl)-7-(1-ethyl-propoxy)-5-methyl-pyrazolo[1,5-a]-pyrimidine;

8-(4-chloro-2,6-dimethyl-phenyl)-4-(1-ethyl-propoxy)-2-methyl-pyrazolo[1,5-a]-[1,3,5]triazine;

(1-ethyl-propyl)-[2-methyl-8-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a][1,3,5]triazin-4-yl]-amine;

4-(1-ethyl-propoxy)-2-methyl-8-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a][1,3,5]-triazine;

4-(1-ethyl-propoxy)-2,7-dimethyl-8-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a][1,3,5]-triazine;

[2,7-dimethyl-8-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a][1,3,5]triazin-4-yl]-(1-ethyl-propyl)-amine;

[1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrazolo[4,3-b]pyridin-7-yl]-(1-ethyl-propyl)-amine;

7-(1-ethyl-propoxy)-1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrazolo[4,3-b]-pyridine;

7-(1-ethyl-propoxy)-5-methyl-3-(2,4,6-trimethyl-phenyl)-isothiazolo[4,5-b]-pyridine;

(1-ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-isothiazolo[4,5-b]pyridin-7-yl]-amine;

1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-7-(1-ethyl-propoxy)-1H-pyrrolo[3,2-b]pyridine;

1,5-dimethyl-3-(2,6-dimethyl-4-chloro-phenyl)-7-(1-ethyl-propoxy)-1H-pyrrolo[3,2-b]pyridine;

2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-7-(1-ethyl-propoxy)-1H-pyrrolo[3,2-b]pyridine;

2,5-dimethyl-3-(2,6-dimethyl-4-chloro-phenyl)-7-(1-ethyl-propoxy)-1H-pyrrolo[3,2-b]pyridine;

7-(1-cyclopropylmethyl-propoxy)-1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridine;

7-(1-cyclopropylmethyl-propoxy)-1,5-dimethyl-3-(2,6-dimethyl-4-chloro-phenyl)-1H-pyrrolo[3,2-b]pyridine;

1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-7-(tetrahydro-furan-3-yloxy)-1H-pyrrolo[3,2-b]pyridine;

1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-7-(S)-(tetrahydro-furan-3-yloxy)-1H-pyrrolo[3,2-b]pyridine;

1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-7-(1-propyl-butoxy)-1H-pyrrolo[3,2-b]pyridine;

7-sec-butylsulfanyl-1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridine;

cyclopropylmethyl-[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-b]pyrimidin-4-yl]-propyl-amine;

2-[1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridin-7-ylamino]-butan-1-ol;

1,5-dimethyl-7-thiazolidin-3-yl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridine;

[1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridin-7-yl]-ethyl-(2,2,2-trifluoro-ethyl)-amine;

cyclopropylmethyl-[1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridin-7-yl]-propyl-amine;

(1-ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-isoxazolo[4,5-b]pyridin-7-yl]-amine; and

7-(1-ethyl-propoxy)-5-methyl-3-(2,4,6-trimethyl-phenyl)-isoxazolo[4,5-b]-pyridine.

This invention also relates to a pharmaceutical composition for the treatment, prevention or inhibition of (a) a disorder the treatment of which can be effected or facilitated by antagonizing CRF, including but not limited to disorders induced or facilitated by CRF, or (b) a disorder selected from inflammatory disorders such as rheumatoid arthritis and osteoarthritis, pain, asthma, psoriasis and allergies; generalized anxiety disorder; panic; phobias; obsessive-compulsive disorder; post-traumatic stress disorder; sleep disorders induced by stress; pain perception such as fibromyalgia; mood disorders such as depression, including major depression, single episode

depression, recurrent depression, child abuse induced depression, mood disorders associated with premenstrual syndrome, and postpartum depression; dysthemia; bipolar disorders; cyclothymia; chronic fatigue syndrome; stress-induced headache; cancer; irritable bowel syndrome, Crohn's disease; spastic colon; post operative ileus; ulcer; diarrhea; stress-induced fever; human immunodeficiency virus (HIV) infections; neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease; gastrointestinal diseases; eating disorders such as anorexia and bulimia nervosa; hemorrhagic stress; chemical dependencies and addictions (e.g., dependencies on alcohol, nicotine, cocaine, heroin, benzodiazepines, or other drugs); drug and alcohol withdrawal symptoms; stress-induced psychotic episodes; euthyroid sick syndrome; syndrome of inappropriate antidiarrhetic hormone (ADH); obesity; infertility; head traumas; spinal cord trauma; ischemic neuronal damage (e.g., cerebral ischemia such as cerebral hippocampal ischemia); excitotoxic neuronal damage; epilepsy; stroke; immune dysfunctions including stress induced immune dysfunctions (e.g., porcine stress syndrome, bovine shipping fever, equine paroxysmal fibrillation, and dysfunctions induced by confinement in chickens, sheering stress in sheep or human-animal interaction related stress in dogs); muscular spasms; urinary incontinence; senile dementia of the Alzheimer's type; multiinfract dementia; amyotrophic lateral sclerosis; hypertension; tachycardia; congestive heart failure; osteoporosis; premature birth; and hypoglycemia in a mammal, including a human, comprising an amount of a compound of the formula I, or a pharmaceutically acceptable salt thereof, that is effective in the treatment of such disorder, and a pharmaceutically acceptable carrier.

The invention also relates to a method for the treatment, prevention or inhibition of (a) a disorder the treatment of which can be effected or facilitated by antagonizing CRF, including but not limited to disorders induced or facilitator by CRF, or (b) a disorder selected from inflammatory disorders such as rheumatoid arthritis and osteoarthritis, pain, asthma, psoriasis and allergies; generalized anxiety disorder; panic; phobias; obsessive-compulsive disorder; post-traumatic stress disorder; sleep disorders induced by stress; pain perception such as fibromyalgia; mood disorders such as depression, including major depression, single episode depression, recurrent depression, child abuse induced depression, mood disorders associated with premenstrual syndrome, and postpartum depression; dysthemia; bipolar disorders;

cyclothymia; chronic fatigue syndrome; stress-induced headache; cancer; irritable bowel syndrome; Crohn's disease; spastic colon; post operative ileus; ulcer; diarrhea; stress-induced fever; human immunodeficiency virus (HIV) infections; neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease; gastrointestinal diseases; eating disorders such as anorexia and bulimia nervosa; hemorrhagic stress; stress-induced psychotic episodes; euthyroid sick syndrome; syndrome of inappropriate antidiarrhetic hormone (ADH); obesity; infertility; head traumas; spinal cord trauma; ischemic neuronal damage (e.g., cerebral ischemia such as cerebral hippocampal ischemia); excitotoxic neuronal damage; epilepsy; stroke; immune dysfunctions including stress induced immune dysfunctions (e.g., porcine stress syndrome, bovine shipping fever, equine paroxysmal fibrillation, and dysfunctions induced by confinement in chickens, sheering stress in sheep or human-animal interaction related stress in dogs); muscular spasms; urinary incontinence; senile dementia of the Alzheimer's type; multiinfarct dementia; amyotrophic lateral sclerosis; chemical dependencies and addictions (e.g., dependencies on alcohol, nicotine, cocaine, heroin, benzodiazepines, or other drugs); drug and alcohol withdrawal symptoms; hypertension; tachycardia; congestive heart failure; osteoporosis; premature birth; and hypoglycemia in a mammal, including a human, comprising administering to a subject in need of said treatment an amount of a compound of the formula I, or a pharmaceutically acceptable salt thereof, that is effective in treating such disorder.

This invention also relates to a method of treating or preventing a disorder or condition, the treatment or prevention of which can be effected or facilitated by inhibiting CRH binding protein, in a mammal, including a human, comprising administering to said mammal a CRH binding protein inhibiting amount of a compound of the formula I, or a pharmaceutically acceptable salt thereof.

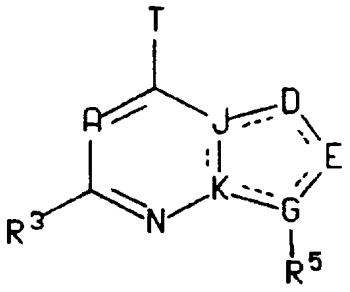
This invention also relates to a pharmaceutical composition for treating or preventing a disorder or conditon, the treatment or prevention of which can be effected or facilitated by inhibiting CRH binding protein in a mammal, including a human, comprising a CRH binding protein inhibiting amount of a compound of the formula I, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

This invention includes all optical isomers and other stereoisomers of compounds of the formula I. When such compounds contain one or more chiral

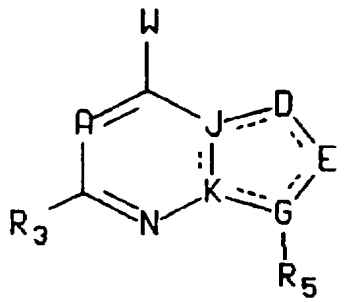
centers, it is understood that the invention includes the racemic mixtures as well as all individual enantiomers and diastereomers of such compounds, and mixtures thereof.

Detailed Description of the Invention

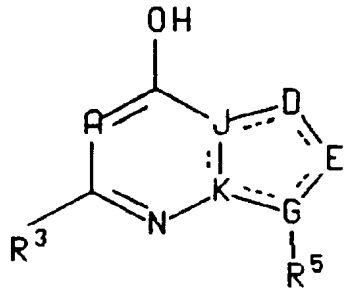
The following compounds having the formulas II, III, IV and V are useful as intermediates in the synthesis of compounds of the formula I.



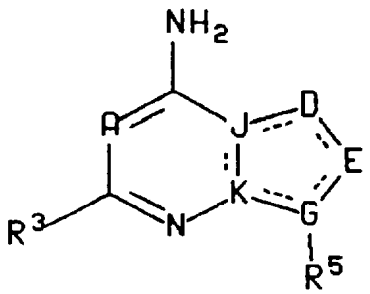
II



III



IV

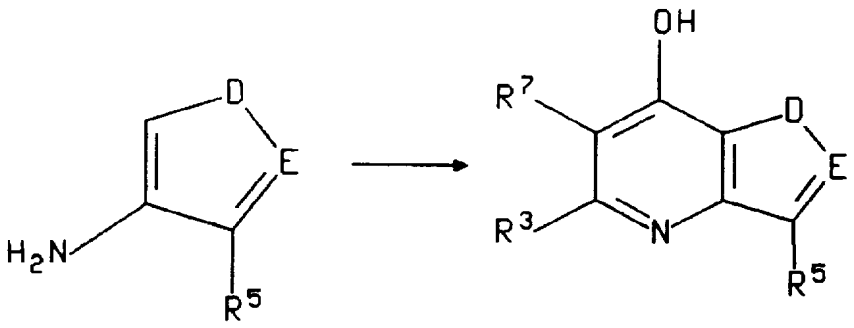


V

wherein T is chloro, bromo, iodo or $-OSO_2CF_3$; W is cyano, formyl, or $-COO(C_0-C_4$ alkyl) and A, J, K, D, E, G, R^3, and R^5 are as defined above with reference to formula I.

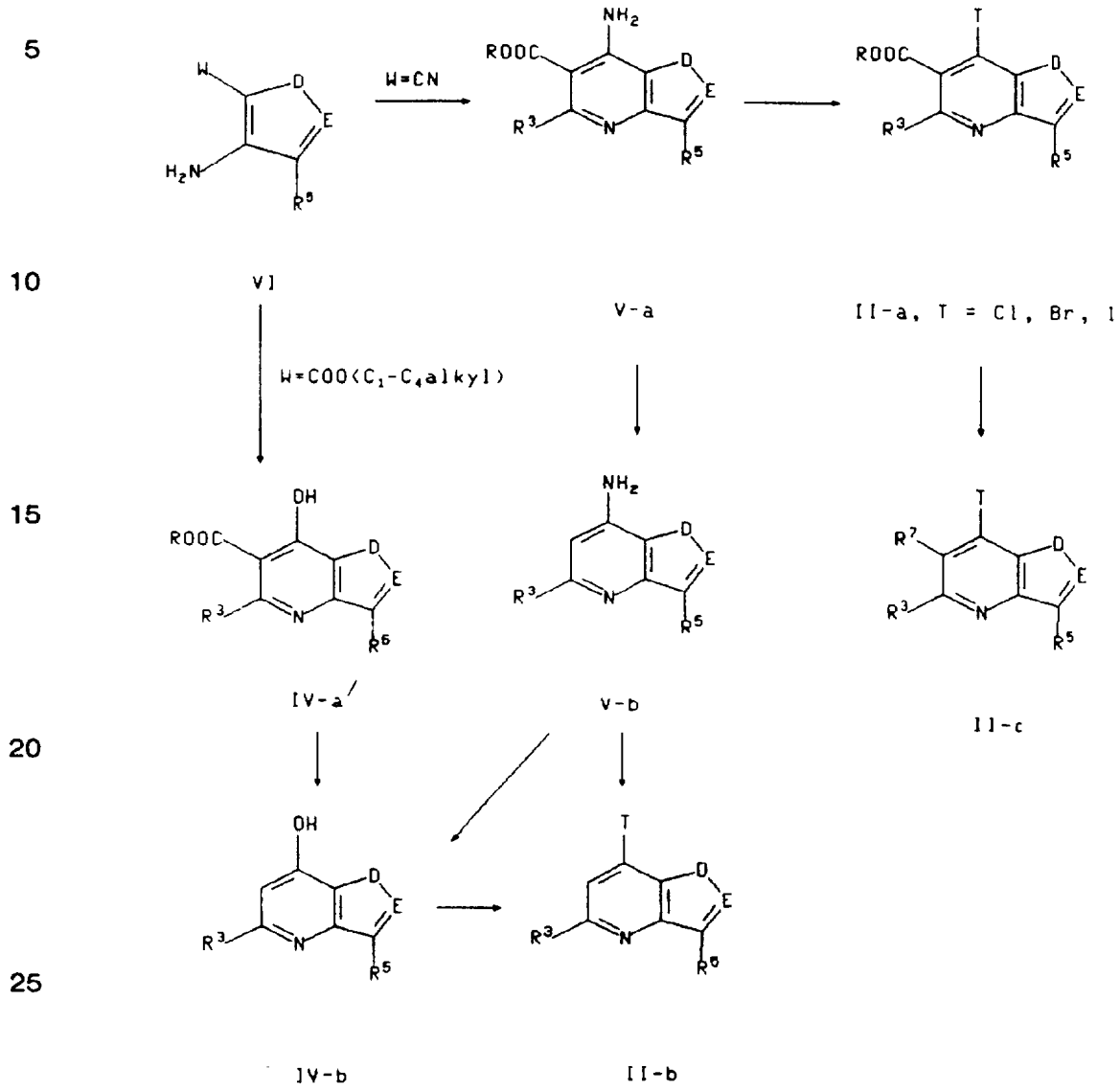
Compounds of the formula I may be prepared as described below. In the reaction schemes and discussion that follow, A, B, D, E, G, J, K, R^3, R^5 and structural formulae I, II, III, IV, and V are defined as above.

SCHEME 1

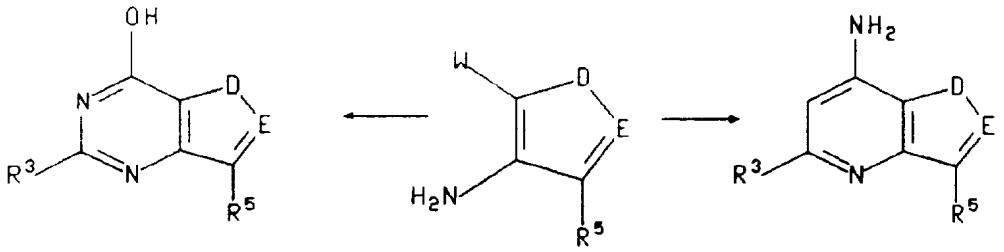


VI-a IV-a

SCHEME 2



SCHEME 3

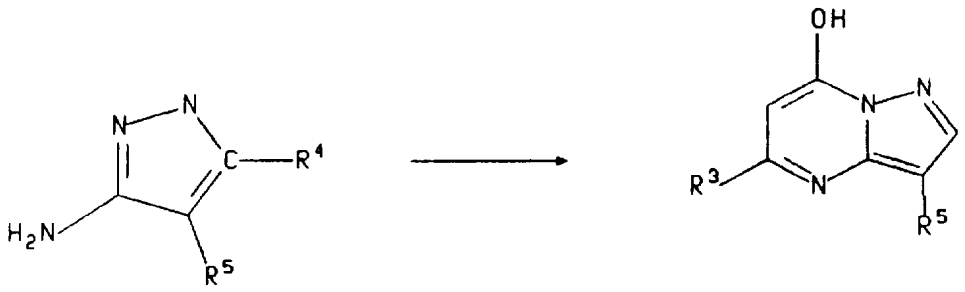


IV-c

VI
(W=CN, -COO(C_1-C_4 alkyl), -$CONH_2$)

V-b

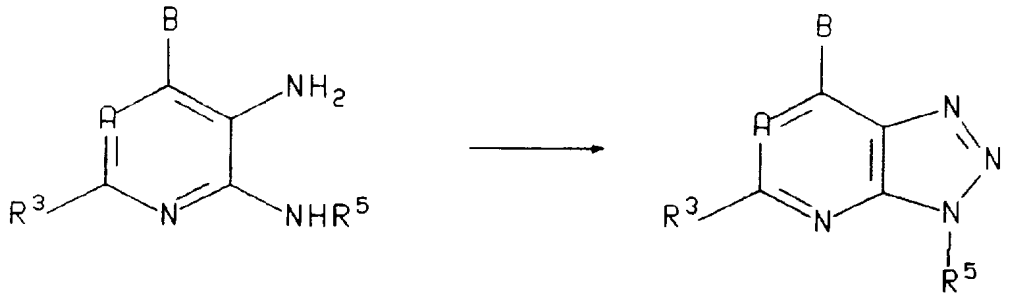
SCHEME 4



IX

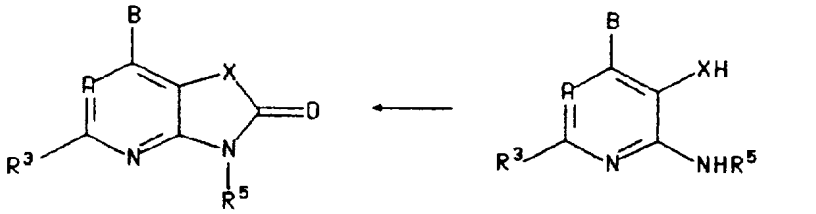
IV-d

SCHEME 5



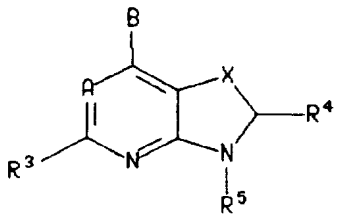
X I-N

SCHEME 6



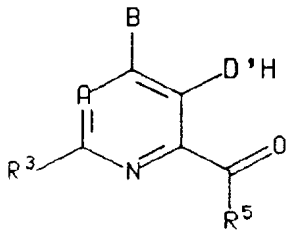
I-K, X=S or O, Y=O
I-U, X=S or O, Y=S

XI



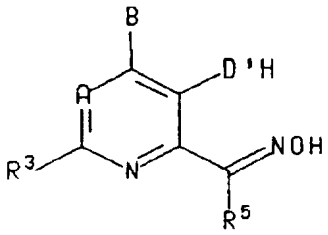
I-L, X=S
I-M, X=O

SCHEME 7

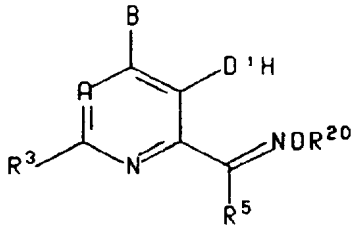


XII

D' = NR^8, O, S

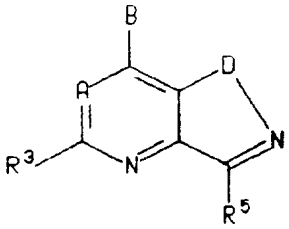


XIII



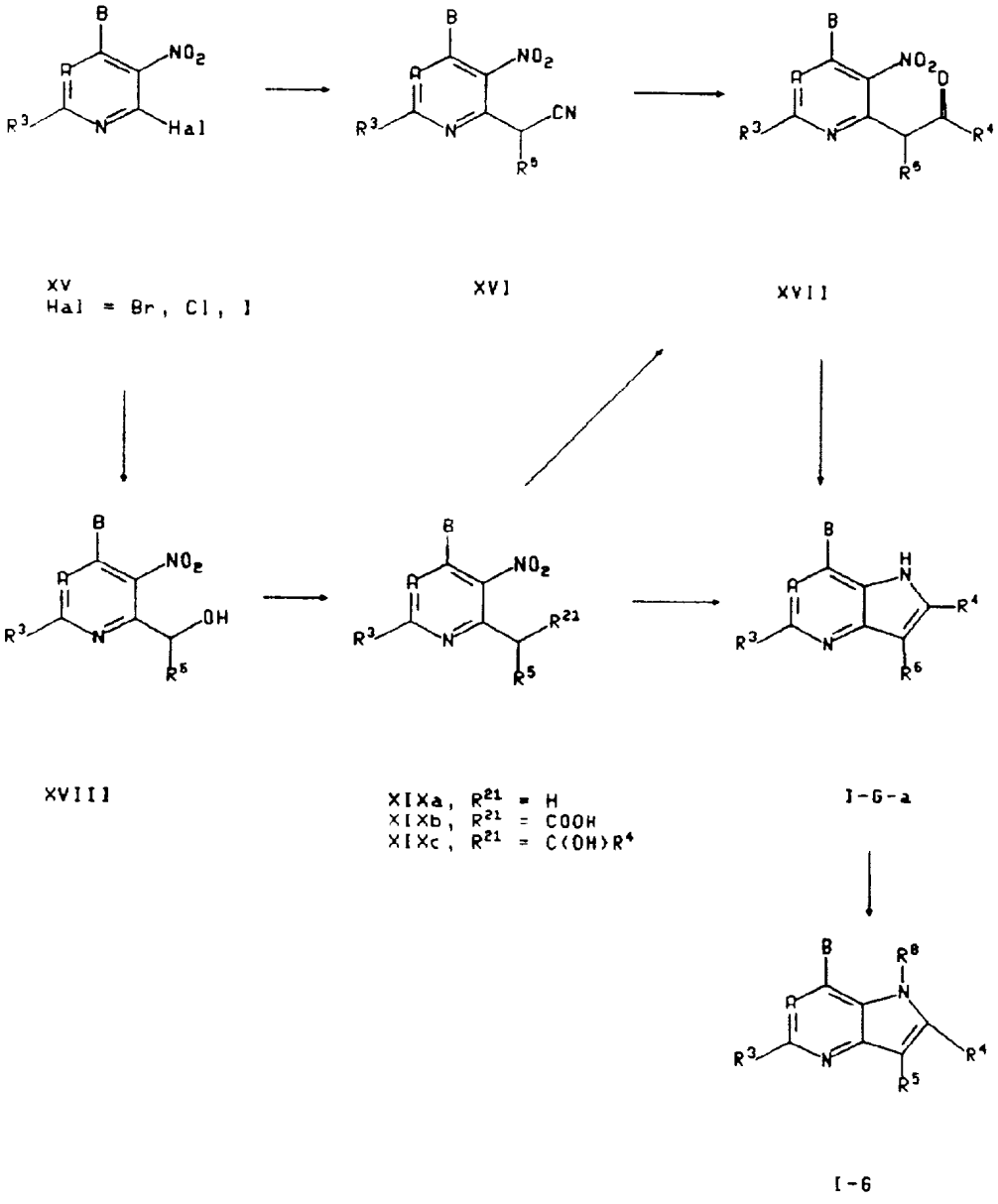
XIV

R^{20} = Acetate or CF_3CO

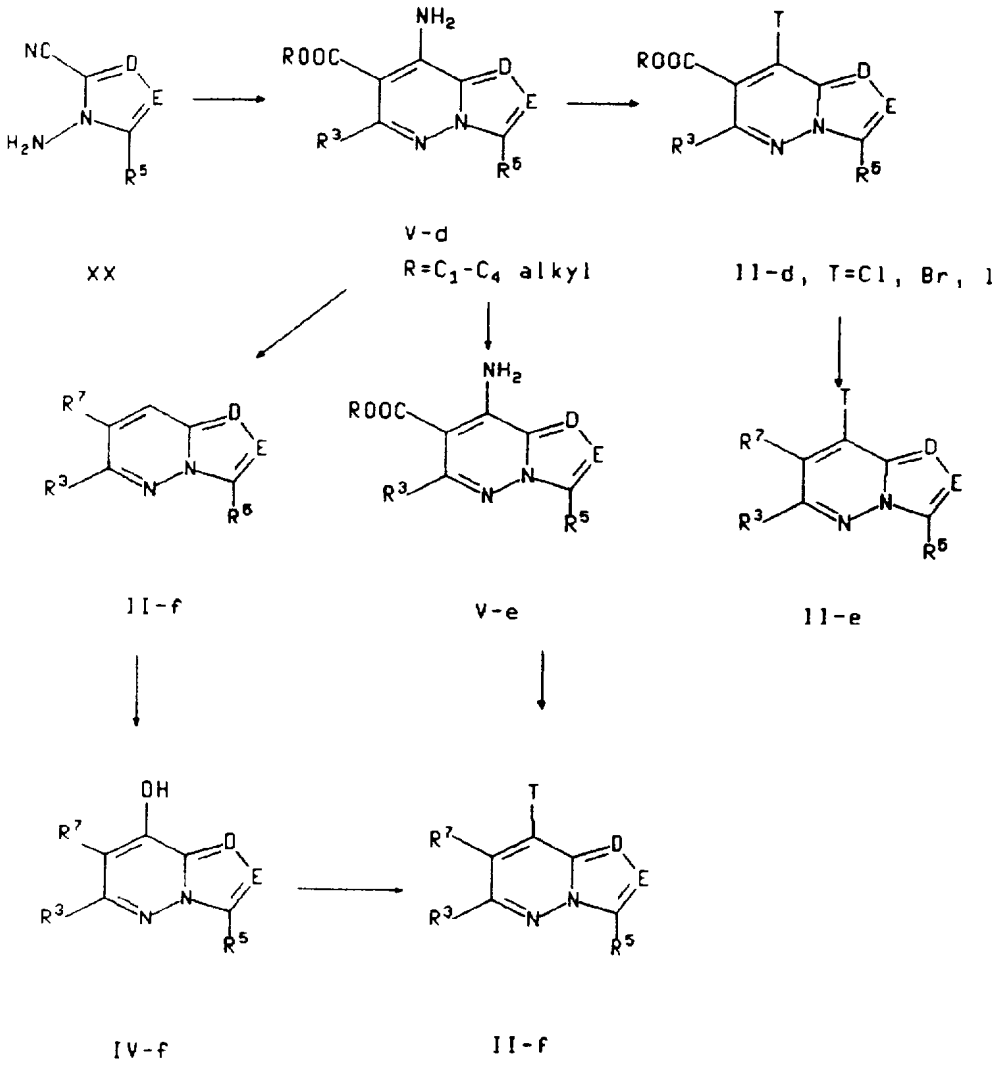


I-V, D = NR^8
I-W, D = O
I-X, D = S

SCHEME 8


XV
Hal = Br, Cl, I
XVI
XVII
XVIII
XIXa, R21 = H
XIXb, R21 = COOH
XIXc, R21 = C(OH)R4
I-G-a
I-G

SCHEME 9



Compounds of the formula I wherein B is $-NR^1R^2$ or $-NHCR^1R^2R^{11}$ may be prepared by reacting a compound of the formula II wherein T is chloro, bromo, or iodo with a compound of the formula BH, in the presence of a base, with or without an organometallic compound such as Cu(I)X, wherein X is chloro, bromo or iodo, or an acid (such as p-TsOH (Ts=Tosyl) or another sterically hindered phenol) or an equivalent agent known to those of skill in the art. Suitable solvents for this reaction include DMSO, NMP, dimethylacetamide and THF. An excess of BH may be used as both the reagent and the base. Other bases such as potassium or sodium carbonate, a trialkylamine, a potassium or sodium (C_1-C_4 alkoxide) and sodium hydride may also be used. When R^7 is an electron withdrawing group such as -COO(C_1-C_4alkyl) or CN, the reaction generally is carried out at a temperature between about room temperature and about 100°C. When R^7 is a non-electron withdrawing group, the reaction temperature can generally range from about 50°C to about 270°C and the pressure can generally range from about 4 psi to about 300 psi. A pressure reactor may be used.

Alternatively, the compounds of formula I may be prepared by reacting a compound of the formula II wherein T is bromo or iodo with 1 equivalent or an excess of BH and a base such as sodium or potassium carbonate or a sodium or potassium (C_1-C_4 alkoxide), in the presence of a palladium (II) or a palladium (0) catalyst such as $Pd(OAc)_2$ or $Pd(PPh_3)_4$, together with a racemic or chiral phosphino agent such as 2,2-bis(diphenylphosphino)-1,1-binaphthyl (BINAP). Alternatively, premade Pd(II)(BINAP) may be used in an appropriate inert (i.e., inert with respect to the reaction at hand) solvent such as toluene, xylene, dioxane or sulfolane, at a temperature from about room temperature to about 180°C, preferably at about reflux temperature.

Compounds of the formula I wherein B is $-OCR^1R^2R^{11}$, $-SCR^1R^2R^{11}$, or $-NHCR^1R^2R^{11}$ may be prepared by reacting compounds in the formula II wherein T is chloro, bromo or iodo with a compound of the formula BH in the presence of a base which is capable of deprotonation of BH (e.g., sodium or potassium hydride, or an organometallic base such as sodium diisopropylamide, sodium bis(trimethylsilyl)amide, lithium diisopropylamide, lithium bis(trimethylsilyl)amide, a sodium C_1-C_4 alkoxide or n-butyllithium), in an inert organic solvent such as tetrahydrofuran, acetonitrile, dimethylsulfoxide, acetone, a C_2-C_5 alcohol, chloroform, benzene, xylene, toluene, N,N-dimethylformamide (DMF), methylene chloride, 1-methyl-2-pyrrolidinone or a mixture of

two or more of the above solvents (e.g., DMSO and THF), at a temperature from about 0°C to about 180°C, preferably from about 50°C to about 180°C.

Compounds of the formula I wherein B is $-CR^1R^2R^{11}$, $-C(C=CR^2R^{12})R^1$, $-CR^2R^{11}NHR^1$, $-CR^2R^{11}OR^1$, $-CR^2R^{11}SR^1$, or $-C(O)R^2$ may be prepared from compounds of the formula III wherein W is cyano, formyl or carboxy, as described below.

Reacting compounds of formula III wherein W is cyano with a Grignard reagent containing the group R^2 in an inert solvent such as THF, glyme, ether or dioxane, will yield corresponding compounds of formula I wherein B is $-COR^2$. Further reaction of the compounds of formula I wherein B is COR^2 with a Grignard reagent containing R^1, using a solvent such as those referred to above, will yield the corresponding the compounds of formula I wherein B is $-CR^1R^2OH$. Reacting compounds of formula III wherein W is formyl with a Grignard reagent containing the group R^2, in an ethereal solvent such as those referred to above, will yield the corresponding compounds of formula I wherein B is $-CHR^2OH$.

Compounds of formula I wherein B is $-CR^1R^2R^{11}$ or $-C(C=CR^2R^{11})R^1$ may be prepared by conventional methods. Thus, reaction of a compound of the formula I wherein B is $-CR^{1'}R^{2'}OH$, (wherein $R^{1'}$ and $R^{2'}$ are defined as R^1 and R^2, respectively, except that $R^{1'}$ may not be R^1 and $R^{2'}$ may not be R^2), with an acid such as concentrated sulfuric acid in acetic acid, or a Burgess inner salt such as (carboxysulfamoyl)triethylammonium hydroxide methyl ester, will yield a compound of the formula I wherein B is $-C(=CR^2R^{11})R^1$. Hydrogenation of a compound of formula I wherein B is $-C(=CR^2R^{11})R^1$ using palladium on carbon (Pd/C) or a platinum dioxide catalyst in a (C_1-C_4)alkanol solvent will yield a compound of the formula I wherein B is $-CHR^1R^2$. Reaction of a compound of the formula I wherein B is $-CR^1R^2OH$ with diethylaminosulfur trifluoride or triphenylphosphine/carbon tetrachloride in an inert solvent such as carbon tetrachloride will afford a compound of the formula I wherein B is $-CR^1R^2F$ or $-CR^1R^2Cl$, respectively.

Reduction of a compound of formula I wherein B is $-COR^2$ with sodium borohydride, in an inert solvent such as a (C_1-C_4)alkanol, will yield a compound of the formula I wherein B is $-CHR^2OH$. Alkylation of a compound of the formula I wherein B is $-CHR^2OH$ with an alkyl halide (such as alkyl iodide) in the presence of a base such as sodium hydride (NaH) at room temperature, in an inert solvent such as toluene, THF,

dioxane or ether, will yield the corresponding compound of the formula I wherein B is $-CHR^2OR^1$.

Compounds of the formula I wherein B is $-CR^2R^{10}NHR^1$ may be prepared by conventional methods such as reductive amination of the corresponding compounds of the formula I wherein B is $-C(O)R^2$ with an appropriate amine and reducing agent (such as sodium cyanoborohydride, sodium triacetoxyborohydride, or lithium aluminum tetrahydride) in an appropriate inert solvent such as a C_1-C_4 alkanol or acetic acid.

Conversion of compounds in formula I wherein B is $-C(O)R^2$ into compounds in formula I wherein B is $-C(S)R^2$ can be accomplished using standard methods well known in the art (e.g., using Lawesson's Reagent or diphosphorus pentasulfide (P_2S_5)). Reduction of compounds of the formula I wherein B is $-C(S)R^2$ with a reducing agent such as sodium borohydride or lithium aluminum tetrahydride gives the corresponding compounds of the formula I wherein B is $-CHR^2SH$. Alkylation of compounds of the formula I wherein B is $-CHR^2SH$ with alkyl halide (such as alkyl iodide) in the presence of a base such as sodium hydride in an inert solvent such as THF, DMF or toluene at about room temperature will afford the corresponding compounds of the formula I wherein B is $-CHR^2SR^1$.

Compounds in formula II may be prepared from compounds of the formula IV or V as described below.

Compounds of formula II wherein T is chloro, bromo or iodo can be prepared by reacting compounds of the formula IV with from one equivalent to an excess of POT_3 (wherein T is chloro, bromo or iodo) in the presence or absence of a di(C_1-C_4 alkyl)aniline, preferably diethylaniline, with or without a solvent (such as dichloroethane, DMF, dimethylsulfoxide (DMSO) or acetamide), at a temperature from about room temperature to about 180°C, preferably from about 100°C to about 150°C. Alternatively, compounds of formula II wherein T is chloro, bromo or iodo can be prepared by reacting the corresponding compounds of formula II wherein T is $-O-SO_2CF_3$ with a sodium or potassium halide, in an appropriate inert solvent such as THF, sulfolane, DMSO, DMF or acetonitrile, at a temperature from about 60°C to about 180°C. Compounds of formula II wherein T is $-OSO_2CF_3$ can be prepared by reacting compounds of formula IV with Tf_2O in the presence of a base such as triethylamine or pyridine, in an appropriate inert solvent such as THF, methylene chloride, dioxane,

ether or toluene, at an temperature from about 0°C to about 50°C, preferably from about 0°C to about room temperature.

Alternatively, compounds of formula II wherein T is chloro, bromo or iodo may be prepared by reacting compounds of formula V with a (C_1-C_7 alkyl)-nitrite and Cu(I)T_2 (wherein T is chloro, bromo or iodo) in an appropriate inert solvent such as acetonitrile, acetone, methylene chloride, THF, dioxane, benzene, toluene, dichloroethane, DMF, DMSO or N-methylpyrrolidinone (NMP) at a temperature from about room temperature to about 150°C, preferably from about 40°C to about 100°C.

Compounds of the formula IV may be prepared by reacting the appropriate compounds of formula V with sodium nitrite ($NaNO_2$) in an aqueous acid such as sulfuric acid, acetic acid or phosporic acid, with or without an organic solvent, preferably in acetonitrite (CH_3CN) or acetone.

Compounds of formula III wherein W is cyano can be prepared by reacting the corresponding compounds of formula II wherein T is chloro, bromo or iodo with potassium cyanide, copper cyanide, sodium cyanide or a di(C_1-C_4alkyl)aluminum cyanide in an appropriate inert solvent such as dimethylsulfoxide, DMF, toluene or xylene, at temperature from about room temperature to about 180°C, preferably from about 60°C to about 150°C, with or without Pd(II)OAc or Pd(0)(PPh_3)$_4$.

Compounds of formula III wherein W is -CHO or -COOH may be prepared by reacting compounds in formula II wherein T is bromo or iodo with an organolithium reagent such as t-BuLi, s-BuLi, or n-BuLi in an appropriate inert solvent such as THF, dioxane, ether, benzene or methylene chloride, at temperature from about -120°C to about room temperature, preferably from about -110°C to about -60°C, followed by quenching with an appropriate electrophile such as DMF or CO_2 (gas or dry ice), to give compounds of formula III wherein W is -CHO and -COOH, respectively.

It is understood that the general organic chemistry knowledge can be applied to change the steps of the reaction sequences described herein depending on the feasibility of the reaction. For example, a protecting group may be used at any stage of the various syntheses described above at which it is workable, or an ester group may be reduced to the corresponding C_1-C_4 alkyl group at any convenient stage. Compounds of formulas I - XVIII, wherein R^3 is chloro, bromo, -COO(C_1-C_4 alkyl) or -COOH, can be converted to the corresponding compounds wherein R^3 is (C_1-C_4 alkyl), -O(C_1-C_4 alkyl), fluoro, -S(C_1-C_4 alkyl) at any convenient stage, as appropriate, in the

syntheses referred to above using methods described in the literature. Compounds of formula I - XVIII, wherein R^3 is -O(C_1-C_4 alkyl) or -S(C_1-C_4 alkyl) can be prepared by reacting the corresponding compounds wherein R^3 is chloro, bromo or iodo, with a nucleophile such as a C_1-C_4 alkanol or a C_1-C_4 alkanethiol, in the presence of an organic or inorganic base. Suitable bases for such a reaction include sodium and sodium hydride. Compounds of formulas I - XVIII wherein R^3 is fluoro can be prepared by reacting the corresponding compounds wherein R^3 is chloro with tetrabutylammonium fluoride in a suitable inert solvent such as DMSO, methylene chloride, or tetrahydrofuran. Tetrahydrofuran is preferred.

Reduction of an ester or carboxylic acid using lithium aluminum tetrahydride/aluminum trichloride ($LiAlH_4/AlCl_3$) in an appropriate inert solvent such as THF, ether, or dioxane, at temperature from about 100°C to about room temperature, will afford the corresponding compound wherein R^3 is CH_3. Conversion of compounds wherein B is -COOH to the corresponding compounds wherein B is -CO(C_1-C_3 alkyl) may be accomplished using standard alkylation methods well known in art. Reduction of compounds wherein B is -CO(C_1-C_3 alkyl) using standard methods well known in the art will afford the corresponding compounds wherein R^3 is one of various (C_1-C_4 alkyl) derivatives.

Compounds of the formula IV-a wherein A is CR^7 and G, J, and K are carbon may be prepared by heating compounds of formula VI-a with an appropriate compound of the formula $R^3C(O)CR^7COO(C_1$-C_4 alkyl) under acid or Lewis acid conditions with or without a solvent, as shown in Scheme 1. Examples of such reaction conditions are: a) heating in polyphosphoric acid; b) heating in toluene, benzene or xylene in the presence of acid catalyst (such as p-TsOH, sulfuric acid or HCl(g)) using a Dean-Stark trap; or c) heating in an appropriate solvent such as dichloroethane, diphenylether (Ph_2O) or Dowtherm A in the presence of a Lewis acid such as $SnCl_4$, $ZnCl_2$/HCl or $AlCl_3$.

Compounds of formula VI-a may be prepared using methods described in the scientific literature. (See Gazz. Chim. Ital.; 111, p167-172(1981); Chem. Pharm. Bull.; 24, 3001-3010 (1976); J. Org. Chem., 38, 1777-1780 (1973); Chem. Abstr. 68, 68982f (1968); Aust. J. Chem.; 22, 563-572(1969); J. Chem. Soc. Perkin Trans.2, p1954, p1957 (1972); J. Heterocycl. Chem.; FR, 19, 443-445(1982); J. Heterocycl. Chem., 22,

1496-1502 (1985); Tetrahedron, 47, 4639-4644 (1991); J. Heterocycl. Chem., 28, 2053-2055 (1991); J. Heterocycl. Chem., 29, 251-153 (1992).

Compounds of formula V-a and IV-a', wherein R is C_1-C_4alkyl can be prepared by heating compounds of formula VI wherein W is -CN and -COO(C_1-C_4 alkyl), respectively, with an appropriate $R^3C(O)CH_2COO(C_1$-C_4 alkyl) in the presence of a Lewis acid such as $SnCl_4$, $AlCl_3$, $TiCl_3$ or $ZnCl_2$, in a inert solvent such as dichloroethane, at about the reflux temperature, as shown in Scheme 2. Base hydrolysis of a compound of the formula V-a or IV-a' with sodium hydroxide in H_2O/(C_1-C_4 alcohol) at reflux, or with lithium hydroxide in water/THF or water/dioxane at temperature from about room temperature to about the reflux temperature, followed by decarboxylation by heating in a oil bath at a temperature from about 140-180°C will give a compound of formula V-b or IV-b, respectively. Compounds of the formulas V-a and IV-a' may be converted into compounds of the formula II-a, and compounds of the formulas V-b and IV-b may be converted into compounds of the formula II-b by the procedures described above. The conversion of compounds of the formula II-a into those of the formula II-c can be accomplished using procedures analogous to those described above for converting compounds wherein R^3 is -COO(C_1-C_4 alkyl) into those wherein R^3 is C_1-C_4 alkyl.

Compounds of formula VI may be prepared using methods described in the literature. (See Liebigs Ann Chem., 1534-1546, 1979; Gazz. Chim. Ital., 97, 25-33, 1967; Gazz. Chim. Ital., 120, 725-730, 1990; Eur. J. Med. Chem. Chim. Ther., 26, 143-158, 1991; J. Heterocycl. Che., Fr. 19, 443-445, 1982; J. Heterocycl. Chem., 22, 1496-1502, 1985; J. Heterocycl. Chem., 31, 305-312, 1994; J. Heterocycl. Chem., 24, 243-245, 1987; J. Org. Chem. 57, 3713-3716, 1992; Liebigs Ann Chem., 1702-1710, 1984; Chem. Pharm. Bull., 34, 701-702, 1986; Pharmazie, 48, 849-853, 1993; Bull. Soc. Chim. Belg., 103, 181-184, 1994; and Indian. J. Chem. Sect. B, 33, 436-440, 1994).

Compounds of formula IV-c wherein A is N and G, J, and K are carbon may be prepared, as shown in Scheme 3, by reacting compounds of formula VI with $(R^3CO)_2O$, R^3COOH or $R^3CO(OC_1$-C_2 alkyl)$_3$, in acetic acid or an appropriate inert organic solvent such as toluene, dioxane, acetonitrile, methylene chloride or chloroform, at a temperature from about 25°C to about 150°C, preferably at about the reflux temperature, followed by heating in 85% phosphoric acid or an aqueous acid such as acetic acid, hydrochloric acid or sulfuric acid, preferably in 50-85% phosphoric acid. Compounds of formula V-b may be prepared, as shown in Scheme 3, by heating the

corresponding compounds of formula VI with excess of the appropriate compound of the formula R^3CONH_2.

Compounds of formula IV-d may be prepared, as shown in Scheme 4, by reacting compounds of formula IX with an appropriate reagent having the formula $R^3C(O)CHR^7COO(C_1\text{-}C_4alkyl)$ in an R^3COOH solvent at temperature from about 60°C to about 180°C, preferably at about the reflux temperature.

Compounds of formula I-N may be prepared, as shown in Scheme 5, by reacting the corresponding compounds of formula X with a (C_1-C_7 alkyl)nitrite, with or without $CuBr_2$, $CuCl_2$, or CuI_2, in an appropriate inert solvent such as acetonitrile, acetone, methylene chloride, chloroform, benzene or toluene, preferably acetonitrile at a temperature from about 25°C to about 150°C, preferably from about 60°C to 100°C.

Compounds of formula I-L and I-M may be prepared, as shown in Scheme 6, by reacting compounds of formula XI wherein X is S or O with a compound of the formula R^4CHO or $R^4CH(OC_1\text{-}C_2$ alkyl)$_2$ and an acid catalyst such as p-TsOH, HCl, HBr, H_2SO_4, or HCl, in toluene, xylene or benzene, preferably toluene, with from one to ten equivalents of water, at temperature from about 70°C to about 160°C, using a Dean-Stark trap or in the presence of anhydrous sodium sulfate.

Compounds of formula I-K and I-U may be prepared by reacting the corresponding compounds of formula XI with triphosgene and thiophosgene, respectively, and a base such as triethylamine or pyridine in an appropriate inert solvent such as methylene chloride, THF, dioxane, ether, benzene or chloroform, preferably methylene chloride or dry THF, at temperature from about 0°C to about 25°C.

Compounds of formula I-V, I-W, and I-X may be prepared, as illustrated in Scheme 7, starting from compounds of formula XII. Compounds of formula XIII may be prepared by reacting the corresponding compounds of formula XII with hydroxylamine in acidic (e.g., in trifluoroacetic acid) or basic (e.g., in NaOAc or NaOH and hydroxylamine hydrochloride in a C_1-C_4 alcohol/water mixture) conditions at temperatures from about 25°C to about 150°C, preferably at about the reflux temperature. Compounds of formula XIV can be prepared by heating the corresponding compounds of formula XIII in acetic anhydride, trifluoroacetic anhydride or Tf_2O, with or without a solvent such as acetic acid or methylene chloride, in the presence of an appropriate amine base such as triethylamine or pyridine. Compounds of formula I-V, I-W and I-X may be prepared by heating the corresponding compounds

of formula XIV and pyridine in an inert solvent such as DMF, DMSO, NMP, sulfolane or acetamide at a temperature from about 80°C to about 180°C.

Compounds of formula I-G may be prepared, as illustrated in Scheme 8, by reducing the corresponding compounds of formula XVII. This reduction can be performed using standard methods known in literature for reduction of a nitro group to an amino group. Such methods include hydrogenation or reduction by iron in acetic acid. Cyclization may occur upon reduction or heating in an appropriate solvent such as a C_1-C_4 alcohol, acetonitrile, toluene, THF, methylene chloride or acetic acid.

The conversion of compounds of formula XVI into those of formula XVII can be accomplished using methods analogous to those described above for the conversion of compounds of formula V wherein W is cyano into compounds of formula I wherein B is a group having a carbon atom directly attached to the bicyclic ring. The best method for conversion of a cyano group to a -COOH group is acid hydrolysis, for example, heating the cyano compound in 50-85% phosphoric acid or 50-90% acetic acid, preferably phosphoric acid. The best method for converting a cyano group into a -CO(C_1-C_4 alkyl) is reacting the cyano compound with a Grignard reagent at a temperature from about 0°C to about 25°C in ether, THF or dioxane. The best method for converting a cyano group into a -CHO group is a diisobutylaluminum hydride reduction in THF, dioxane, or ether at a temperature from about -78°C to about 25°C, preferably from about -78°C to about -40°C.

Compounds of the formula XVI may be prepared by reacting compounds of formula XV, wherein Hal is chloro, bromo or iodo, with a sodium, potassium, or lithium salt of R^5CH_2CN in an appropriate inert solvent such as toluene, benzene, a C_1-C_5 alcohol, THF, DMSO, dioxane, or pyridine, with or without a Pd(II) or Pd(0) catalyst, at a temperature from about -78°C to about 130°C.

Alternatively, compounds of formula XVII may be prepared by subjecting compounds of the formula XV to halogen-metal exchange (e.g., using an organo lithium agent such as tBuLi, s-BuLi or BuLi at -78°C in ether, THF, or dioxane), followed by quenching with an electorphile such as R^5CHO, to give compounds of formula XVIII. Compounds of formula XIX-a may be prepared by reacting the corresponding compounds of formula XVIII with thionyl chloride, followed by reduction. Reacting compounds of formula XIX-a with a base (such as organolithium agent (e.g., lithium diisopropylamide or BuLi), followed by quenching with an electrophile (such as carbon

dioxide) will yield the corresponding compounds of formula XIX-b wherein R^{21} is -COOH. Compounds of formula XIX-c wherein R^{21} is -C(OH)R^4 may be prepared in a similar fashion by quenching the appropriate compound of formula XIX-a with an electrophile of the formula C_1-C_3 alkyl-CHO. Compounds of formula XVII can be prepared by reacting compounds of formula XIX-c with PCC (pyridinium chlorochromate) using standard PCC oxidation methods that are well known in the art.

Compounds of formula II-d, II-e and II-f, wherein T is chloro, bromo or iodo and D, E, R^3, R^5 and R^7 are as defined to those in formula I may be prepared, as shown in Scheme 9, by methods analogous to those described in Scheme 2. Alternatively, Compounds of formula II-d to II-f may be prepared using procedures analogous to those described in the literature (See: Pharm. Bull, 5, 229-231, 1957; Monatsh Chem, 100, 671-678, 1969; J. Heterocycl. Chem., 3, 218-220, 1966, Chem. Pharm. Bull,. 23, 2891-2895, 1975; J. Heterocycl. Chem., 8, 1-6, 1971; Justus Liebigs Ann. Chem, 735, 35-44, 1970; Tetrahedron Lett, 1479, 1968; and Chem. Abstr., 1939, 4988.)

The acid addition salts of compounds of the formula can be prepared in a conventional manner by treating a solution or suspension of the corresponding free base with one chemical equivalent of a pharmaceutically acceptable acid. Conventional concentration or crystallization techniques can be employed to isolate the salts. Illustrative of suitable acids are acetic, lactic, succinic, maleic, tartaric, citric, gluconic, ascorbic, benzoic, cinnamic, fumaric, sulfuric, phosphoric, hydrochloric, hydrobromic, hydroiodic, sulfamic, sulfonic acids such as methanesulfonic, benzene sulfonic, p-toluenesulfonic, and related acids.

The compounds of formula I and their pharmaceutically acceptable salts (hereinafter referred to, collectively, as "the active compounds of this invention") may be administered alone or in combination with pharmaceutically acceptable carriers, in either single or multiple doses. Suitable pharmaceutical carriers include inert solid diluents or fillers, sterile aqueous solutions, oils (e.g., sesame oil, peanut oil) and various organic solvents. The pharmaceutical compositions formed by combining the novel compounds of formula I and pharmaceutically acceptable carriers can then be readily administered in a variety of dosage forms such as tablets, oil gel, powders, lozenges, syrups, injectable solutions and the like. These pharmaceutical compositions can, if desired, contain additional ingredients such as flavorings, binders, excipients and the like. Thus, for purposes of oral administration, tablets containing various excipients

such as sodium citrate, calcium carbonate and calcium phosphate may be employed along with various disintegrants such as starch, methylcellulose, alginic acid and certain complex silicates, together with binding agents such as polyvinylpyrrolidone, sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often useful for tabletting purposes. Solid compositions of a similar type may also be employed as fillers in soft and hard filled gelatin capsules. Preferred materials for this include lactose or milk sugar and high molecular weight polyethylene glycols. When aqueous suspensions or elixirs are desired for oral administration, the essential active ingredient therein may be combined with various sweetening or flavoring agents, coloring matter or dyes and, if desired, emulsifying or suspending agents, together with diluents such as water, ethanol, propylene glycol, glycerin and combinations thereof.

For parenteral administration, solutions containing an active compound of this invention or a pharmaceutically acceptable salt thereof in sesame or peanut oil, aqueous propylene glycol, or in sterile aqueous solution may be employed. Such aqueous solutions should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. The sterile aqueous media employed are all readily available by standard techniques known to those skilled in the art.

The effective dosages for the active compounds of this invention will depend on the intended route of administration and factors such as the age and weight of the patient, as generally known to a physician. The dosages will also depend on the particular illness to be treated. For instance, the daily dosage for stress-induced illnesses, inflammatory disorders, Alzheimer's disease, gastro-intestinal diseases, anorexia nervosa, hemorrhagic stress and drug and alcohol withdrawal symptoms will generally range from about 0.1 to about 50 mg/kg body weight of the patient to be treated.

Methods that may be used to determine the CRF antagonist activity of the active compounds of this invention and their pharmaceutically acceptable salts are described in Endocrinology, 116, 1653-1659 (1985) and Peptides, 10, 179-188 (1985). The binding activities for compounds of the formula I, expressed as IC_{50} values, generally range from about 0.5 nanomolar to about 10 micromolar. Methods that can be used

to determine the CRF binding protein inhibiting activity of compounds of the formula I are described in Brain Research, (1997), 745 (1,2),248-255.

The present invention is illustrated by the following examples. It will be understood, however, that the invention is not limited to the specific details of these examples. Melting points are uncorrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) and C^{13} nuclear magnetic resonance spectra (C^{13} NMR) were measured for solutions in deuterochloroform ($CDCl_3$) and peak positions are expressed in parts per million (ppm) downfield from tetramethylsilane (TMS). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad.

The following abbreviations are used in the Examples: Ph=phenyl; iPr=isopropyl.

EXAMPLE 1

7-(1-Ethyl-propoxy)-5-methyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidine

A solution of 3-pentanol (140 mg, 1.5 mmol) in 1 ml of dry THF was treated with 60% sodium hydride in oil (28 mg, 0.7 mmol) and stirred at room temperature for 10 min. A solution of 7-chloro-5-methyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidine (75 mg, 0.262 mmol) in 1 ml of dry THF was added and the resulting mixture was heated at reflux for 5 hours. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was washed with brine, dried and concentrated to give the crude material. The residue was purified through silica gel column chromatography using 3:7 chcloroform:hexane as eluent to give 75mg (88%) of the title compound. ^{1}H NMR ($CDCl_3$) δ 7.97(s,1H), 6.97(s,2H), 6.03(s,1H), 4.56(m,1H), 2.53(s,3H), 2.32(s,3H), 2.13(s,6H), 2.10(m,4H), 1.09(t,6H) ppm.

EXAMPLE 2

[2,5-Dimethyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-(1-ethyl-propyl)-amine

A mixture of 7-Chloro-2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a] pyrimidine (60 mg, 0.2 mmol) and 1-ethylpropylamine (4 ml) in 1ml of N-methylpyrrolidinone was heated at 125°C oil bath for 15 hrs. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried and concentrated and purified through silica gel column chromatography using 20% ethyl acetate in hexane as eluent to give 35 mg of the title compound as a yellow solid. ^{1}H NMR ($CDCl_3$) δ

6.96(s,2H), 6.00(d,1H), 5.77(s,1H), 3.47 (m,1H), 2.43(s,3H), 2.32(s,3H), 2.22(s,3H), 2.05(s,6H), 1.5-1.9(m,4H), 1.04(t,6H) ppm.

EXAMPLE 3

(1-Ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-amine

A mixture of 7-chloro-5-methyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a] pyrimidine (190 mg, 0.63 mmol) and 1-ethylpropylamine (4 ml) in 1ml of N-methylpyrrolidinone was heated at 125°C oil bath for 15 hours. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried and concentrated and purified through silica gel column chromatography using chloroform to 2% methanol in chloroform as eluent to give 195 mg (87%) of the title compound as a green solid. ^{1}H NMR ($CDCl_3$) δ 7.87(s,1H), 6.97(s,2H), 6.12(d,1H), 5.85(s,1H), 3.52(m,1H), 2.48(s,3H), 2.09(s,3H), 2.16(s,6H), 1.6-1.9(m,4H), 1.05(t,6H)ppm.

EXAMPLE 4

7-(1-Ethyl-propoxy)-2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo [1,5-a]pyrimidine

To a suspension of 60% Sodium hydride in oil (160 mg) in 4 ml of DMSO was added 3-pentanol (853 mg), and then 7-chloro-2,5-dimethyl-3-(2,4,6-trimethyl-phenyl) -pyrazolo[1,5-a]pyrimidine (580 mg) at room temperature. The mixture was heated at 88°C overnight. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried and concentrated and purified through silica gel column chromatography using 10% hexane in chloroform as eluent to give the title compound as an orange oil. ^{1}H NMR ($CDCl_3$) δ 6.96(s,2H), 5.95(s,1H), 4.52(m,1H), 2.48(s,3H), 2.33(s,3H), 2.27(s,3H), 2.03(s,6H), 1.75-2.00(m,4H), 1.08(t,6H)ppm.

EXAMPLE 5

[2,5-Dimethyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-ethyl-propyl-amine

A mixture of 7-chloro-5-methyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a] pyrimidine (200 mg, 0.66 mmol) and N-propylethylamine (2 ml) in 1ml of N-methylpyrrolidinone was heated at 135°C oil bath for 4 hours. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried and concentrated and purified through silica gel column chromatography using hexane to 10% ethyl acetate in hexane as eluent to give 150 mg of the title compound as a clear

green oil. ^{1}H NMR ($CDCl_3$) δ 6.95(s,2H), 5.80(s,1H), 3.85(q,2H), 3.67(dd,2H), 2.41(s,3H), 2.32(s,3H), 2.21(s,3H), 2.03(s,6H), 1.76(m,2H), 1.29(t,3H), 0.98(t,3H) ppm.

EXAMPLE 6

[6-Bromo-5-bromomethyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b] pyridin-7-yl]-(1-ethyl-propyl)-amine

To a solution of butyl nitrite (119 mg, 1.15 mmol) and CuBr2 (205 mg, 0.919 mmol) in 16 ml of acetonitrile was added N4-(1-ethyl-propyl)-6-methyl-N2-(2,4,6-trimethyl-phenyl)-pyridine-2,3,4-triamine (250 mg, 0.766 mmol). The resulting mixture was heated at 65°C for 1.5 hrs. The mixture was cooled to room temperature and 2N HCl (16 ml) was added. The mixture was neutralized with 2N NaOH and extracted with ethyl acetate. The organic layer was washed with brine, dried and concentrated to give a brown oil. The oil residue was purified through silica gel column chromatography using 1:1 hexane :ethyl acetate as eluent to give 61mg of [6-bromo-5-bromomethyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b] pyridin-7-yl]-(1-ethyl-propyl)-amine as white crystals. Mp 123-125°C. ^{1}H NMR ($CDCl_3$) δ 7.06(s,2H), 5.53(d,1H), 5.22(m,1H), 4.67(s,2H), 2.39(s,3H), 1.96(s,6H), 1.6-1.9(m,4H), 1.06(t,6H)ppm and 103 mg of [6-bromo-5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b]pyridin-7-yl]-(1-ethyl-propyl)-amine as white solid. Mp 115-117°C. ^{1}H NMR ($CDCl_3$) δ 7.04(s,2H), 5.36(d,1H), 5.21(m,1H), 2.64(s,3H), 2.38(s,3H), 1.95(s,6H), 1.6-1.9(m,4H), 1.05(t,6H)ppm.

EXAMPLE 7

(1-Ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b] pyridin-7-yl]-amine

A mixture of butyl nitrite (119 mg, 1.15 mmol) and N4-(1-ethyl-propyl)-6-methyl-N2-(2,4,6-trimethyl-phenyl)-pyridine-2,3,4-triamine (250 mg, 0.766 mmol) in anhydrous acetonitrile (16 ml) was heated at 65°C for 2 hours. The mixture was cooled to room temperature and 2N HCl (16 ml) was added. The mixture was neutralized with 2N NaOH and extracted with ethyl acetate. The organic layer was washed with brine, dried and concentrated to give 250 mg of the crude product as a brown oil. The oil residue was purified through silica gel column using chloroform as eluent to give 201 mg of the title compound as golden yellow solid. Mp 131-133°C. ^{1}H NMR ($CDCl_3$) δ 7.022(s,2H), 6.20(s,1H), 5.44(d,1H), 3.65(m,1H), 2.50(s,3H), 2.36(s,3H), 1.96(s,6H), 1.5-1.8(m,4H), 1.03(t,6H)ppm.

EXAMPLE 8

[6-Bromo-5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b]pyridin -7-yl]-(1-ethyl-propyl)-methyl-amine

To a solution of [6-bromo-5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo [4,5-b]pyridin-7-yl]-(1-ethyl-propyl)-amine (50 mg, 0.12 mmol) in dry THF)1.5 ml) was added 2.5 M n-BuLi in hexane (0.14 ml) at -78°C. After stirring at -78°C for 10 min, 0.5 ml of methyl iodide was added at that temperature then allowed to warmed to room temperature and stirred for 15 min for 2 hours. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was washed with brine, dried and concentrated to give 46 mg of brown oil. The residue was purified through silica gel coliumn chromatography using 1:1 chloroform:hexane as eluent to give the title compound as a golden oil. ^{1}H NMR ($CDCl_3$) δ 7.04(s,2H), 4.35(m,1H), 3.32(s,3H), 2.70(s,3H), 2.38(s,3H), 1.94(s,6H), 1.7-2.0(m,4H), 1.01(t,6H)ppm.

EXAMPLE 9

7-(1-Ethyl-propoxy)-5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3] triazolo[4,5-b]pyridine

A mixture of 4-(1-Ethyl-propoxy)-6-methyl-N2-(2,4,6-trimethyl-phenyl)-pyridine-2,3-diamine (50 mg, 0.153 mmol) and butyl nitrite (24 mg, 0.229 mmol) in 4 ml acetonitrile was heated at 65°C for 2 hours. An additional 0.13 ml of butyl nitrite was added and the resulting mixture was heated at 65°C for 2 hours. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried and concentrated to give 58 mg of brown oil. The oil was purified through silica gel column chromatography using 5% ethyl acetate in hexane as eluent to give 46 mg (88%) of the title compound as a light yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.04(s,2H), 6.60(s,1H), 5.26(m,1H), 2.57(s,3H), 2.38(s,3H), 1.94(s,6H), 1.8-2.0(m, 4H), 1.07(t,6H) ppm.

EXAMPLE 10

4-(1-Ethyl-propoxy)-2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidine

To a solution of 3-pentanol (0.09 ml, 0.883 mmol) in dry THF was added 60% NaH in oil (20 mg, 0.500 mmol) and stirred for 5 min. A solution of 4-chloro-2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidine (50 mg, 0.166 mmol) in dry THF was added to the reaction mixture and the resulting mixture was heated at reflux for 2 hr. The mixture was quenched with water, extracted with ethyl acetate. The

organic layer was washed with brine, separated, dried and concentrated to dryness to give the title compound as a white solid. ^{1}H NMR ($CDCl_3$) δ 6.92(s,3H), 5.43(m,1H), 4.02(s,3H), 2.56(s,3H), 2.29(s,3H), 2.08(s,6H), 1.80(m,4H), 0.99(t,6H).

The title compounds of Examples 11-14 were prepared by the method analogous to that described in Example 10 starting from of 4-chloro-2-methyl-5-substituted-7-(substituted-phenyl)-5H-pyrrolo[3,2-d]pyrimidine or 7-chloro-5-methyl-1-substituted-3-(substituted-phenyl)-1H-pyrrolo[3,2-b]pyridine and an appropriate alcohol or thiol and a base.

EXAMPLE 11

(+)-2,5-Dimethyl-4-(tetrahydro-furan-3-yloxy)-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidine.

^{1}H NMR ($CDCl_3$) δ 6.95(s,1H), 6.92(s,2H), 5.88(m,1H), 3.9-4.08(m,4H), 4.01(s,3H), 2.56(s,3H), 2.29(s,3H), 2.2-2.4(m,2H), 2.07(s,6H) ppm.

EXAMPLE 12

2,5-Dimethyl-4-(S)-(tetrahydro-furan-3-yloxy)-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidine.

^{1}H NMR ($CDCl_3$) δ 6.95(s,1H), 6.92(s,2H), 5.88(m,1H), 3.9-4.08(m,4H), 4.01(s,3H), 2.56(s,3H), 2.29(s,3H), 2.2-2.4(m,2H), 2.07(s,6H) ppm.

EXAMPLE 13

2,5-Dimethyl-4-(1-propyl-butoxy)-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidine.

^{1}H NMR ($CDCl_3$) δ 6.93(s,2H), 6.92(s,1H), 5.58(m,1H), 4.02(s,3H), 2.56(s,3H), 2.29(s,3H), 2.09(s,6H), 1.6-1.8(m,4H), 1.4-1.6(m,4H), 0.96(t,6H)ppm.

EXAMPLE 14

4-sec-Butylsulfanyl-2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidine.

^{1}H NMR ($CDCl_3$) δ 6.97(s,1H), 6.94(s,2H), 4.34(m,1H), 4.13(s,3H), 2.63(s,3H), 2.30(s,3H), 2.07(s,6H), 1.7-1.9(m,2H), 1.48(d,3H), 1.09(t,3H) ppm.

The title compounds of Examples 15-18 were prepared using the following procedure.

PROCEDURE FOR EXAMPLES. 15 - 18

A mixture of 4-chloro-2-methyl-5-substituted-7-(substituted-phenyl)-5H-pyrrolo[3,2-d]pyrimidine or 7-bromo-1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-

pyrrolo[3,2-b]pyridine-6-carboxylic acid methyl ester (1 mmol) and an appropriate amine in DMSO (2 ml) was heated in 130°C oil bath until all the starting material was consumed. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried and concentrated to dryness to give the corresponding 4-alkylamino-2-methyl-5-substituted-7-(substituted-phenyl)-5H-pyrrolo[3,2-d]pyrimidineor 7-alkylamino-1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridine-6-carboxylic acid methyl ester derivative. Silica gel column chromatography may be used for purification.

EXAMPLE 15

[2,5-Dimethyl-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidin-4-yl]-(1-ethyl-propyl)-amine

^{1}H NMR ($CDCl_3$) δ 6.91(s,2H), 6.76(s,1H), 4.61(d,1H,NH), 4.33(m,1H), 4.04(s,3H), 2.49(s,3H), 2.28(s,3H), 2.09(s,6H), 1.72(m,2H), 1.60(m,2H), 0.98(t,6H)ppm.

EXAMPLE 16

Butyl-[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidin-4-yl]-ethyl-amine

^{1}H NMR ($CDCl_3$) δ 6.96(s,1H), 6.92(s,2H), 3.93(s,3H), 3.44(q,2H), 3.40(m,2H), 2.57(s,3H), 2.29(s,3H), 2.09(s,6H), 1.57(m,2H), 1.30(m,2H), 1.14(t,3H), 0.88(t,3H)ppm.

EXAMPLE 17

2,5-Dimethyl-4-thiazolidin-3-yl-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidine

^{1}H NMR ($CDCl_3$) δ 7.03(s,1H), 6.92(s,2H), 4.78(s,2H), 4.02(s,3H), 3.96(m,2H), 3.18(m,2H), 2.56(s,3H), 2.29(,3H), 2.06(s,6H) ppm.

EXAMPLE 18

7-(1-Ethyl-propylamino)-1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridine-6-carboxylic acid methyl ester

^{1}H NMR ($CDCl_3$) δ 6.98(s,1H), 6.93(s,2H), 5.40(d,1H), 3.97(s,3H), 3.91(s,3H), 3.35(m,1H), 2.57(s,3H), 2.30(s,3H), 2.09(s,6H), 1.52(m,4H), 0.87(t,6H) ppm.

EXAMPLE 19

7-(1-Ethyl-propylamino)-1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]-pyridine-6-carboxylic acid

A mixture of 7-(1-ethyl-propylamino)-1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridine-6-carboxylic acid methyl ester and NaOH in a 1:1 mixture of

MeOH/water was heated at reflux over night. The resulting mixture was acidified with 2N HCl to a pH of 4-5, and extracted with chloroform. The organic layer was dried and concentrated to give the title compound.

^{1}H NMR ($CDCl_3$) δ 7.02(s,1H), 6.82(s,2H), 3.98(s,3H), 3.78(m,1H), 2.59(s,3H), 2.07(s,3H), 2.00(s,6H), 1.64(m,4H), 0.90(t,6H) ppm.

EXAMPLE 20

[1,5-Dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridin-7-yl]-(1-ethyl-propyl)-amine

A mixture of 7-(1-ethyl-propylamino)-1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridine-6-carboxylic acid was heated in 150-160°C oil bath until all the starting material was consumed. ^{1}H NMR ($CDCl_3$) δ 6.94(s,2H), 6.87(s,1H), 6.15(s,1H), 6.10(d,1H), 4.24(s,3H), 3.50(m,1H), 2.64(s,3H), 2.30(s,3H), 2.05(s,6H), 1.77(m,4H), 1.02(t,6H)ppm.

PREPARATION A

4-(2,4,6-Trimethyl-phenyl)-2H-pyrazol-3-ylamine

A mixture of 3-oxo-2-(2,4,6-trimethyl-phenyl)-propionitrile (2.300 g, 12.3 mmol), hydrazine hydrate (0.93 g) and glacial acetic acid (1.55 ml) in 20 ml benzene was heated at reflux for 4.5 hours. Reaction mixture was cooled to room temperature and 50 ml of 18.5% HCl in water was added. The benzene layer was separated and reextracted with 18.5% HCl. The aqueous layer were combined and neutralized with ammonium hydroxide and stirred at rt. overnight. Precipitate formed and was filtered to yield the title compound (0.256 g) as a yellow solid. The benzene layer was concentrated and purified through silica gel column using 5% methanol in chloroform as eluent to give an additional 1.450 g of the title compound; ^{1}H NMR ($CDCl_3$) δ 7.24(s,1H), 6.95(s,2H), 4.75(brs,2H), 2.32(s,3H),2.13(s,6H) ppm.

PREPARATION B

5-Methyl-4-(2,4,6-trimethyl-phenyl)-2H-pyrazol-3-ylamine

The title compound was prepared as white solid by the method analogous to that described in preparation A starting from 3-oxo-2-(2,4,6-trimethyl-phenyl)-butyronitrile. ^{1}H NMR ($CDCl_3$) δ 7.7(brs,1H), 6.96(s,2H), 2.32(s,3H), 2.13(s,3H), 2.06(s,6H) ppm.

PREPARATION C

2,5-Dimethyl-3-(2,4,6-trimethyl-phenyl)-4H-pyrazolo[1,5-a]pyrimidin-7-one

Methyl acetoacetate (0.38 ml) was added to a solution of 5-methyl-4-(2,4,6-trimethyl-phenyl)-2H-pyrazol-3-ylamine (641 mg, 2.98 mmol) in 4 ml of acetic acid. The reaction mixture was heated at reflux for overnight. The mixture was concentrated to dryness and the residue was purified through silica gel column chromatography using 5% methanol in chloroform as eluent to give 560 mg (65.5%) of the title compound as a white solid; ^{1}H NMR (DMSO-d_6) δ 11.7(s,1H), 6.98(s,2H), 5.52(s,1H), 2.29(s,3H), 2.20(s,3H), 1.97(s,3H), 1.95(s,6H) ppm.

PREPARATION D

5-Methyl-3-(2,4,6-trimethyl-phenyl)-4H-pyrazolo[1,5-a]pyrimidin-7-one

Methyl acetoacetate (0.7 ml) was added to a solution of 4-(2,4,6-Trimethyl-phenyl)-2H-pyrazol-3-ylamine (1.120 g, 5.57 mmol) in 5 ml of acetic acid and the resulting mixture was heated at reflux for two days. Reaction mixture cooled and a white solid formed. Ethanol (6 ml) was added and stirred at room temperature overnight, filtered to give white solid which was recrystallized from ethanol to give 673 mg(45.2%) of the title compound as white crystals. ^{1}H NMR (DMSO-d_6) δ 11.9(s,1H), 7.7(s,1H), 6.95(s,2H), 5.55(s,1H), 2.25(s,3H), 2.20(s,3H), 2.0(s,6H)ppm.

PREPARATION E

7-Chloro-5-methyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidine

A suspension of 5-methyl-3-(2,4,6-trimethyl-phenyl)-4H-pyrazolo[1,5-a]pyrimidin -7-one (590 mg, 2.2 mmol) in 9 ml of $POCl_3$ was treated with diethylaniline (0.7 ml) and the resulting mixture was stirred at reflux for 15 hours. The reaction mixture was concentrated to dryness. The residue was treated with ice water and stirred for 20 min, then extracted with chloroform. The organic layer was dried and concentrated to yield an orange oil which crystallized upon standing. The material was purified through silica gel column chromatography using chloroform as eluent to give 590 mg (94%) of the title compound as a yellow solid; ^{1}H NMR ($CDCl_3$) δ 8.08(s,1H), 6.98(s,2H), 6.86(s,1H), 2.56(s,3H), 2.33(s,3H), 2.09(s,6H) ppm.

PREPARATION F

7-Chloro-2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidine

The title compound was prepared as an oil by the method analogous to that described in preparation E starting from 2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-4H-pyrazolo[1,5-a]pyrimidin-7-one. ^{1}H NMR ($CDCl_3$) δ 6.98(s,2H), 6.77(s,1H), 2.51(s,3H), 2.33(s,3H), 2.31(s,3H), 1.99(s,6H) ppm.

PREPARATION G

2,5-Dimethyl-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidin-4-ol

A mixture of 3-amino-1-methyl-4-(2,4,6-trimethylphenyl)-1H-pyrrole-2-carbonitrile (0.4 mmol) and acetic anhydride (0.043 ml) in acetic acid (0.01 ml) was heated at reflux untill all the starting material was consumed. The reaction mixture was concentrated to dryness. The residue was quenched with water and extracted with ethyl acetate. The organic extracts was washed with brine and concentrated to dryness. The residue was suspended in 0.5 ml of 85% phosphoric acid and heated at 130°C for 1 hour. The mixture was cooled and poured into ice-water, stirred until solid formed. The solid was filtered to give 2,5-dimethyl-7-(2,4,6-trimethylphenyl)-5H-pyrrolo[3,2-d]pyrimidin-4-ol. The alternative way for the work up is via extraction technique. The ice water was extracted with chloroform. The organic layer was dried and concentrated to dryness to give the desired product.

^{1}H NMR δ ($CDCl_3$) 6.92(s,2H), 6.88(s,1H), 4.14(s,3H), 2.43(s,3H), 2.29(s,3H), 2.09(s,6H).

The following compounds can be prepared in a similar manner:

2,5-Dimethyl-7-(2,6-dimethyl-4-chloro-phenyl)-5H-pyrrolo[3,2-d]pyrimidin-4-ol;

2,5-Dimethyl-7-(2,6-dimethyl-4-bromo-phenyl)-5H-pyrrolo[3,2-d]pyrimidin-4-ol;

2-Methyl-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidin-4-ol;

2-Methyl-7-(2,6-dimethyl-4-chloro-phenyl)-5H-pyrrolo[3,2-d]pyrimidin-4-ol;

2-Methyl-7-(2, 6-drimethyl-4-bromo-phenyl)-5H-pyrrolo[3,2-d]pyrimidin-4-ol; and

2-Methyl-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidin-4-ol;

PREPARATION H

4-Chloro-2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidine

A mixture of 2, 5-dimethyl-7-(2,4,6-trimethylphenyl)-5H-pyrrolo[3,2-d]pyrimidin-4-ol (1 mmol) in $POCl_3$ (1.3 ml) was heated at reflux until all the starting material were

consumed (about 1-3 hours). The mixture was concentrated to dryness. The residue was porued into ice-water and extracted with ethyl acetate. The organic layer was dried and concentrated to dryness to give the title compound.

^{1}H NMR ($CDCl_3$) δ 7.18(s,1H), 6.95(s,2H), 4.16(s,3H), 2.69(s,3H), 2.31(s,3H), 2.05(s,6H) ppm.

PREPARATION I

7-Amino-1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridine-6-carboxylic acid methyl ester

A mixture of 3-amino-1-methyl-4-(2,4,6-trimethylphenyl)-1H-pyrrole-2-carbonitrile (2 mmol), methyl acetoacetate (4 mmol) and $SnCl_4$ (4 mmol) in 1, 2-dichloroethane was heated at reflux for about six hours until all the starting material were consumed. The mixture was quenched with acetone, basified with saturated $NaHCO_3$, then filtered through Celite®. The filtrate was concentrated to dryness. The residue was quenched with water and extracted with chloroform. The chloroform layer was washed with brine, dried and concentrated to dryness to give 7-amino-1,5-dimethyl -3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridine-6-carboxylic acid methyl ester. The product may be purified by trituration.

^{1}H NMR ($CDCl_3$) δ 6.91(s,2H), 6.85(s,1H), 6.28(brs,2H), 4.10(s,3H), 3.89(s,3H), 2.62(s,3H), 2.28(s,3H), 2.09(s,6H) ppm.

PREPARATION J

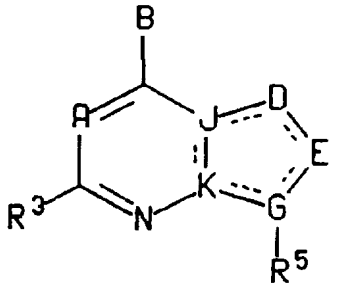
7-Bromo-1,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-1H-pyrrolo[3,2-b]pyridine-6-carboxylic acid methyl ester

A mixture 7-amino-1, 5-dimethyl-3-(2, 4, 6-trimethylphenyl)-1H-pyrrolo[3, 2-b]pyridine-6-carboxylic acid methyl ester (1 mmol), n-butylnitrite (BuONO) (1.5 mmol) and $CuBr_2$ in acetonitrile was heated at 60-70°C until all starting material was consumed. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried and concentrated to dryness to give the title compound.

^{1}H NMR ($CDCl_3$) δ 7.09(s,1H), 6.94(s,2H), 4.16(s,3H), 3.98(s,3H), 2.52(s,3H), 2.30(s,3H), 2.04(s,6H) ppm. 2,7-dibromo-1,5-dimethyl-3-(2,4,6-trimethyl-phenyl) -1H-pyrrolo[3,2-b]pyridine-6-carboxylic acid methyl ester was also produced as a minor component. ^{1}H NMR ($CDCl_3$) δ 7.00(s,2H), 4.45(s,3H), 4.03(s,3H), 2.51(s,3H), 2.34(s,3H), 2.12(s,6H) ppm.

CLAIMS

1. A compound of the formula



I

or a pharmaceutically acceptable salt thereof, wherein

the dashed lines represent optional double bonds;

A is nitrogen or CR^7;

B is $-NR^1R^2$, $-CR^1R^2R^{10}$ $-C(=CR^2R^{11})R^1$, $-NHCR^1R^2R^{10}$, $-OCR^1R^2R^{10}$, $-SCR^1R^2R^{10}$, $-CR^2R^{10}NHR^1$, $-CR^2R^{10}OR^1$, $-CR^2R^{10}SR^1$ or $-COR^2$;

J and K are each independently nitrogen or carbon and both J and K are not nitrogens ;

D and E are each selected, independently, from nitrogen, CR^4, C=O, C=S, sulfur, oxygen, CR^4R^6 and NR^8 ;

G is nitrogen or carbon;

the ring containing D, E, G, K, and J in formula I may be a saturated or unsaturated 5-membered ring and may optionally contain one or two double bonds and may optionally contain from one to three heteroatoms in the ring and may optionally have one or two C=O or C=S groups;

R^1 is C_1-C_6 alkyl optionally substituted with one or two substituents independently selected from hydroxy, fluoro, chloro, bromo, iodo, -O-(C_1-C_4 alkyl), CF_3, -C(=O)O-(C_1-C_4alkyl), -OC(=O)(C_1-C_4 alkyl), -OC(=O)N(C_1-C_4 alkyl)(C_1-C_2 alkyl), -NHCO(C_1-C_4 alkyl), -COOH, -COO(C_1-C_4 alkyl), -CONH(C_1-C_4 alkyl), -CON(C_1-C_4 alkyl)(C_1-C_2 alkyl), -S(C_1-C_4 alkyl), -CN, $-NO_2$, -SO(C_1-C_4 alkyl), $-SO_2$(C_1-C_4 alkyl), $-SO_2$NH(C_1-C_4 alkyl) and $-SO_2$N(C_1-C_4 alkyl)(C_1-C_2 alkyl), wherein each of the C_1-C_4 alkyl groups in the foregoing R^1 groups may optionally contain one or two double or triple bonds;

R^2 is C_1-C_{12} alkyl which may optionally contain from one to three double or triple bonds, aryl or (C_1-C_4 alkylene)aryl, wherein said aryl and the aryl moiety of said (C_1-C_4

alkylene)aryl is selected from phenyl, naphthyl, thienyl, benzothienyl, pyridyl, quinolyl, pyrazinyl, pyrimidinyl, imidazolyl, furanyl, benzofuranyl, benzothiazolyl, isothiazolyl, pyrazolyl, pyrrolyl, indolyl, pyrrolopyridyl, oxazolyl and benzoxazolyl; C_3-C_8 cycloalkyl or (C_1-C_6 alkylene)(C_3-C_8 cycloalkyl), wherein one or two of the carbon atoms of said cycloalkyl and the 5 to 8 membered cycloalkyl moieties of said (C_1-C_6 alkylene)(C_3-C_8 cycloalkyl) may optionally and independently be replaced by an oxygen or sulfur atom or by NZ^2 wherein Z^2 is selected from hydrogen, C_1-C_4 alkyl, benzyl and C_1-C_4 alkanoyl, and wherein each of the foregoing R^2 groups may optionally be substituted with from one to three substituents independently selected from chloro, fluoro, hydroxy and C_1-C_4 alkyl, or with one substituent selected from bromo, iodo, C_1-C_6 alkoxy, -OC(=O)(C_1-C_6 alkyl), -OC(=O)N(C_1-C_4 alkyl)(C_1-C_2 alkyl), -S(C_1-C_6 alkyl), amino, -NH(C_1-C_2 alkyl), -N(C_1-C_2 alkyl)(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)-CO-(C_1-C_4 alkyl), -NHCO(C_1-C_4 alkyl), -COOH, -COO(C_1-C_4 alkyl), -CONH(C_1-C_4 alkyl), -CON(C_1-C_4 alkyl)(C_1-C_2 alkyl), -SH, -CN, -NO_2, -SO(C_1-C_4 alkyl), -SO_2(C_1-C_4 alkyl), -SO_2NH(C_1-C_4 alkyl) and -SO_2N(C_1-C_4 alkyl)(C_1-C_2 alkyl);

-NR^1R^2 or $CR^1R^2R^{10}$ may form a saturated 3 to 8 membered carbocyclic ring which may optionally contain from one to three double bonds and wherein one or two of the ring carbon atoms of such 5 to 8 membered rings may optionally and independently be replaced by an oxygen or sulfur atom or by NZ^3 wherein Z^3 is hydrogen, C_1-C_4 alkyl, benzyl or C_1-C_4 alkanoyl;

R^3 is hydrogen, C_1-C_4 alkyl, -O(C_1-C_4 alkyl), chloro, fluoro, bromo, iodo, (C_1-C_2 alkylene)-O-(C_1-C_2 alkyl), (C_1-C_2 alkylene)-OH, or -S(C_1-C_4 alkyl);

each R^4 is, independently, hydrogen, (C_1-C_6 alkyl), fluoro, chloro, bromo, iodo, hydroxy, cyano, amino, (C_1-C_2 alkylene)-OH, CF_3, CH_2SCH_3, nitro, -O(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)(C_1-C_2 alkyl), -S(C_1-C_4 alkyl), -CO(C_1-C_4 alkyl), -C(=O)H or -C(=O)O(C_1-C_4alkyl);

R^6 is hydrogen, methyl or ethyl;

R^8 is hydrogen or C_1-C_4 alkyl;

R^5 is phenyl, pyridyl, pyrazinyl, pyrimidyl, pyridazinyl and wherein each of the foregoing R^5 groups is substituted with from one to four substituents R^{13} wherein one to three of said substituents may be selected, independently, from fluoro, chloro, C_1-C_6 alkyl and -O(C_1-C_6 alkyl) and one of said substituents may be selected from bromo, iodo, formyl, OH, (C_1-C_4 alkylene)-OH, (C_1-C_4alkylene)-O-(C_1-C_2 alkyl), -CN, -CF_3, -NO_2,

$-NH_2$, $-NH(C_1-C_4$ alkyl), $-N(C_1-C_2$ alkyl)$(C_1-C_6$ alkyl), $-OCO(C_1-C_4$ alkyl), $(C_1-C_4$ alkylene)$-O-(C_1-C_4$ alkyl), $-S(C_1-C_6$ alkyl), $(C_1-C_4$ alkylene$)-S-(C_1-C_4$ alkyl), $-C(=O)O(C_1-C_4$ alkyl), $-C(=O)(C_1-C_4$ alkyl), -COOH, $-SO_2NH(C_1-C_4$ alkyl), $-SO_2N(C_1-C_2$ alkyl$)(C_1-C_4$ alkyl), $-SO_2NH_2$, $-NHSO_2(C_1-C_4$ alkyl), $-S(C_1-C_6$ alkyl) and $-SO_2(C_1-C_6$ alkyl), and wherein each of the C_1-C_4 alkyl and C_1-C_6 alkyl moieties in the foregoing R^5 groups may optionally have one or two double bonds;

R^7 is hydrogen, C_1-C_4 alkyl, halo (e.g., chloro, fluoro, iodo or bromo), hydroxy, $-O(C_1-C_4$ alkyl), $-C(=O)(C_1-C_4$ alkyl), $-C(=O)O(C_1-C_4$ alkyl), $-OCF_3$, $-CF_3$, $-CH_2OH$ or $-CH_2O(C_1-C_2$ alkyl);

R^{10} is hydrogen, hydroxy, methoxy or fluoro;

R^{11} is hydrogen or C_1-C_4 alkyl; and

with the proviso that: a) when both J and K are carbons and D is CR^4 and E is nitrogen, then G can not be nitrogen; (b) when both J and K are carbons and D and G are nitrogens, then E can not be CR^4 or C=O or C=S; (c) when both J and K are carbons and D and E are carbons, then G can not be nitrogen; (d) when G is carbon, it must be double banded to E; and (e) in the ring containing J, K, D, E and G, there can not be two double bonds adjacent to each other;

and the pharmaceutically acceptable salts of such compounds.

2. Compounds according to claim 1 wherein B is $-NR^1R^2$, $-NHCHR^1R^2$, $-OCHR^1R^2$ and R^1 is C_1-C_6 alkyl, which may optionally be substituted with one fluoro, or C_1-C_4 alkoxy group and which may optionally contain one double or triple bond; and R^2 is C_1-C_4 alkyl or $(C_1-C_2$ alkyl$)$-CO-$(C_1-C_2$ alkyl) which may optionally contain one double or triple bond.

3. Compounds according to claim 1, wherein B is $-CHR^1R^2$, $-NR^1R^2$, $-NHCHR^1R^2$, $-OCHR^1R^2$, $-SCHR^1R^2$; and R^1 is C_1-C_6 alkyl, which may optionally be substituted with one hydroxy, fluoro, CF_3, cyclopropyl or C_1-C_4 alkoxy group and which may optionally contain one double or triple bond; and R^2 is benzyl or C_1-C_6 alkyl, which may optionally contain one double or triple bond, wherein said C_1-C_6 alkyl and the phenyl moiety of said benzyl may optionally be substituted with one fluoro, hydroxy, CF_3, cyclopropyl, C_1-C_2 alkyl, C_1-C_2 alkoxy or chloro group.

4. Compounds according to claim 1 wherein R^3 is methyl.

5. Compounds according to claim 1 wherein R^4, R^6, R^8, R^9, and R^{12} are hydrogen or methyl.

6. Compounds according to claim 1 wherein R^5 is di- or tri-substituted phenyl in which the two or three substitutents are independently selected from C_1-C_4 alkyl, O-(C_1-C_4 alkyl), (C_1-C_4 alkylene)-O-(C_1-C_4alkyl), CF_3, OCF_3, CHO, (C_1-C_4alkylene)-OH, cyano, chloro, fluoro, bromo and iodo, wherein each of the forgoing (C_1-C_4) alkyl groups may optionally contain one double or triple bond.

7. Compounds 1 wherein R^3 is methyl, ethyl, chloro or methoxy; and each of R^4, R^6, R^8, R^9, and R^{12} is, independently, hydrogen, methyl or ethyl.

8. Compounds wherein R^5 is di- or tri-substituted pyridyl, or pyrimidyl in which the two or three substitutents are independently selected from C_1-C_4 alkyl, O-(C_1-C_4 alkyl), (C_1-C_4 alkylene)-O-(C_1-C_4 alkyl), CF_3, OCF_3, CHO, (C_1-C_4 alkylene)-OH, cyano, chloro, fluoro, bromo and iodo, wherein each of the forgoing (C_1-C_4) alkyl groups may optionally contain one double or triple bond.

9. Compounds according to claim 1 wherein A is N, CH or CCH_3.

10. Compounds according to claim 1 wherein A is CH, J and K are carbon and D, E, and G are nitrogen.

11. Compounds according to claim 1 wherein J and D are nitrogen, and K and G are carbon, and E is CH, CCH_3 or CC_2H_5.

12. Compounds according to claim 1 wherein J and K are carbon, and D--EG is O-$C(CH_3)$=C, O-CH=C, S-$C(CH_3)$=C, S-CH=C, N(CH_3)-$C(CH_3)$=C, NHC(CH_3)=C, NHC(CH_3CH_2)=C, N(CH_3)-CH=C, O-N=C, S-N=C, N(CH_3)-N=C, O-CH_2N or S-CH_2N.

13. A compound according to claim 1 wherein B is -CHR^1R^2, -$NCHR^1R^2$ or -$OCHR^1R^2$, and the CHR^1R^2 group of B is a cyclopentane ring, a tetrahydrofuran ring or a tetrahydrothienyl ring.

14. A compound according to claim 1 wherein the NR^1R^2 group of B is a five membered saturated or unsaturated heterocyclic ring.

15. A compound according to claim 14 wherein the NR^1R^2 is a pyrrolo ring, a pyrrolidino ring, a thiazolidino ring or a morpholino ring.

16. A pharmaceutical composition for the treatment, prevention or inhibition of (a) a disorder the treatment of which can be effected or facilitated by antagonizing CRF, including but not limited to disorders induced or facilitated by CRF, or (b) a disorder selected from inflammatory disorders such as rheumatoid arthritis and osteoarthritis, pain, asthma, psoriasis and allergies; generalized anxiety disorder; panic;

phobias; obsessive-compulsive disorder; post-traumatic stress disorder; sleep disorders induced by stress; pain perception such as fibromyalgia; mood disorders such as depression, including major depression, single episode depression, recurrent, depression, child abuse induced depression, mood disorders associated with premenstrual syndrome, and postpartum depression; dysthemia; bipolar disorders; cyclothymia; chronic fatigue syndrome; stress-induced headache; cancer; irritable bowel syndrome, Crohn's disease; spastic colon; post operative ileus; ulcer;diarrhea; stress-induced fever; human immunodeficiency virus (HIV) infections; neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease; gastrointestinal diseases; eating disorders such as anorexia and bulimia nervosa; hemorrhagic stress; chemical dependencies and addictions (e.g., dependencies on alcohol, cocaine, heroin, benzodiazepines, or other drugs); drug and alcohol withdrawal symptoms; stress-induced psychotic episodes; euthyroid sick syndrome; syndrome of inappropriate antidiarrhetic hormone (ADH); obesity; infertility; head traumas; spinal cord trauma; ischemic neuronal damage (e.g., cerebral ischemia such as cerebral hippocampal ischemia); excitotoxic neuronal damage; epilepsy; stroke; immune dysfunctions including stress induced immune dysfunctions (e.g., porcine stress syndrome, bovine shipping fever, equine paroxysmal fibrillation, and dysfunctions induced by confinement in chickens, sheering stress in sheep or human-animal interaction related stress in dogs); muscular spasms; urinary incontinence; senile dementia of the Alzheimer's type; multiinfarct dementia; amyotrophic lateral sclerosis; hypertension; tachycardia; congestive heart failure; osteoporosis; premature birth; and hypoglycemia in a mammal, comprising an amount of a compound according to claim 1 that is effective in the treatment of such disorder, and a pharmaceutically acceptable carrier.

17. A method for the treatment, prevention or inhibition of (a) a disorder the treatment of which can be effected or facilitated by antagonizing CRF, including but not limited to disorders induced or facilitated by CRF, or (b) a disorder selected from inflammatory disorders such as rheumatoid arthritis and osteoarthritis, pain, asthma, psoriasis and allergies; generalized anxiety disorder; panic; phobias; obsessive-compulsive disorder; post-traumatic stress disorder; sleep disorders induced by stress; pain perception such as fibromyalgia; mood disorders such as depression, including major depression, single episode depression, recurrent premenstrual syndrome, and

postpartum depression; dysthemia; bipolar disorders; cyclothymia; chronic fatigue syndrome; stress-induced headache; cancer; irritable bowel syndrome, Crohn's disease; spastic colon; post operative ileus; ulcer; diarrhea; stress-induced fever; human immunodeficiency virus (HIV) infections; neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease; gastrointestinal diseases; eating disorders such as anorexia and bulimia nervosa; hemorrhagic stress; chemical dependencies and addictions (e.g., dependencies on alcohol, cocaine, heroin, benzodiazepines, or other drugs); drug and alcohol withdrawal symptoms; stress-induced psychotic episodes; euthyroid sick syndrome; syndrome of inappropriate antidiarrhetic hormone (ADH); obesity; infertility; head traumas; spinal cord trauma; ischemic neuronal damage (e.g., cerebral ischemia such as cerebral hippocampal ischemia); excitotoxic neuronal damage; epilepsy; stroke; immune dysfunctions including stress induced immune dysfunctions (e.g., porcine stress syndrome, bovine shipping fever, equine paroxysmal fibrillation, and dysfunctions induced by confinement in chickens, sheering stress in sheep or human-animal interaction related stress in dogs); muscular spasms; urinary incontinence; senile dementia of the Alzheimer's type; multiinfarct dementia; amyotrophic lateral sclerosis; hypertension; tachycardia; congestive heart failure; osteoporosis; premature birth; and hypoglycemia in a mammal, comprising administering to a subject in need of said treatment an amount of a compound according to claim 1, that is effective in treating such disorder.

18. A method of treating or preventing a disorder or condition, the treatment or prevention of which can be effected or facilitated by inhibiting CRH binding protein in a mammal, comprising administering to said mammal a CRH binding protein inhibiting amount of a compound according to claim 1.

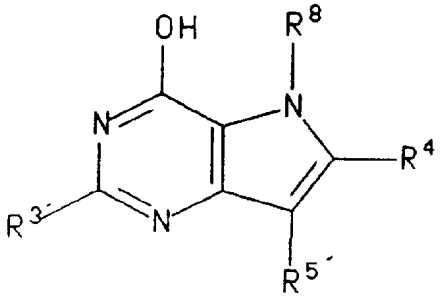
19. A pharmaceutical composition for treating or preventing a disorder or condition, the treatment or prevention of which can be effected or facilitated by inhibiting CRH binding protein in a mammal, comprising a CRH binding protein inhibiting amount of a compound according to claim 1 and a pharmaceutically acceptable carrier.

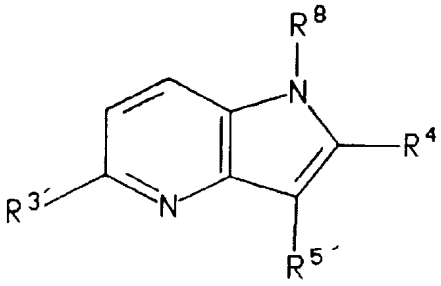
20. A compound according to claim 11 or 12 wherein A is N or CH, R^3 is methyl and each R^4, R^6, R^8, R^9 and R^{12} is, independently, hydrogen or methyl.

21. A compound according to claim 20, wherein R^5 is di- or tri-substituted phenyl, wherein the two or three substitutents are independently selected from C_1-C_4

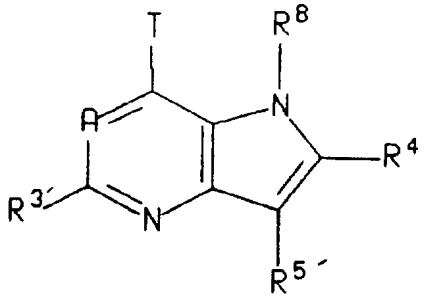
alkyl, O-(C_1-C_4 alkyl), (C_1-C_4 alkylene)-O-(C_1-C_4alkyl), CF_3, OCF_3, CHO, (C_1-C_4alkylene)-OH, cyano, chloro, fluoro, bromo and iodo, wherein each of the forgoing (C_1-C_4) alkyl groups may optionally contain one double or triple bond.

22. A compound of the formula





or



wherein $R^{3'}$ is C_1-C_4 alkyl, $R^{7'}$ is hydrogen, methyl, chloro, bromo, -COOH or -COO(C_1-C_4 alkyl), T is chloro, bromo, iodo or triflate, R^8 is hydrogen or C_1-C_4 alkyl and R^4 is hydrogen, (C_1-C_6 alkyl), fluoro, chloro, bromo, iodo, hydroxy, cyano, amino, (C_1-C_2 alkylene)-OH, CF_3, CH_2SCH_3, nitro, -O(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)(C_1-C_2 alkyl), -S(C_1-C_4 alkyl), -CO(C_1-C_4 alkyl), -C(=O)H or -C(=O)O(C_1-C_4alkyl);

23. A compound according to claim 1 wherein said compound is:

7-(1-ethyl-propoxy)-5-methyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidine;

[2,5-Dimethyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-(1-ethyl-propyl)-amine;

(1-Ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-amine;

7-(1-Ethyl-propoxy)-2,5-dimethyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidine;

[2,5-Dimethyl-3-(2,4,6-trimethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-ethyl-propyl-amine;

[6-Bromo-5-bromomethyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b]pyridin-7-yl]-(1-ethyl-propyl)-amine;

(1-Ethyl-propyl)-[5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b]pyridin-7-yl]-amine;

[6-Bromo-5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b]pyridin-7-yl]-(1-ethyl-propyl)-methyl-amine;

7-(1-Ethyl-propoxy)-5-methyl-3-(2,4,6-trimethyl-phenyl)-3H-[1,2,3]triazolo[4,5-b]pyridine;

4-(1-Ethyl-propoxy)-2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidine;

(±)-2,5-Dimethyl-4-(tetrahydro-furan-3-yloxy)-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo-[3,2-d]pyrimidine;

2,5-Dimethyl-4-(S)-(tetrahydro-furan-3-yloxy)-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo-[3,2-d]pyrimidine;

2,5-Dimethyl-4-(1-propyl-butoxy)-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidine; or

4-sec-Butylsulfanyl-2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-5H-pyrrolo[3,2-d]pyrimidine;

or a pharmaceutically acceptable salt of such compound.

INTERNATIONAL SEARCH REPORT

Intern. ial Application No
PCT/IB 97/00922

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D487/04 C07D471/04 A61K31/505 A61K31/435
//(C07D487/04,239:00,231:00),(C07D471/04,249:00,221:00),
(C07D487/04,239:00,209:00)

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 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 33750 A (PFIZER) 14 December 1995 see claims 1,13 ---	1,16
P,X	WO 96 35689 A (NEUROGEN) 14 November 1996 see page 3, line 18 - line 25; claim 1; examples IE,II,ID -----	1,16,22

☐ 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
27 October 1997	04. 11. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Alfaro Faus, I

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

INTERNATIONAL SEARCH REPORT

In ational application No.
PCT/IB 97/00922

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 17 and 18
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr. ial Application No
PCT/IB 97/00922

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9533750 A	14-12-95	AU 2453095 A	04-01-96
		BR 9502708 A	30-04-96
		EP 0764166 A	26-03-97
		FI 964894 A	05-12-96
		HU 75774 A	28-05-97
		NO 965237 A	06-02-97
WO 9635689 A	14-11-96	US 5644057 A	01-07-97
		AU 5679096 A	29-11-96
		CA 2194756 A	14-11-96
		EP 0770080 A	02-05-97

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 February 2022 (17.02.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/036123 A1

(51) International Patent Classification:
A61B 18/14 (2006.01) *A61B 18/04* (2006.01)
A61B 18/02 (2006.01)

(21) International Application Number:
PCT/US2021/045780

(22) International Filing Date:
12 August 2021 (12.08.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/064,863 12 August 2020 (12.08.2020) US

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

(72) Inventors: **BARNES, Christopher**; 548 Market Street, Suite 74589, San Francisco, California 94105 (US). **KARPF, David**; 548 Market Street, Suite 74589, San Francisco, California 94105 (US). **NOOR, Mustafa**; 548 Market Street, Suite 74589, San Francisco, California 94105 (US).

(74) Agent: **LAMIQUIZ, Jiarong L.**; WILSON SONSINI, 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, IT, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, 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).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(54) **Title:** METHODS AND COMPOSITIONS FOR TREATING POLYCYSTIC OVARY SYNDROME

(57) **Abstract:** Polycystic ovary syndrome (PCOS) is characterized by elevated levels of androgens, cysts in the ovaries, and irregular periods. Women with PCOS present with additional symptoms, including hirsutism, alopecia, acne, infertility, weight gain, fatigue, depression and mood changes. The present disclosure provides new compounds, salts, compositions and uses thereof in the treatment of PCOS due to elevated adrenal androgens. Further, the present disclosure provides methods for treating PCOS due to elevated adrenal androgens.

WO 2022/036123 A1

METHODS AND COMPOSITIONS FOR TREATING POLYCYSTIC OVARY SYNDROME

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/064,863, filed August 12, 2020; which is referenced herein in its entirety.

BACKGROUND

[0002] Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders affecting an estimated 12% of reproductive aged women. Women with PCOS may have polycystic ovarian morphologic features, ovulatory dysfunction in the form of infrequent or prolonged menstrual periods, and hyperandrogenism in the form of excess male hormone levels. This complex disorder has both environmental influences, such as obesity and insulin resistance, and inappropriate endocrine signaling from the hypothalamus and pituitary glands as contributors. Hyperandrogenism presents clinically as hirsutism and acne and biochemically with elevated serum androgen levels. Androgen and androgen precursors can be produced and secreted by the ovaries and also by the adrenal cortices in about the same amount in response to stimulation by pituitary derived luteinizing hormone (LH) to the ovaries and adrenocorticotropic hormone (ACTH) to the adrenal gland, respectively. Emerging data indicate that PCOS patients can be categorized according to whether the source of excessive androgen is primarily from the ovaries, namely, functional ovarian hyperandrogenism (FOH), or the adrenal glands, namely, functional adrenal hyperandrogenism (FAH), both (FOH and FAH), or neither and attributable to either insulin resistance /obesity or of unknown origin. A majority or about two-thirds of cases have functionally typical PCOS (PCOS-T) that is due to typical FOH, characterized by hyperresponsiveness of 17-hydroxyprogesterone (17-OHP). About one fifth of cases have functionally atypical FOH (PCOS-A), lacking 17-OHP hyperresponsiveness. Within these two FOH populations, about one-third may have both FOH and FAH. About 8% of PCOS cases are attributed to either obesity and the remainder are unknown or idiopathic in nature. Only about 3-5% PCOS cases are due to isolated FAH with androgen responsiveness to ACTH.

SUMMARY

[0003] Adrenal androgen excess in PCOS appears to occur independently of ovarian androgen excess, suggesting it may represent an intrinsic, and possible primary source of abnormal synthesis of adrenal androgens. Adrenal androgen excess of PCOS may not result from deficiencies in specific enzymatic steps, rather it may represent an altered pituitary responsivity to corticotropin releasing factor (CRF) and adrenocorticotropic hormone (ACTH).

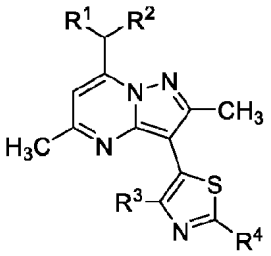
[0004] In an aspect, the present disclosure provides methods for treating polycystic ovary syndrome with functional ovarian hyperandrogenism and functional adrenal hyperandrogenism (PCOS-FOH + FAH) in a subject in need thereof, comprising administering a CRF1 antagonist or pharmaceutically acceptable salt thereof.

[0005] In another aspect, the present disclosure provides methods for treating polycystic ovary syndrome with functional adrenal hyperandrogenism (PCOS-FAH) in a subject in need thereof, comprising administering a CRF1 antagonist or pharmaceutically acceptable salt thereof.

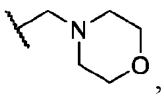
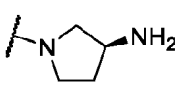
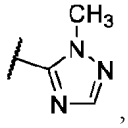
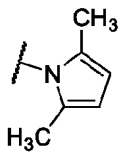
[0006] In another aspect, the present disclosure provides methods for treating polycystic ovary syndrome (PCOS) in a subject in need thereof, comprising administering a CRF1 antagonist or pharmaceutically acceptable salt thereof.

[0007] In another aspect, the present disclosure provides methods for treating polycystic ovary syndrome (PCOS) in a subject in need thereof, comprising accessing a source of excessive androgen; and administering a CRF1 antagonist or pharmaceutically acceptable salt thereof. In some embodiments, the cosyntropin is administered at a dose about 1 $\mu g/m^2$. In some embodiments, the cosyntropin is administered at a dose about 10 $\mu g/m^2$. In some embodiments, the cosyntropin is administered at a dose about 250 $\mu g/m^2$. In some embodiments, the source of excessive androgen is adrenal glands. In some embodiments, the source of excessive androgen is ovaries.

[0008] In some embodiments, the CRF_1 antagonist is a compound of Formula (I):

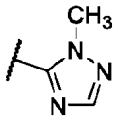


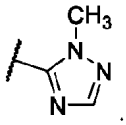
Formula (I)

or a pharmaceutically acceptable salt thereof, wherein: R^1 and R^2 are independently ethyl or *n*-propyl; R^3 is hydrogen, F, Cl, Br, methyl, trifluoromethyl, or methoxy; R^4 is hydrogen, Br, $-NR^aR^b$, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , , , or ; R^a and R^b are independently hydrogen, C_1-C_3alkyl, $-CH_2CH_2NH_2$, $-CH_2CH_2NHC(O)OC(CH_3)_3$, or $-CH_2CH_2NHCH_2CH_2CH_3$.

[0009] In some embodiments, R^3 is F, Cl, Br, methyl, or trifluoromethyl.

[0010] In some embodiments, R^3 is Cl or Br.

[0011] In some embodiments, R^4 is $-NR^aR^b$, pyridin-4-yl, morpholin-4-yl, or .

[0012] In some embodiments, R^4 is morpholin-4-yl or .

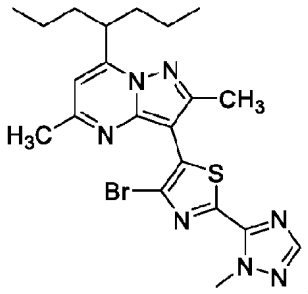
[0013] In some embodiments, R^4 is $-NR^aR^b$, and R^a and R^b are independently C_1-C_3alkyl.

[0014] In some embodiments, the CRF_1 antagonist or a pharmaceutically acceptable salt thereof is 3-[4-bromo-2-(2-methyl-2*H*-[1,2,4]triazol-3-yl)-thiazol-5-yl]-2,5-dimethyl-7-(1-propyl-butyl)- pyrazolo[1,5-*a*]pyrimidine.

[0015] In some embodiments, the CRF_1 antagonist or a pharmaceutically acceptable salt thereof is 3-(4-bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine.

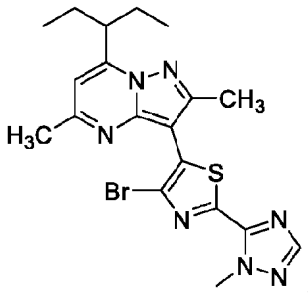
[0016] In some embodiments, the CRF_1 antagonist or a pharmaceutically acceptable salt thereof is 3-(4-chloro-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine.

[0017] In some embodiments, the CRF_1 antagonist is



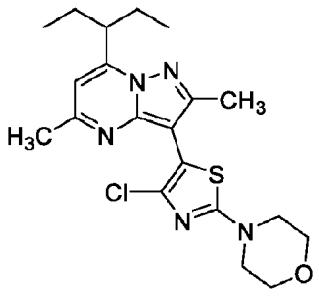
, or a pharmaceutically acceptable salt thereof.

[0018] In some embodiments, the CRF_1 antagonist is



, or a pharmaceutically acceptable salt thereof.

[0019] In some embodiments, the compound is



, or a pharmaceutically acceptable salt thereof.

[0020] In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt thereof is selected from the group consisting of: Antalarmin hydrochloride, CP-154,526, CP-376395 hydrochloride, NBI 27914 hydrochloride, NBI 35965 hydrochloride, NGD 98-2 hydrochloride, Pexacerfont, R 121919 hydrochloride, SSR125543 (crinecerfont), and SN003.

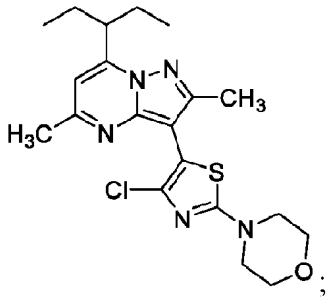
[0021] In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 5 mg to about 400 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 300 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 200 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 150 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 100 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 75 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 50 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 25 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 10 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt thereof is administered as a pharmaceutical composition.

[0022] In some embodiments, an adrenocorticotropic hormone (ACTH) level is reduced by at least 5% from baseline. In some embodiments, an adrenocorticotropic hormone (ACTH) level is reduced by at least 10% from baseline. In some embodiments, a dehydroepiandrosterone sulfate (DHEAS) level is reduced by at least 5% from baseline. In some embodiments, a dehydroepiandrosterone sulfate (DHEAS) level is reduced by at least 10% from baseline. In some embodiments, the androstenedione (A4) level is reduced by at least 5% from baseline. In some embodiments, the androstenedione (A4) level is reduced by at least 10% from baseline. In some embodiments, the 1β-hydroxyandrostenedione (11OHA4) level is reduced by at least 5%

from baseline. In some embodiments, the 1β-hydroxyandrostenedione (11OHA4) level is reduced by at least 10% from baseline. In some embodiments, the 11β-hydroxytestosterone (11OHT) level is reduced by at least 5% from baseline. In some embodiments, the 11β-hydroxytestosterone (11OHT) level is reduced by at least 10% from baseline.

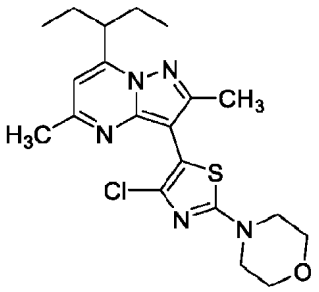
[0023] In some embodiments, a reduced ACTH, DHEAS, A4, 11OHA4 or 11OHT level from baseline is maintained for at least 24 hours. In some embodiments, a reduced ACTH, DHEAS, A4, 11OHA4 or 11OHT level from baseline is maintained for at least 4 weeks.

[0024] In another aspect, the present disclosure provides methods for treating polycystic ovary syndrome with functional adrenal hyperandrogenism (PCOS-FAH) in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 3:



; or a pharmaceutically acceptable salt or solvate thereof.

[0025] In another aspect, the present disclosure provides methods for treating polycystic ovary syndrome with functional ovarian hyperandrogenism and functional adrenal hyperandrogenism (PCOS-FOH+FAH) in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 3:



; or a pharmaceutically acceptable salt or solvate thereof.

[0026] In some embodiments, the Compound 3 is administered at a dose between about 1 mg/day and about 400 mg/day.

[0027] In some embodiments, the pharmaceutical composition comprises about 25 mg of Compound 3, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 50 mg of Compound 3, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises between about 75 mg of Compound 3, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises about 100 mg of Compound 3, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments,

the pharmaceutical composition comprises about 200 mg of Compound 3, or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition comprises between about 1 mg and about 300 mg of Compound 3, or a pharmaceutically acceptable salt or solvate thereof.

[0028] In some embodiments, the pharmaceutical composition is in the form of microparticles. In some embodiments, the average size of the microparticles is between about 1 μm to about 20 μm. In some embodiments, the average size of the microparticles is between about 5 μm to about 15 μm. In some embodiments, the average size of the microparticles is less than about 10 μm.

[0029] In some embodiments, the pharmaceutical composition is in the form of a capsule or a tablet. In some embodiments, the capsule is a hard gelatin capsule. In some embodiments, the capsule is a soft gelatin capsule.

[0030] 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. 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.

[0031] In some embodiments, the subject is a pediatric patient. In some embodiments, the subject is from about 9 years old to about 18 years old. In some embodiments, the subject is from about 8 years of age to about 55 years of age. In some embodiments, the subject is an adult patient.

[0032] In some embodiments, the subject is treated for a period of about 2 weeks to about 36 weeks. In some embodiments, the subject is treated for a period of about 1 month to 12 months. In some embodiments, the subject is treated for a period of about 10 months to 50 years.

[0033] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0034] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

DETAILED DESCRIPTION

[0035] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

Polycystic Ovary Syndrome (PCOS)

[0036] Polycystic ovarian syndrome (PCOS) is a heterogeneous disorder with multiple phenotypes and is one of the most common endocrine metabolic disorders in reproductive aged women. PCOS was first diagnosed and described as a syndrome of oligo-amenorrhea and polycystic ovaries that was variably accompanied by hirsutism, acne, and obesity. There are various diagnostic criteria for adolescent and adult patients considering the core diagnostic features, such as hyperandrogenism, persistent ovulatory dysfunction, and polycystic ovarian morphology (PCOM). The exact etiology of this disorder is not entirely clear, however, both genetic and environmental factors seem to play roles in causing the disorder. Generally, PCOS presents as a phenotype reflecting a self-perpetuating vicious cycle involving neuroendocrine, metabolic, and ovarian dysfunction and is characterized by excessive ovarian and/or adrenal androgen secretion. For example, intrinsic ovarian factors such as altered steroidogenesis and factors external to the ovary such as hyperinsulinemia contribute to the excessive ovarian androgen production. The classic ovarian phenotype of enlarged ovaries with string-of-pearl morphology and theca interstitial hyperplasia reflects androgen exposure; this morphology has also been observed in women with congenital adrenal hyperplasia (CAH).

[0037] In order to attempt to understand the pathophysiology of PCOS, it is important to understand the molecular basis of steroidogenesis and androgen source, production and physiology. Under normal circumstances in women, the ovaries and adrenal glands contribute about equally to testosterone production. Approximately half of testosterone originates from direct testosterone secretion by the ovaries and adrenal glands, whereas half is produced by

peripheral conversion of circulating androstenedione, which itself arises from approximately equal ovarian and adrenal secretion. Androgen production is not under direct negative feedback regulation by the neuroendocrine system in females, as is the case for estradiol and cortisol secretion. Androgens are secreted by both the ovaries and adrenal glands in response to their respective tropic hormones, LH and ACTH. The zona reticularis of the adrenal gland resembles the theca cell compartment of the ovary in its expression of the core enzymatic pattern for androgen production.

[0038] PCOS patients can be functionally categorized as distinct subtypes based on the source of androgen excess listed in Table 1 below. Androgen excess can be derived from the ovaries, the adrenal glands, both, or neither. Primary PCOS is the most common variety of PCOS without any known cause and a proposed theory suggests that functional ovarian hyperandrogenism (FOH) along with disturbance of hypothalamo-pituitary-ovarian (HPO) axis function may be the underlying cause. Further, functional adrenal hyperandrogenism (FAH) other than HPO axis disturbance may be explained for a certain population of PCOS patients. The PCOS patients with primary FAH may experience an average 50% increase in adrenal volume that correlates with hyperandrogenemia severity.

[0039] Table 1 Functional classification of PCOS based on source of androgen excess

PCOS Functional Type	Source of Androgen	GnRHag Test 17OHP Response	DAST Testosterone Response	ACTH test DHEA Response	Prevalence Among PCOS
PCOS-T	Primary FOH (typical FOH)	High[a]	High in 92.5%	High in 28% (associated FAH)	67%[c]
PCOS-A	Primary FOH (atypical FOH)	Normal[b]	High	High in 30% (associated FAH)	20%
	Primary FAH (isolated FAH)	Normal	Normal	High	5%
	PCOS without FOH or FAH (PCOS-A of obesity or idiopathic PCOS-A)	Normal	Normal	Normal	8%

[0040] Test procedures to determine the source of androgen are listed below in Table 2. The ovarian hyperandrogenism of PCOS is demonstrated directly by the GnRHag test or the hCG test and in combination with the dexamethasone androgen-suppression test (DAST). The GnRHag test determines the coordinated function of the ovarian follicle. Leuprolide acetate 10 µg/kg sc (or a comparable dose of any other short-acting GnRHag) stimulates endogenous LH and FSH release that peaks at 3–4 hours and persists for 24 hours; this in turn stimulates increased secretion of sex steroids and their precursors, with serum levels peaking at 18–24 hours. In the absence of evidence of a steroidogenic block, an elevated 17OHP response is typical of PCOS. Ovarian steroidogenic enzyme deficiency, which is rare, can be detected by an abnormal pattern of steroid intermediates in response to the test. hCG is an LH analog: 5000 IU intramuscularly stimulates steroidogenic responses comparable with those of a GnRHag test at 24 hours. DAST indirectly tests for ovarian source of androgens by suppressing ACTH-dependent androgen production of androgens. In the presence of normal adrenocortical

suppression an inappropriately elevated serum testosterone post-DAST indicates an ACTH-independent source of androgen, which is ordinarily of ovarian origin.

[0041] Adrenal hyperandrogenism may be demonstrated by a rapid ACTH test: cosyntropin is administered iv and peak steroid responses occur at 15–60 minutes. An elevated DHEA post this test indicates an ACTH-dependent source of androgen, which is ordinarily of adrenal origin. The test may be performed using cosyntropin 250 μg, this is a supramaximal dose. Lower doses of cosyntropin may be used (10 μg/m2) which elicit a similar peak response. A low-dose ACTH test (1.0-μg cosyntropin) may be used and may be more physiologic. It usually elicits nearly as great a peak response that promptly wanes, and in PCOS, does not elicit such a wide spectrum of elevated steroid intermediates as do larger doses.

[0042] Table 2 Methods to determine source of female androgen excess

Test	Rationale	Method	Outcome Measures	Interpretation*
GnRHag	Endogenous LH and FSH release stimulates coordinated function of ovarian follicles	Leuprolide acetate 10 μg/kg sc (for maximum stimulation)	Ovarian steroid secretion peaks at 20–24 h	17OHP >152 ng/dL without steroidogenic block indicates typical FOH (PCOS-T)
hCG	Exogenous administration of LH analog stimulates theca-interstitial cells	hCG 3000 IU/m² (for maximum stimulation)	Ovarian steroid secretion peaks at 24 h	17OHP >152 ng/dL without steroidogenic block indicates typical FOH (PCOS-T)
LDAST	Long DAST: dexamethasone profoundly suppresses adrenal androgens over several days	Dexamethasone 0.5 mg QID per os × 4–5 d	Free testosterone, DHEAS, cortisol; sample early morning @ 5	Free testosterone ≥5 pg/mL with DHEAS <70 and cortisol <1 μg/dL characteristic of FOH
SDAST	Short DAST: dexamethasone rapidly suppresses adrenal testosterone and cortisol	Dexamethasone 0.25 mg/m² per os at 12 noon	Total testosterone, cortisol sample 4 PM (4 h)	Total testosterone >26 ng/mL, cortisol <5 μg/dL suggests FOH
ACTH	Exogenous ACTH stimulates adrenal steroidogenesis	Cosyntropin ≥10 μg/m² (for maximum stimulation)	DHEA,17OHP, steroid intermediates, cortisol peak at 30–60 min	DHEA 1500–3000 μg/dL without steroidogenic block indicates FAH

Corticotropin releasing factor

[0043] Currently, no single universal treatment for PCOS is available. As a result, current treatments tend to be individualized and adapted to the actual needs of the individual patient. Furthermore, treatment may be symptom-oriented. For example, targets for pharmacological treatment may include androgen excess. For PCOS patients with elevated adrenal androgens, FOH+FAH and PCOS-FAH patients, in some embodiments, pharmacological treatment, such as corticotropin releasing factor (CRF) receptor antagonist targeting ACTH production may be used.

[0044] 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.

[0045] 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.

[0046] 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.

[0047] 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, and congenital adrenal hyperplasia.

[0048] 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. The middle layer, the zona reticularis is responsible for the production of androgens such as DHEA, DHEAS and androstenedione, (A4) the precursor to testosterone (T) and dihydrotestosterone (DHT). This layer is also responsible for the production of the 11-oxyandrogens, 11β-hydroxyandrostenedione (11OHA4) and 11β-hydroxytestosterone (11OHT), which are major bioactive androgens, particularly in women. Due to an unknown cause in PCOS patients with elevated androgens, the zone reticularis displays a hyperresponsiveness to ACTH resulting excessive levels of DHEA, DHEAS, 11OHA4, 11OHT and A4. The excessive ACTH stimulation causes hypertrophy of the zona reticularis resulting in

adrenal hyperplasia and clinically manifests with physical features typical of PCOS and hyperandrogenism. Reducing the stimulatory effect on the pituitary's ACTH secretion by inhibiting the CRF1 receptor is expected is reduce excessive androgen synthesis from the ACTH responsive zone of the adrenal gland in PCOS. The normalization of elevated androgen levels in the short term would be expected to ameliorate cardinal features of PCOS - hirsutism, acne and menstrual irregularities – over the long term.

Certain Definitions

[0049] 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.

[0050] 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.

[0051] The term "about" is used to indicate that a value includes 10% level of error for the device or method being employed to determine the value.

[0052] 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."

[0053] "Alkyl" refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, containing no unsaturation, and preferably having from one to five carbon atoms (i.e. C_1-C_5alkyl). In some embodiments, an alkyl comprises one to four carbon atoms (i.e., C_1-C_4alkyl).). In some embodiments, an alkyl comprises one to three carbon atoms (i.e., C_1-C_3alkyl).). In some embodiments, an alkyl comprises one to two carbon atoms (i.e., C_1-C_2alkyl). In some embodiments, an alkyl comprises one carbon atom (i.e., C_1alkyl). In certain embodiments, the alkyl group is selected from methyl, ethyl, 1-propyl (*n*-propyl), 1-methylethyl (iso-propyl), 1-butyl (*n*-butyl), 1-methylpropyl (sec-butyl), 2-methylpropyl (isobutyl), 1,1-dimethylethyl (tert-butyl), or 1-pentyl (n-pentyl). The alkyl is attached to the rest of the molecule by a single bond. Unless stated otherwise specifically in the specification, an alkyl group is optionally substituted by one or more substituents such as those described herein.

[0054] 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.

[0055] "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.

[0056] 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.

[0057] The term "pharmaceutical composition" means a composition comprising at least one active ingredient, such as a steroid or a pharmaceutically acceptable salt thereof or a CRF_1 antagonist or a pharmaceutically acceptable salt thereof, 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.

[0058] The term "supraphysiologic amount" describes glucocorticoid dose levels that are above the daily glucocorticoid requirement (production rate) found in healthy individuals.

[0059] The term "physiologic amount" describes glucocorticoid dose levels that meet the daily glucocorticoid requirement (production rate) found in healthy individuals.

[0060] The term "hydrocortisone equivalents" as used herein is understood by a person skilled in the art to be the conversion calculations needed to be considered when substituting one glucocorticoid for another as the potency and duration of action of various glucocorticoids may vary. Hence, the term "hydrocortisone equivalents" is the standard used for comparison of glucocorticoid potency.

[0061] 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).

[0062] 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.

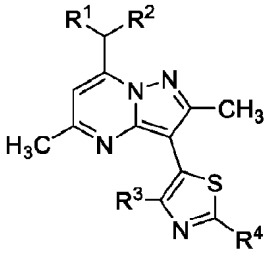
Compounds

[0063] Disclosed herein are CRF_1 antagonists or pharmaceutically acceptable salt thereof such as Antalarmin hydrochloride, CP-154,526, CP-376395 hydrochloride, NBI 27914 hydrochloride, NBI 35965 hydrochloride, NGD 98-2 hydrochloride, Pexacerfont, R 121919 hydrochloride, SN003, and SSR125543 (crinecerfont).

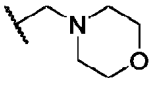
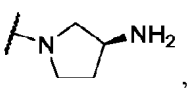
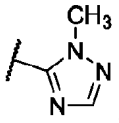
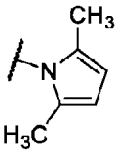
[0064] In one aspect, the CRF_1 antagonist or pharmaceutically acceptable salt thereof is selected from the group consisting of: *n*-butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine hydrochloride (Antalarmin hydrochloride), *n*-butyl-N-ethyl-2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine hydrochloride (Pfizer CP154526), N-(1-Ethylpropyl)-3,6-dimethyl-2-(2,4,6-trimethylphenox y)-4-pyridinamine hydrochloride (Pfizer CP376395 hydrochloride), 5-Chloro-N-(cyclopropylmethyl)-2-methyl-*n*-propyl-N'-(2,4,6-trichlorophenyl)-4,6-pyrimidinediamine hydrochloride (NBI27914 hydrochloride), (7S)-6-(Cyclopropylmethyl)-2-(2,4-dichlorophenyl)-7-ethyl-7,8-dihydro-4-methyl-6H-1,3,6,8a-tetraazaacenaphthylene hydrochloride (NBI35965 hydrochloride), N-(1-Ethylpropyl)-3-methoxy-5-[2-methoxy-4-

(trifluoromethoxy)phenyl]-6-methyl-2-pyrazinamine hydrochloride (NGD 98-2 hydrochloride), 8-(6-Methoxy-2-methyl-3-pyridinyl)-2,7-dimethyl-N-[(1R)-1-methylpropyl]pyrazolo[1,5-a]-1,3,5-triazin-4-amine (Pexacerfont), 3-[6-(Dimethylamino)-4-methyl-3-pyridinyl]-2,5-dimethyl-N,N-dipropylpyrazolo[1,5-a]pyrimidin-7-amine hydrochloride (R 121919 hydrochloride), and N-(4-Methoxy-2-methylphenyl)-1-[1-(methoxymethyl)propyl]-6-methyl-1H-1,2,3-triazolo[4,5-c]pyridine-4-amine (SN003), (S)-4-(2-chloro-4-methoxy-5-methylphenyl)-N-(2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl)-5-methyl-N-(prop-2-yn-1-yl)thiazol-2-amine (SSR125543).

[0065] Disclosed herein is a compound of Formula (I):



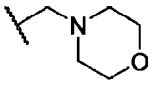
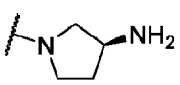
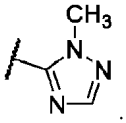
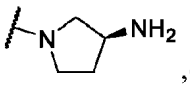
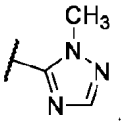
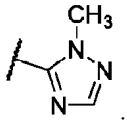
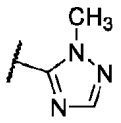
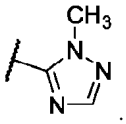
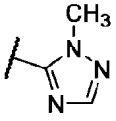
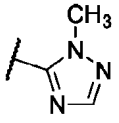
Formula (I)

or a pharmaceutically acceptable salt thereof, wherein R^1 and R^2 are independently ethyl or *n*-propyl; R^3 is H, Cl, Br, methyl, trifluoromethyl, or methoxy; R^4 is H, Br, $-NR^aR^b$, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , , , or ; and

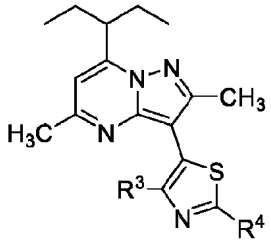
R^a and R^b are independently hydrogen, C_1-C_3alkyl, $-CH_2CH_2NH_2$, $-CH_2CH_2NHC(O)OC(CH_3)_3$, or $-CH_2CH_2NHCH_2CH_2CH_3$.

[0066] In some embodiments, R^1 and R^2 are independently ethyl or *n*-propyl. In some embodiments, R^1 is ethyl and R^2 is *n*-propyl. In some embodiments, R^1 is *n*-propyl and R^2 is ethyl. In some embodiments, R^1 and R^2 are both ethyl. In some embodiments, R^1 and R^2 are both *n*-propyl.

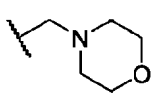
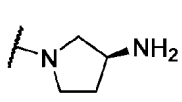
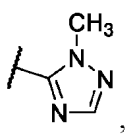
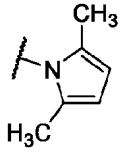
[0067] In some embodiments, R^3 is hydrogen, Cl, Br, methyl, or trifluoromethyl. In some embodiments, R^3 is hydrogen, Cl, Br, or methyl. In some embodiments, R^3 is hydrogen, Cl, or Br. In some embodiments, R^3 is hydrogen. In some embodiments, R^3 is Cl. In some embodiments, R^3 is Br.

[0068] In some embodiments, R^4 is hydrogen, Br, $-NR^aR^b$, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , ,or . In some embodiments, R^4 is Br, $-NR^aR^b$, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, ,or . In some embodiments, R^4 is $-NR^aR^b$, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, or . In some embodiments, R^4 is $-NR^aR^b$, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, or . In some embodiments, R^4 is $-NR^aR^b$,pyridin-4-yl, morpholin-4-yl, or . In some embodiments, R^4 is morpholin-4-yl or . In some embodiments, R^4 is morpholin-4-yl. In some embodiments, R^4 is . In some embodiments, R^4 is $-NR^aR^b$ and R^a and R^b are independently C_1-C_3alkyl.

[0069] Disclosed herein is a compound of Formula (II):

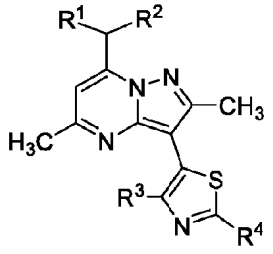


Formula (II)

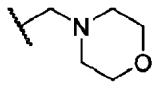
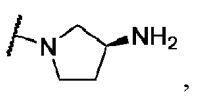
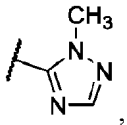
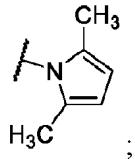
or a pharmaceutically acceptable salt thereof, wherein R^3 is H, Cl, Br, methyl, trifluoromethyl, or methoxy; R^4 is H, Br, $-NR^aR^b$, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , , , or ; and R^a and R^b are independently

hydrogen, C_1-C_3alkyl, $-CH_2CH_2NH_2$, $-CH_2CH_2NHC(O)OC(CH_3)_3$, or $-CH_2CH_2NHCH_2CH_2CH_3$–$CH_2CH_2NHCH_2CH_2CH_3$.

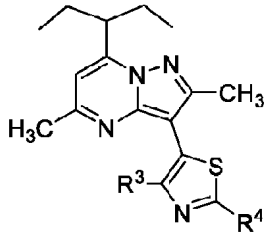
[0070] Disclosed herein is a compound of Formula (III):



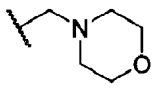
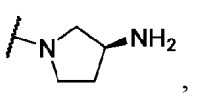
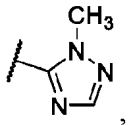
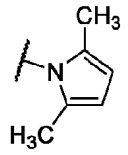
Formula (III)

or a pharmaceutically acceptable salt thereof, wherein R^1 and R^2 are *n*-propyl; R^3 is H, Cl, Br, methyl, trifluoromethyl, or methoxy; R^4 is H, Br, $-NR^aR^b$, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , , , or ; and R^a and R^b are independently hydrogen, C_1-C_3alkyl, $-CH_2CH_2NH_2$, $-CH_2CH_2NHC(O)OC(CH_3)_3$, or $-CH_2CH_2NHCH_2CH_2CH_3$.

[0071] Disclosed herein is a compound of Formula (IV):

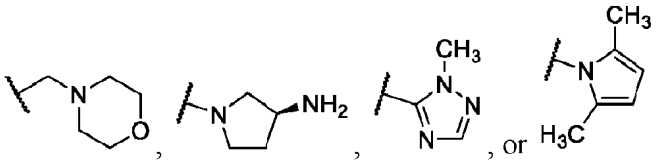
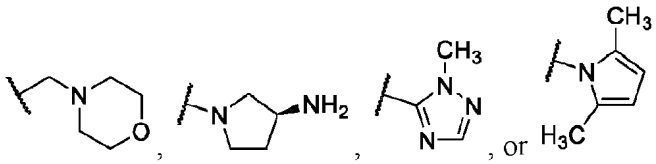
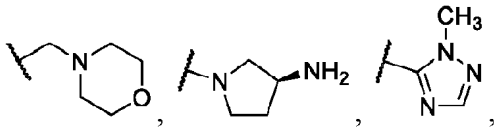
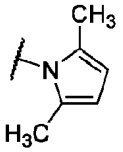
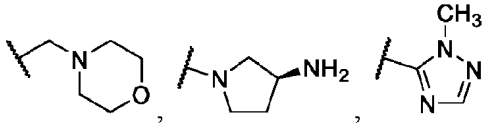
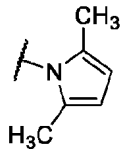
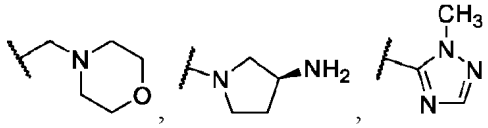


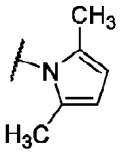
Formula IV

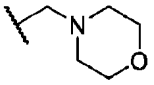
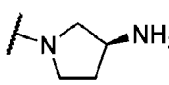
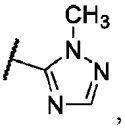
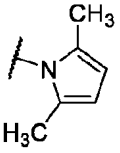
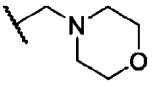
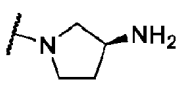
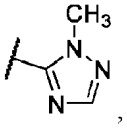
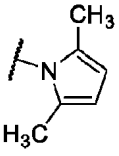
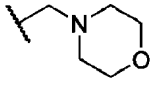
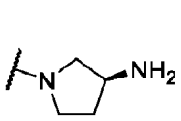
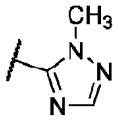
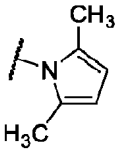
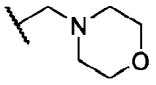
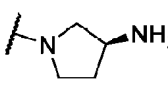
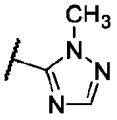
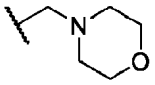
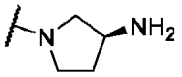
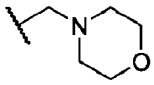
or a pharmaceutically acceptable salt thereof, wherein R^3 is Cl, Br, methyl, trifluoromethyl, or methoxy; R^4 is H, Br, $-NR^aR^b$, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , , , or ; and R^a and R^b are independently hydrogen, C_1-C_3alkyl, $-CH_2CH_2NH_2$, $-CH_2CH_2NHC(O)OC(CH_3)_3$, or $-CH_2CH_2NHCH_2CH_2CH_3$.

[0072] For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^3 may be hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy. For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^3 may be Cl, Br, methyl, trifluoromethyl, or methoxy. For a compound or pharmaceutically acceptable salt of Formula

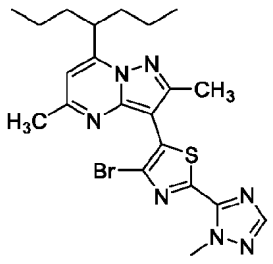
(I), (II), (III), and (IV), R^3 may be Cl, Br, methyl, or trifluoromethyl. For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^3 may be Cl, Br, or methyl. For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^3 may be Cl. For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^3 may be Br. For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^3 may be methyl.

[0073] For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^4 is Br, $-NR^aR^b$, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^4 is Br, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^4 is Br, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , or . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^4 is Br, acetamido, pyridin-4-yl, morpholin-4-yl, , or . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^4 is Br, pyridin-4-yl, morpholin-4-yl, , or



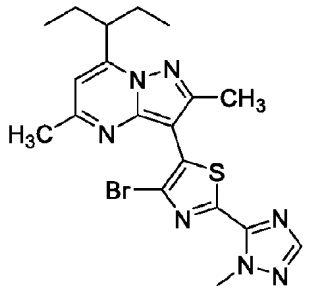
. For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^4 is pyridin-4-yl, morpholin-4-yl, , , , or . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^4 is morpholin-4-yl, , , , or . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^4 is , , , or . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^4 is , , or . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^4 is , or . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^4 is . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R^4 is morpholin-4-yl.

[0074] Disclosed herein is 3-[4-bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl]-2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-a]pyrimidine (Compound 1) or a pharmaceutically acceptable salt thereof:



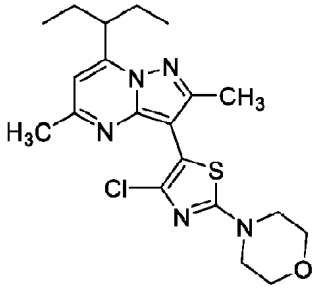
Compound 1.

[0075] Disclosed herein is 3-(4-bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine (Compound 2) or a pharmaceutically acceptable salt thereof:



Compound 2.

[0076] 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) (Compound 3) or a pharmaceutically acceptable salt thereof:



Compound 3.

[0077] 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 3. 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 3.

[0078] In one aspect, the CRF_1 antagonist or pharmaceutically acceptable salt thereof may be an astressin. An astressin generally refers to a nonselective corticotropin releasing hormone antagonist that reduces the synthesis of ACTH and cortisol.

Pharmaceutical Compositions

[0079] Disclosed herein are pharmaceutical compositions comprising a compound or pharmaceutically acceptable salt described herein. In certain embodiments, the composition comprises a steroid or a pharmaceutically acceptable salt thereof and a CRF_1 antagonist or pharmaceutically acceptable salt thereof as disclosed above. In some embodiments, the steroid or a pharmaceutically acceptable salt thereof is exogenous glucocorticoids (GC) or a pharmaceutically acceptable salt thereof.

Dosage Form

[0080] 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 a compound or pharmaceutically acceptable salt described herein 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.

[0081] 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.

[0082] 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

[0083] 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.

[0084] 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.

[0085] In some embodiments, the capsule comprises one or more pharmaceutically acceptable excipients. In some embodiments, the capsule is free of additional excipients.

[0086] 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

[0087] 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.

[0088] In some embodiments, the pharmaceutical composition is formulated as a tablet.

[0089] 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 a compound or pharmaceutically acceptable salt described herein 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 a compound or pharmaceutically acceptable salt described herein, 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 a compound or pharmaceutically acceptable salt described herein, 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 a compound or pharmaceutically acceptable salt described herein. 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.

[0090] 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, less than about 200 mg, less than about 100 mg or less than 50 mg. In some embodiments, the tablet has a dose strength of more than about 10 mg, 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 100 mg. In some

embodiments, the tablet size is less than 200 mg for a dose strength of 50 mg. In some embodiments, the tablet size is less than 50 mg for a dose strength of 10 mg.

[0091] 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 about 90% of the tablet is dissolved in less than 60 minutes in conventional dissolution media. In some embodiments, more than about 100% of the tablet is dissolved in less than 60 minutes in conventional dissolution media.

[0092] In some embodiments, the tablet is produced at a commercial scale.

[0093] In some embodiments, the tablet comprises one or more pharmaceutically acceptable excipients.

[0094] 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 a compound or pharmaceutically acceptable salt described herein. 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

[0095] 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.

[0096] 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

[0097] In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 500 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 400 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 300 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 200 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 100 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 90 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 80 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 70 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 60 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 50 mg of a compound or pharmaceutically acceptable salt

described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 40 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 30 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 20 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 10 mg of a compound or pharmaceutically acceptable salt described herein.

[0098] In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 500 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 400 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 300 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 200 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 100 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 90 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 80 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 70 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 60 mg of a compound or pharmaceutically acceptable salt described herein f. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 50 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 40 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg

and about 30 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 20 mg of a compound or pharmaceutically acceptable salt described herein.

Particle size

[0099] In some embodiments, the pharmaceutical composition, in the form of a tablet or a capsule, comprises a compound or pharmaceutically acceptable salt described herein, in the form of microparticles. In some embodiments, the microparticles have an average size from about 1 μm to about 100 μm. In some embodiments, the microparticles have an average size from about 1 μm to about 50 μm. In some embodiments, the microparticles have an average size from about 1 μm to about 30 μm. In some embodiments, the microparticles have an average size from about 1 μm to about 20 μm. In some embodiments, the microparticles have an average size from about 5 μm to about 15 μm. In some embodiments, the microparticles have an average size from about 1 μm to about 10 μm. In some embodiments, the microparticles have an average size from about 3 μm to about 10 μm. In some embodiments, the microparticles have an average size from about 4 μm to about 9 μm.

Methods of Treatment

[0100] Disclosed herein are methods of treating polycystic ovary syndrome (PCOS) in a subject in need thereof, comprising administering a compound or pharmaceutically acceptable salt described herein. In some embodiments, the subject in need thereof has PCOS-FAH. In some embodiments, the subject in need thereof has PCOS-FOH+FAH. In some embodiments, the methods described herein result in the reduction of a level of a hormone. Such hormones include deoxycorticosterone, 11-deoxycortisol, cortisol, corticosterone, pregnenolone, 17α-hydroxy pregnenolone, progesterone, 17-OHP, dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulfate (DHEAS), androstenediol, androstenedione (A4), testosterone (T), dihydrotestosterone (DHT), estrone, estradiol, estriol, 11β-hydroxyandrostenedione (11OHA4), 11β-hydroxytestosterone (11OHT), 11-ketoandrostenedione (11KA4), 11-ketotestosterone (11KT), 11β-hydroxy-5α-androstenedione (11OHDHA4), 11-keto-5α-androstenedione (11KDHA4), 11β-hydroxydihydrotestosterone (11OHDHT), 11-ketodihydrotestosterone (11KDHT) and ACTH. In some embodiments, the methods described herein result in the reduction of 17-OHP levels. In some embodiments, the methods described herein result in the reduction of A4 levels. In some embodiments, the methods described herein result in the reduction of ACTH levels. In some embodiments, the methods described herein result in the reduction of DHEA levels. In some embodiments, the methods described herein result in the reduction of DHEAS levels. In some embodiments, the methods described herein

result in the reduction of testosterone (T) levels. In some embodiments, the methods described herein result in the reduction of DHT levels. In some embodiments, the methods described herein result in the reduction of 11OHA4 levels. In some embodiments, the methods described herein result in the reduction of 11OHT levels. In some embodiments, the methods described herein result in the reduction of 11KA4 levels. In some embodiments, the methods described herein result in the reduction of 11KT levels.

[0101] Further, in some embodiments, the methods described herein result in the maintenance of the reduction of 17-OHP, A4, ACTH, DHEA, DHEAS, T, DHT, 11OHA4, 11OHT, 11KA4,and/or 11KT levels. In some embodiments, the reductions of 17-OHP, A4, ACTH, DHEA, DHEAS, T, DHT, 11OHA4, 11OHT, 11KA4 and/or 11KT levels may last for at least 24 hours, 36 hours, 48 hours, 60 hours, 72 hours, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, 21 weeks, 22 weeks, 23 weeks, 24 weeks, 25 weeks, 26 weeks, 27 weeks, 28 weeks, 29 weeks, 30 weeks, 31 weeks, 32 weeks, 33 weeks, 34 weeks, 35 weeks, 36 weeks, 37 weeks, 38 weeks, 39 weeks, 40 weeks, 41 weeks, 42 weeks, 43 weeks, 44 weeks, 45 weeks, 46 weeks, 47 weeks, 48 weeks, 49 weeks, 50 weeks, 51 weeks, 52 weeks, 2 years, 5 years, 10 years, 15 years, 20 years, 25 years, 30 years, 35 years, 40 years, 45 years, 50 years, 55 years, 60 years, 65 years, 70 years, 75 years, 80 years, 85 years, 90 years, 95 years, or 100 years.

[0102] In some embodiments, the methods described herein result in the reduction of DHEAS levels. In some embodiments, the DHEAS level is reduced by at least about 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% from baseline. The baseline level of the DHEAS may be measured in a subject before beginning the treatment disclosed herein. In some embodiments, the DHEAS level is reduced by at least 5% from baseline. In some embodiments, the reduced DHEAS level from baseline may be maintained for at least about 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, 48 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, 21 weeks, 22 weeks, 23 weeks, 24 weeks, 25 weeks, 26 weeks, 27 weeks, 28 weeks, 29 weeks, or 30 weeks.

[0103] Further, in some embodiments, the methods described herein result in the maintenance of the reduction of 17-OHP, A4, ACTH, T, DHT, 11OHA4, 11OHT, 11KA4 and/or 11KT levels. In some embodiments, the reductions of 17-OHP, A4, ACTH T, DHT, 11OHA4, 11OHT, 11KA4 and/or 11KT levels may last for at least 24 hours, 36 hours, 48 hours, 60 hours, 72

hours, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, 21 weeks, 22 weeks, 23 weeks, 24 weeks, 25 weeks, 26 weeks, 27 weeks, 28 weeks, 29 weeks, 30 weeks, 31 weeks, 32 weeks, 33 weeks, 34 weeks, 35 weeks, 36 weeks, 37 weeks, 38 weeks, 39 weeks, 40 weeks, 41 weeks, 42 weeks, 43 weeks, 44 weeks, 45 weeks, 46 weeks, 47 weeks, 48 weeks, 49 weeks, 50 weeks, 51 weeks, 52 weeks, 2 years, 5 years, 10 years, 15 years, 20 years, 25 years, 30 years, 35 years, 40 years, 45 years, 50 years, 55 years, 60 years, 65 years, 70 years, 75 years, 80 years, 85 years, 90 years, 95 years, or 100 years.

[0104] In some embodiments, disclosed herein are methods for treating PCOS in a subject in need thereof, and the methods further comprise accessing a source of excessive androgen; and administering a CRF_1 antagonist or pharmaceutically acceptable salt thereof. In some embodiments, accessing a source of excessive androgen comprises measuring the primary source of excessive androgen produced in the subject suffering PCOS. In some embodiments, the primary source is the ovaries. In some embodiments, the primary source is the adrenal glands. In some embodiments, the primary source is both the ovaries and the adrenal glands. In some embodiments, the primary source is an organ that is able to produce androgen.

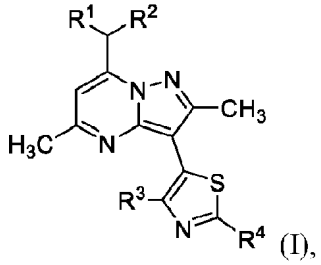
[0105] In some embodiments, cosyntropin or any other suitable hormones or chemical compounds may be administered to a subject for ACTH response in order to test for adrenal hyperandrogenism. In some embodiments, the cosyntropin is administered at a dose at least about 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 250or $\mu g/m^2$. In some embodiments, the cosyntropin is administered at a dose at about the range between the doses disclosed herein. In some embodiments, the cosyntropin is administered at a dose at most about 250, 200, 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, 5, 4, 3, 2, 1 $\mu g/m^2$.

[0106] In some embodiments, the subject will be treated for a period of about 1 week to about 40 weeks. In some embodiments, the subject will be treated for a period of about 2 weeks to about 39 weeks. In some embodiments, the subject will be treated for a period of about 3 weeks to about 38 weeks. In some embodiments, the subject will be treated for a period of about 4 weeks to about 36 weeks. In some embodiments, the subject will be treated for a period of about 1 month to 12 months. In some embodiments, the subject will be treated for a period of about 10 months to 10 years. In some embodiments, the subject will be treated for a period of about 1 months to 10 years. In some embodiments, the subject will be treated for a period of about 10 months to 20 years. In some embodiments, the subject will be treated for a period of about 1 months to 20 years. In some embodiments, the subject will be treated for a period of about 10

months to 30 years. In some embodiments, the subject will be treated for a period of about 1 months to 30 years. In some embodiments, the subject will be treated for a period of about 10 months to 40 years. In some embodiments, the subject will be treated for a period of about 1 months to 40 years. In some embodiments, the subject will be treated for a period of about 10 months to 50 years. In some embodiments, the subject will be treated for a period of about 1 months to 50 years.

[0107] In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt thereof is selected from the group consisting of: Antalarmin hydrochloride, CP-154,526, CP-376395 hydrochloride, NBI 27914 hydrochloride, NBI 35965 hydrochloride, NGD 98-2 hydrochloride, Pexacerfont, R 121919 hydrochloride, SSR125543 (crinecerfont), AND SN003.

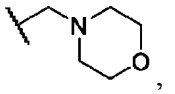
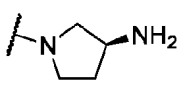
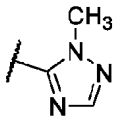
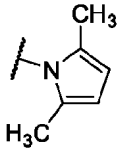
[0108] In some embodiments, the CRF_1 antagonist is a compound of Formula (I):


(I),

or a pharmaceutically acceptable salt thereof, wherein:

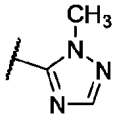
R^1 and R^2 are independently ethyl or *n*-propyl;

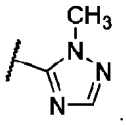
R^3 is hydrogen, F, Cl, Br, methyl, trifluoromethyl, or methoxy; and

R^4 is hydrogen, Br, $-NR^aR^b$, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , , , or ; and

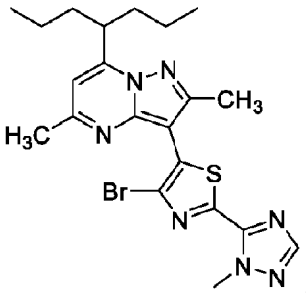
R^a and R^b are independently hydrogen, C_1-C_3alkyl, $-CH_2CH_2NH_2$, $-CH_2CH_2NHC(O)OC(CH_3)_3$–$CH_2CH_2NHC(O)OC(CH_3)_3$, or $-CH_2CH_2NHCH_2CH_2CH_3$–.

[0109] In some embodiments, R^3 is F, Cl, Br, methyl, or trifluoromethyl. In some embodiments, R^3 is Cl, Br, or methyl.

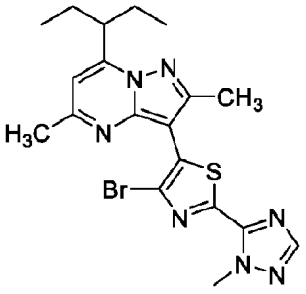
[0110] In some embodiments, R^4 is Br, $-NR^aR^b$–NR^aR^b, pyridin-4-yl, morpholin-4-yl, or . In some embodiments, R^4 is morpholin-4-yl or


. In some embodiments, R^4 is hydrogen, Br, $-NR^aR^b$ and R^a and R^b are independently C_1-C_3alkyl.

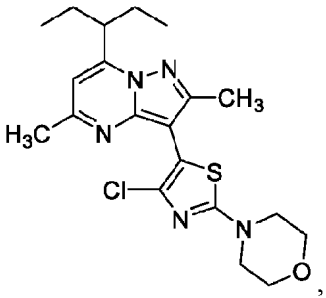
[0111] In some embodiments, the compound is


, or a pharmaceutically acceptable salt thereof.

[0112] In some embodiments, the compound is


, or a pharmaceutically acceptable salt thereof.

[0113] In some embodiments, the compound is


, or a pharmaceutically acceptable salt thereof.

[0114] In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 10 mg to about 200 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 200 mg total daily dose to the subject.

[0115] In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 150 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 100 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 50 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is

administered in a dose of about 40 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 30 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 25 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 20 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 15 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 10 mg total daily dose to the subject. In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 5 mg total daily dose to the subject.

[0116] In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt is in the form of microparticles. In some embodiments, the average size of the microparticles is between about 1 μm to about 20 μm. In some embodiments, the average size of the microparticles is between about 5 μm to about 15 μm. In some embodiments, the average size of the microparticles is less than about 10 μ.

[0117] In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt thereof is administered as a pharmaceutical composition. In some embodiments, the steroid or a pharmaceutically acceptable salt thereof is administered as a pharmaceutical composition.

[0118] In some embodiments, the pharmaceutical composition is in the form of a capsule or a tablet. 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.

[0119] 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.

[0120] In some embodiments, CRF_1 antagonist or pharmaceutically acceptable salt 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, CRF_1 antagonist or pharmaceutically acceptable salt 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, CRF_1 antagonist or pharmaceutically acceptable salt 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, CRF_1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 3 to about 5 hours in a subject.

[0121] In some embodiments, CRF_1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 8 hours in a subject. In some embodiments, CRF_1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 7 hours in a subject. In some embodiments, CRF_1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 6 hours in a subject. In some embodiments, CRF_1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 5 hours in a subject. In some embodiments, CRF_1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 4 hours in a subject. In some embodiments, CRF_1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 3 hours in a subject. In some embodiments, CRF_1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 2 hours in a subject. In some embodiments, CRF_1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 1 hour in a subject.

[0122] In some embodiments, the methods described herein include administration of the pharmaceutical composition comprising CRF_1 antagonist or pharmaceutically acceptable salt, 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 CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof once a day. In some embodiments, the methods described herein administer CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof twice a day.

[0123] In some embodiments, the methods described herein include administration of about 1 mg to about 2000 mg of CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, per day. In some embodiments, CRF_1 antagonist or pharmaceutically acceptable salt is administered at a dose between about 50 mg/day and about 1600 mg/day. In some embodiments, Compound 3 is administered at a dose between about 50 mg/day and about 1500 mg/day. In some embodiments, Compound 3 is administered at a dose between about 50 mg/day and about 1400 mg/day. In some embodiments, Compound 3 is administered at a dose between about 50 mg/day and about 1300

mg/day. In some embodiments, Compound 3 is administered at a dose between about 50 mg/day and about 1200 mg/day. In some embodiments, Compound 3 is administered at a dose between about 50 mg/day and about 1100 mg/day. In some embodiments, Compound 3 is administered at a dose between about 50 mg/day and about 1000 mg/day. In some embodiments, Compound 3 is administered at a dose between about 50 mg/day and about 900 mg/day. In some embodiments, Compound 3 is administered at a dose between about 50 mg/day and about 800 mg/day. In some embodiments, Compound 3 is administered at a dose between about 60 mg/day and about 800 mg/day. In some embodiments, Compound 3 is administered at a dose between about 70 mg/day and about 800 mg/day. In some embodiments, Compound 3 is administered at a dose between about 80 mg/day and about 800 mg/day. In some embodiments, Compound 3 is administered at a dose between about 90 mg/day and about 800 mg/day. In some embodiments, Compound 3 is administered at a dose between about 100 mg/day and about 800 mg/day. In some embodiments, Compound 3 is administered at a dose between about 100 mg/day and about 700 mg/day. In some embodiments, Compound 3 is administered at a dose between about 100 mg/day and about 600 mg/day. In some embodiments, Compound 3 is administered at a dose between 150 mg/day and about 600 mg/day. In some embodiments, Compound 3 is administered at a dose between 200 mg/day and about 600 mg/day. In some embodiments, Compound 3 is administered at a dose between 200 mg/day and about 500 mg/day. In some embodiments, Compound 3 is administered at a dose between 200 mg/day and about 400 mg/day. In some embodiments, Compound 3 is administered at a dose between 25 mg/day and about 200 mg/day.

[0124] In some embodiments, Compound 3 is administered at a dose of about 500 mg/day. In some embodiments, Compound 3 is administered at a dose of about 400 mg/day. In some embodiments, Compound 3 is administered at a dose of about 300 mg/day. In some embodiments, Compound 3 is administered at a dose of about 200 mg/day. In some embodiments, Compound 3 is administered at a dose of about 150 mg/day. In some embodiments, Compound 3 is administered at a dose of about 100 mg/day. In some embodiments, Compound 3 is administered at a dose of about 50 mg/day.

[0125] In some embodiments, about 50 mg to about 1600 mg of CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 100 mg to about 1600 mg of CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 1600 mg of CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 1200 mg of CRF_1

antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 1000 mg of CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 800 mg of CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 100 mg to about 800 mg of CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 800 mg of CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 100 mg to about 600 mg of CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 600 mg of CRF_1 antagonist or pharmaceutically acceptable salt is administered per day. In some embodiments, about 300 mg to about 600 mg of CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 100 mg to about 400 mg of CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 400 mg of CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 300 mg to about 400 mg of CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered each day.

[0126] In some embodiments, less than about 2000 mg CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 1800 mg CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 1600 mg CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 1400 mg CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 1200 mg CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 1000 mg CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 800 mg CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some

embodiments, less than about 600 mg CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 500 mg CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 400 mg CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 300 mg CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 200 mg CRF_1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day.

[0127] 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.

[0128] In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein at bedtime.

[0129] 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.

[0130] In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein in the evening.

[0131] 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.

[0132] In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein in combination with eflornithine and/or retinoids. In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein in combination with contraceptive pills containing androgen receptor blockers such as cyproterone acetate, spironolactone, flutamide, or 5α-reductase inhibitors.

[0133] In some embodiments, the levels of DHEA, DHEAS, A4, 11OHA4, T, 11OHT, DHT and/or ACTH in the subject are determined from a biological sample from the subject. In some embodiments, the biological sample is selected from the group of blood, blood fractions, plasma, serum, urine, other types of bodily secretions, and saliva. In some embodiments, the biological sample is obtained non-invasively.

[0134] In some embodiments, the subject is a pediatric patient. In some embodiments, the subject is an adolescent. In some embodiments, the subject is from about 8 years old to about 18 years old. In some embodiments, the subject is an adult patient. In some embodiments, the subject is from about 18 years old to about 55 years old. In some embodiments, the subject is from about 18 years old to about 50 years old.

[0135] In some embodiments, the steroid and the CRF_1 antagonist are administered concurrently. In some embodiments, the steroid and the CRF_1 antagonist are administered in one pharmaceutical composition. In some embodiments, the steroid and the CRF_1 antagonist are administered concurrently in separate pharmaceutical compositions. In some embodiments, the steroid and the CRF_1 antagonist are administered sequentially.

EXAMPLES

[0136] 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.

[0137] 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 – Reduction of DHEAS level in a subject who has polycystic ovary syndrome (PCOS) and elevated adrenal androgens

[0138] Study Compound 3 in a double-blind, proof-of-concept study, evaluating the safety and efficacy of repeated doses of Compound 3 in adults with elevated adrenal androgens, PCOS-FAH or PCOS-FAH+FOH. After screening, eligible subjects with elevated adrenal androgens documented by elevated DHEAS will be randomized to either a compound 3 dose escalation arm or matching placebo arm.

[0139] The study is planned as a 3-period study with a duration of 4 weeks for Periods 1 and 2 and a duration of 8 weeks in Period 3 for a total treatment duration of 16 weeks. Subjects randomized to the compound 3 dose escalation arm will be treated with escalating doses of 50-mg, 100-mg or 200 mg based on achieving a DHEAS positive response criteria within each treatment period. Subjects not meeting the criteria will be dose escalated to the next highest dose in the preceding period. Subjects meeting the criteria will remain on their current dose level. Placebo subjects will remain on placebo through the full 16 weeks.

[0140] The population are composed of approximately 40 patients, who receive Compound 3 or matching placebo daily for up to 16 weeks. Compound 3 is administered as an oral daily dose. Patients are undergo PK/PD or PD only assessments, approximately every 2 weeks. A follow-up outpatient visit occurs 30 days after the last dose.

[0141] Study Design

- Study Type: Interventional
- Primary Purpose: Treatment
- Study Phase: Phase 2
- Interventional Study Model: Randomized
- Number of Arms: 2
- Masking: Masking of Study drug
- Allocation: Randomized (1:1)
- Enrollment: 40

Arms and Interventions:

Arms	**Assigned Intervention**
Arm 1	Drug: Compound 3 dose escalation (50, 100 and 200-mg)
Arm 2	Drug: Matching Placebo

Outcome Measures

Primary Outcome Measures:

1. Safety and tolerability of Compound 3 in patients with elevated adrenal androgens (adverse effects, serious adverse effects, clinical laboratory parameters, etc.)

[Time Frame: 16 weeks]

Secondary Outcome Measures:

2. Proportion of subjects with:

   a. ≥ 30% change from baseline in dehydroepiandrosterone sulfate (DHEAS)

   b. DHEAS <upper limit of normal (ULN)

   c. DHEAS <75th percentile of normal range (Q3)

[Time Fame: 4 to up to 16 weeks]

3. Pharmacokinetics of compound 3

[Time Frame: 4 weeks]

Exploratory Outcome Measure:

4. Changes in PD markers: Changes in cortisol, ACTH, DHEAS, DHEA, A4, T, 17-OHP, 11OHA4, 11OHT, 11KA4, 11KT

[Time Frame: up to 16 weeks]

Eligibility

- Minimum Age: 18 Years
- Maximum Age: 30 years
- Sex: Female only
- Gender Based: Yes
- Accepts Healthy Volunteers: No
- Criteria: Inclusion

Criteria:

Inclusion Criteria:

- Female subjects 18 to 30 years old, inclusive
- Subjects must have documented PCOS according to the NIH (1990) criteria
- BMI ≤38 kg/m^2
- DHEAS level > age matched reference ULN at Screening
- Evidence of DHEA hyperresponsiveness to ACTH stimulation during Screening

Exclusion Criteria:

- Has a BMI >38kg/m^2
- Has PCOS-FOH
- Has HbA1c > 6.5% or Fasting plasma glucose >126 mg/dL
- Has a history that includes bilateral adrenalectomy or hypopituitarism
- Has a history of allergy or hypersensitivity to tildacerfont or any other CRF_1 receptor antagonist
- Clinically significant unstable medical condition, illness, or chronic disease
- Clinically significant uncontrolled psychiatric disorder
- Clinically significant abnormal laboratory finding or assessment
- 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.

[0142] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

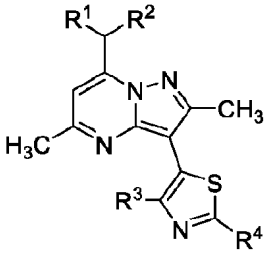
CLAIMS

WHAT IS CLAIMED IS:

1. A method of treating polycystic ovary syndrome with functional ovarian hyperandrogenism and functional adrenal hyperandrogenism (PCOS-FOH + FAH) in a subject in need thereof, comprising administering a CRF1 antagonist or pharmaceutically acceptable salt thereof.

2. A method of treating polycystic ovary syndrome with functional adrenal hyperandrogenism (PCOS-FAH) in a subject in need thereof, comprising administering a CRF1 antagonist or pharmaceutically acceptable salt thereof.

3. The method of any of claims 1-2, wherein said CRF_1 antagonist is a compound of Formula (I):

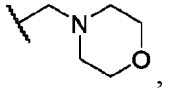
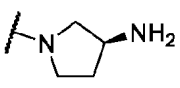
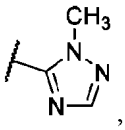
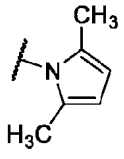


Formula (I)

or a pharmaceutically acceptable salt thereof, wherein:

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

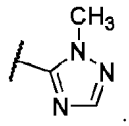
R^3 is hydrogen, F, Cl, Br, methyl, trifluoromethyl, or methoxy;

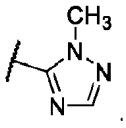
R^4 is hydrogen, Br, $-NR^aR^b$, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , , , or ;

R^a and R^b are independently hydrogen, C_1-C_3alkyl, $-CH_2CH_2NH_2$, $-CH_2CH_2NHC(O)OC(CH_3)_3$, or $-CH_2CH_2NHCH_2CH_2CH_3$.

4. The method of claim 3, wherein R^3 is F, Cl, Br, methyl, or trifluoromethyl.

5. The method of claim 3 or claim 4, wherein R^3 is Cl or Br.

6. The method of any one of claims 4 to 5, wherein R^4 is $-NR^aR^b$, pyridin-4-yl, morpholin-4-yl, or .

7. The method of any one of claims 4 to 6, wherein R^4 is morpholin-4-yl or .

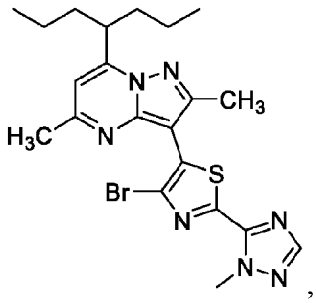
8. The method of any one of claims 4 to 6, wherein R^4 is $-NR^aR^b$, and R^a and R^b are independently C_1-C_3alkyl.

9. The method of claim 4, wherein said CRF_1 antagonist or a pharmaceutically acceptable salt thereof is 3-[4-bromo-2-(2-methyl-2*H*-[1,2,4]triazol-3-yl)-thiazol-5-yl]-2,5-dimethyl-7-(1-propyl-butyl)- pyrazolo[1,5-*a*]pyrimidine.

10. The method of claim 4, wherein said CRF_1 antagonist or a pharmaceutically acceptable salt thereof is 3-(4-bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine.

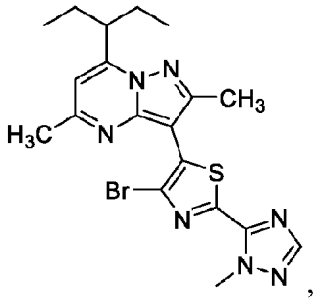
11. The method of claim 4, wherein said CRF_1 antagonist or a pharmaceutically acceptable salt thereof is 3-(4-chloro-2-morpholin-4-yl-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidine.

12. The method of claim 4, wherein said CRF_1 antagonist is



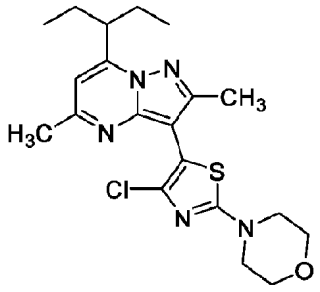
, or a pharmaceutically acceptable salt thereof.

13. The method of claim 4, wherein said CRF_1 antagonist is



, or a pharmaceutically acceptable salt thereof.

14. The method of claim 4, wherein the compound is



, or a pharmaceutically acceptable salt thereof.

15. The method of any of claims 1-2, wherein said CRF_1 antagonist or pharmaceutically acceptable salt thereof is selected from the group consisting of: Antalarmin hydrochloride, CP-154,526, CP-376395 hydrochloride, NBI 27914 hydrochloride, NBI 35965 hydrochloride, NGD 98-2 hydrochloride, Pexacerfont, R 121919 hydrochloride, SSR125543 (crinecerfont), and SN003.

16. The method of any one of claims 1-15, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 5 mg to about 400 mg total daily dose to the subject.

17. The method of any one of claims 1-15, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 300 mg total daily dose to the subject.

18. The method of any one of claims 1-15, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 200 mg total daily dose to the subject.

19. The method of any one of claims 1-15, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 150 mg total daily dose to the subject.

20. The method of any one of claims 1-15, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 100 mg total daily dose to the subject.

21. The method of any one of claims 1-15, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 75 mg total daily dose to the subject.

22. The method of any one of claims 1-15, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 50 mg total daily dose to the subject.

23. The method of any one of claims 1-15, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 25 mg total daily dose to the subject.

24. The method of any one of claims 1-15, wherein the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 10 mg total daily dose to the subject.

25. The method of any one of claims 1-24, wherein the CRF_1 antagonist or pharmaceutically acceptable salt thereof is administered as a pharmaceutical composition.

26. The method of any one of claims 1-25, wherein an adrenocorticotropic hormone (ACTH) level is reduced by at least 5% from baseline.

27. The method of any one of claims 1-25, wherein an adrenocorticotropic hormone (ACTH) level is reduced by at least 10% from baseline.

28. The method of any one of claims 1-25, wherein a dehydroepiandrosterone sulfate (DHEAS) level is reduced by at least 5% from baseline.

29. The method of any one of claims 1-25, wherein a dehydroepiandrosterone sulfate (DHEAS) level is reduced by at least 10% from baseline.

30. The method of any one of claims 1-25, wherein androstenedione (A4) level is reduced by at least 5% from baseline.

31. The method of any one of claims 1-25, wherein androstenedione (A4) level is reduced by at least 10% from baseline.

32. The method of any one of claims 1-25, wherein 1β-hydroxyandrostenedione (11OHA4) level is reduced by at least 5% from baseline.

33. The method of any one of claims 1-25, wherein 1β-hydroxyandrostenedione (11OHA4) level is reduced by at least 10% from baseline.

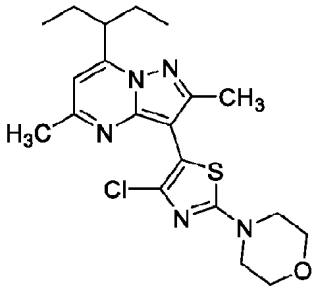
34. The method of any one of claims 1-25, wherein 11β-hydroxytestosterone (11OHT) level is reduced by at least 5% from baseline.

35. The method of any one of claims 1-25, wherein 11β-hydroxytestosterone (11OHT) level is reduced by at least 10% from baseline.

36. The method of any one of claims 25-35, wherein a reduced ACTH, DHEAS, A4, 11OHA4 or 11OHT level from baseline is maintained for at least 24 hours.

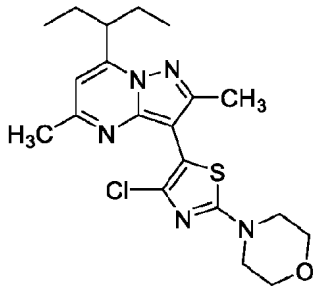
37. The method of any one of claims 25-35, wherein a reduced ACTH, DHEAS, A4, 11OHA4 or 11OHT level from baseline is maintained for at least 4 weeks.

38. A method of treating polycystic ovary syndrome with functional adrenal hyperandrogenism (PCOS-FAH) in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 3:



; or a pharmaceutically acceptable salt or solvate thereof.

39. A method of treating polycystic ovary syndrome with functional ovarian hyperandrogenism and functional adrenal hyperandrogenism (PCOS-FOH+FAH) in a subject in need thereof, comprising administering a pharmaceutical composition comprising Compound 3:



; or a pharmaceutically acceptable salt or solvate thereof.

40. The method of either claim 38 or claim 39, wherein Compound 3 is administered at a dose between about 1 mg/day and about 200 mg/day.

41. The method of any of claims 38-40, wherein the pharmaceutical composition comprises between about 1 mg and about 400 mg of Compound 3, or a pharmaceutically acceptable salt or solvate thereof.

42. The method of claim 41, wherein the pharmaceutical composition comprises about 25 mg of Compound 3, or a pharmaceutically acceptable salt or solvate thereof.

43. The method of claim 41, wherein the pharmaceutical composition comprises about 50 mg of Compound 3, or a pharmaceutically acceptable salt or solvate thereof.

44. The method of claim 41, wherein the pharmaceutical composition comprises between about 75 mg of Compound 3, or a pharmaceutically acceptable salt or solvate thereof.

45. The method of claim 41, wherein the pharmaceutical composition comprises about 100 mg of Compound 3, or a pharmaceutically acceptable salt or solvate thereof.

46. The method of claim 41, wherein the pharmaceutical composition comprises about 200 mg of Compound 3, or a pharmaceutically acceptable salt or solvate thereof.

47. The method of claim 41, wherein the pharmaceutical composition comprises about 300 mg of Compound 3, or a pharmaceutically acceptable salt or solvate thereof.

48. The method of any one of claims 1-47, wherein the pharmaceutical composition is in the form of microparticles.

49. The method of claim 48, wherein the average size of the microparticles is between about 1 μm to about 20 μm.

50. The method of claim 48, wherein the average size of the microparticles is between about 5 μm to about 15 μm.

51. The method of claim 48, wherein the average size of the microparticles is less than about 10 μm.

52. The method of any of claims 1-47, wherein the pharmaceutical composition is in the form of a capsule or a tablet.

53. The method of claim 52, wherein the capsule is a hard gelatin capsule.

54. The method of claim 52, wherein the capsule is a soft gelatin capsule.

55. The method of any one of claims 52-54, 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.

56. The method of any one of claims 52-54, wherein the pharmaceutical composition is free of additional excipients.

57. The method of any one of claims 52-54, wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

58. The method of claim 52, wherein the pharmaceutical composition is in the form of a tablet.

59. The method of claim 58, wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

60. The method of any one of claims 1-59, wherein the subject is a pediatric patient.

61. The method of any one of claims 1-59, wherein the subject is from about 9 years old to about 18 years old.

62. The method of any one of claims 1-59, wherein the subject is from about 8 years of age to about 55 years of age.

63. The method of any one of claims 1-59, wherein the subject is an adult patient.

64. The method of any of claims 1-63, wherein said subject is treated for a period of about 2 weeks to about 36 weeks.

65. The method of any of claims 1-63, wherein said subject is treated for a period of about 1 month to 12 months.

66. The method of any of claims 1-63, wherein said subject is treated for a period of about 10 months to 50 years.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/45780

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61B 18/14; A61B 18/02; A61B 18/04 (2021.01)

CPC - A61N 1/36117; A61B 18/04; A61B 18/1492

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.
A	US 2017/0333126 A1 (Medtronic Ardian Luxembourg S.a.r.l.) 23 November 2017 (23.11.2017); abstract	1-4, 9-15, 38-40
A	US 2010/0222339 A1 (Chen et al.) 02 September 2010 (02.09.2010); abstract, para [0144]	1-4, 9-15, 38-40
A	US 2018/0110554 A1 (Ziva Medicinal, Inc.) 26 April 2018 (26.04.2018); abstract	1-4, 9-15, 38-40
A	WO 2019/036472 A1 (SPRUCE BIOSCIENCES, INC.) 21 February 2019 (21.02.2019); entire document	1-4, 9-15, 38-40
A	US 2013/0045979 A1 (Sanfilippo) 21 February 2013 (21.02.2013); entire document	1-4, 9-15, 38-40

☐ 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
"D" document cited by the applicant in the international application
"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
21 November 2021	DEC 17 2021
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 Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

Form PCT/ISA/210 (second sheet) (July 2019)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/45780

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.: 5-8, 16-37, 41-66
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.

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 January 2021 (28.01.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/016208 A1

(51) International Patent Classification:
A61K 31/56 (2006.01) *A61K 31/5377* (2006.01)
A61K 31/57 (2006.01) *A61P 5/38* (2006.01)
A61K 38/22 (2006.01) *C07D 487/04* (2006.01)
A61K 31/519 (2006.01)

(21) International Application Number:
PCT/US2020/042820

(22) International Filing Date:
20 July 2020 (20.07.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/876,176 19 July 2019 (19.07.2019) US
63/047,822 02 July 2020 (02.07.2020) US

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

(72) Inventors: **GHOSH, Sangita**; 548 Market Street, Suite 74589, San Francisco, California 94105 (US). **BARNES, Christopher**; 548 Market Street, Suite 74589, San Francisco, California 94105 (US). **HUANG, Michael**; 548 Market Street, Suite 74589, San Francisco, California 94105 (US).

(74) Agent: **LAMIQUIZ, Jiarong L.**; WILSON SONSINI, 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, IT, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, 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: METHODS OF TREATING CONGENITAL ADRENAL HYPERPLASIA

(57) Abstract: Patients with congenital adrenal hyperplasia (CAH) need adequate care and treatment in order to lead normal lives. Hence, there is a need for new methods of treating CAH. This disclosure provides new compounds, salts, compositions and uses thereof in the treatment of CAH.

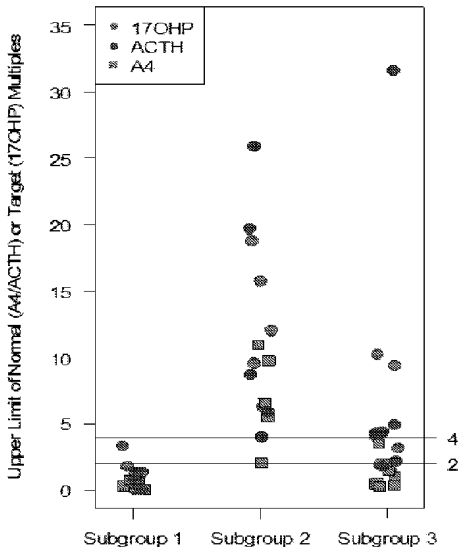


FIG. 1

WO 2021/016208 A1

[Continued on next page]

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))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

METHODS OF TREATING CONGENITAL ADRENAL HYPERPLASIA

CROSS-REFERENCE

[0001] This application claims priority to U.S. Provisional Patent Application No 62/876,176, filed July 19, 2019, and U.S. Provisional Patent Application No 63/047,822, filed on July 2, 2020, each of which is entirely incorporated herein by reference.

BACKGROUND

[0002] Congenital adrenal hyperplasia (CAH) is a group of rare inherited autosomal recessive disorders characterized by a deficiency of one of the enzymes needed to make specific hormones. CAH affects the adrenal glands located at the top of each kidney. Normally, the adrenal glands are responsible for producing three different hormones: 1) glucocorticoids, which gauge the body's response to stress, illness, or injury; 2) mineralocorticoids, which regulate salt and water levels; and 3) androgens, which are male sex hormones. An enzyme deficiency may make the body unable to produce one or more of these hormones, which in turn may result in the overproduction of another type of hormone precursor in order to compensate for the loss.

SUMMARY

[0003] Congenital Adrenal Hyperplasia (CAH) patients need adequate care and treatment in order to lead normal lives. Hence, there is a need for new methods of treating CAH. This disclosure provides new compounds, salts, compositions and uses thereof in the treatment of CAH.

[0004] In an aspect, the present disclosure provides methods for treating Congenital Adrenal Hyperplasia (CAH). The method comprises administering a steroid or a pharmaceutically acceptable salt thereof and a corticotropin-releasing factor type-1 (CRF_1) antagonist or a pharmaceutically acceptable salt thereof to a subject, wherein when said subject has a level of at least one steroid hormone that is less than two times an upper limit of a reference range of said at least one steroid hormone level , a dose of said steroid or a pharmaceutically acceptable salt thereof is reduced compared to a dose of said steroid or a pharmaceutically acceptable salt thereof administered to a CAH patient that does not receive said CRF_1 antagonist or a pharmaceutically acceptable salt thereof.

[0005] In some embodiments, the subject has a level of a second and different steroid hormone that is less than two times an upper limit of a reference range of said second and different steroid hormone level. In some embodiments, the steroid hormone is selected from the group consisting of adrenocorticotropic hormone (ACTH), androstenedione (A4), 17-hydroxyprogesterone (17-OHP), deoxycorticosterone, 11-deoxycortisol, cortisol, corticosterone,

aldosterone, pregnenolone, 17α-hydroxy pregnenolone, progesterone, dehydroepiandrosterone, androstenediol, testosterone, dihydrotestosterone, estrone, estradiol, and estriol.

[0006] In some embodiments, the subject has a level of 17-OHP that is less than two times an upper limit of a reference range of 17-OHP and a level of ACTH that is less than two times an upper limit of a reference range of ACTH. In some embodiments, the subject has a level of 17-OHP that is less than two times an upper limit of a reference range of 17-OHP and a level of A4 that is less than two times an upper limit of a reference range of A4. In some embodiments, the subject has a level of A4 that is less than two times an upper limit of a reference range of A4 and a level of ACTH that is less than two times an upper limit of a reference range of ACTH. In some embodiments, the subject has a level of 17-OHP that is less than 1.75 times an upper limit of a reference range of 17-OHP and a level of ACTH that is less than 1.75 times an upper limit of a reference range of ACTH. In some embodiments, the subject has a level of 17-OHP that is less than 1.75 times an upper limit of a reference range of 17-OHP and a level of A4 that is less than 1.75 times an upper limit of a reference range of A4. In some embodiments, the subject has a level of A4 that is less than 1.75 times an upper limit of a reference range of A4 and a level of ACTH that is less than 1.75 times an upper limit of a reference range of ACTH. In some embodiments, the subject has a level of 17-OHP that is less than 1.5 times an upper limit of a reference range of 17-OHP and a level of ACTH that is less than 1.5 times an upper limit of a reference range of ACTH. In some embodiments, the subject has a level of 17-OHP that is less than 1.5 times an upper limit of a reference range of 17-OHP and a level of A4 that is less than 1.5 times an upper limit of a reference range of A4. In some embodiments, the subject has a level of A4 that is less than 1.5 times an upper limit of a reference range of A4 and a level of ACTH that is less than 1.5 times an upper limit of a reference range of ACTH. In some embodiments, the subject has a level of 17-OHP that is less than 1.25 times an upper limit of a reference range of 17-OHP and a level of ACTH that is less than 1.25 times an upper limit of a reference range of ACTH. In some embodiments, the subject has a level of 17-OHP that is less than 1.25 times an upper limit of a reference range of 17-OHP and a level of A4 that is less than 1.25 times an upper limit of a reference range of A4. In some embodiments, the subject has a level of A4 that is less than 1.25 times an upper limit of a reference range of A4 and a level of ACTH that is less than 1.25 times an upper limit of a reference range of ACTH. In some embodiments, the subject has a level of 17-OHP that is less than one times an upper limit of a reference range of 17-OHP and a level of ACTH that is less than one times an upper limit of a reference range of ACTH. In some embodiments, the subject has a level of 17-OHP that is less than one times an upper limit of a reference range of 17-OHP and a level of A4 that is less than one times an upper limit of a reference range of A4. In some embodiments, the subject has a level of A4 that is less than one

times an upper limit of a reference range of A4 and a level of ACTH that is less than one times an upper limit of a reference range of ACTH.

[0007] In some embodiments, the methods comprise reducing said dose of said steroid or a pharmaceutically acceptable salt thereof in a 5 mg increment.

[0008] In another aspect, the present disclosure provides methods for treating Congenital Adrenal Hyperplasia (CAH). The methods comprise administering a steroid or a pharmaceutically acceptable salt thereof and a corticotropin-releasing factor type-1 (CRF1) antagonist or a pharmaceutically acceptable salt thereof to a subject, wherein when said subject has a level of at least one steroid hormone that is more than an upper limit of a reference range of said at least one steroid hormone level, a dose of said steroid or a pharmaceutically acceptable salt thereof is not reduced compared to a dose of said steroid or a pharmaceutically acceptable salt thereof administered to a CAH patient that does not receive said CRF1 antagonist or a pharmaceutically acceptable salt thereof.

[0009] In some embodiments, the subject has a level of said at least one steroid hormone that is more than two times an upper limit of a reference range of said at least one steroid hormone level. In some embodiments, the subject has a level of said at least one steroid hormone that is more than 1.75 times an upper limit of a reference range of said at least one steroid hormone level. In some embodiments, the subject has a level of said at least one steroid hormone that is more than 1.5 times an upper limit of a reference range of said at least one steroid hormone level. In some embodiments, the subject has a level of said at least one steroid hormone that is more than 1.25 times an upper limit of a reference range of said at least one steroid hormone level.

[0010] In some embodiments, the steroid hormone is selected from the group consisting of adrenocorticotropic hormone (ACTH), androstenedione (A4), 17-hydroxyprogesterone (17-OHP), deoxycorticosterone, 11-deoxycortisol, cortisol, corticosterone, aldosterone, pregnenolone, 17α-hydroxy pregnenolone, progesterone, dehydroepiandrosterone, androstenediol, testosterone, dihydrotestosterone, estrone, estradiol, and estriol.

[0011] In some embodiments, the methods further comprise maintaining said dose of said steroid or a pharmaceutically acceptable salt thereof until said levels of at least two steroid hormones are less than 2 times said upper limit of said reference ranges of said two steroid hormones respectively. In some embodiments, the methods further comprise reducing said dose of said steroid or a pharmaceutically acceptable salt thereof in a 5 mg increment when said levels of at least two steroid hormones are less than 2 times said upper limit of said reference ranges of said two steroid hormones respectively.

[0012] In some embodiments, the at least two steroid hormones comprise 17-OHP and ACTH. In some embodiments, the at least two steroid hormones comprise 17-OHP and A4. In some embodiments, the at least two steroid hormones comprise A4 and ACTH. In some embodiments, the reference range of ACTH or A4 level of said subject is controlled by a factor selected from the group consisting of age, sex, menopausal status, laboratory setting, time of a day and combinations thereof.

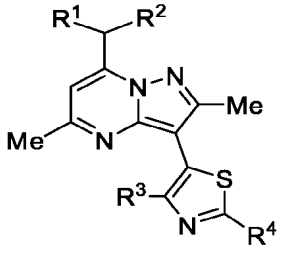
[0013] In some embodiments, the level of ACTH or A4 is 2 times, 1.5 times, 1.25 times, or 1 times said upper limit of said normal range of ACTH or A4 level of said subject. In some embodiments, the amount of said steroid or a pharmaceutically acceptable salt thereof comprises a range of about 10 mg to about 80 mg of hydrocortisone equivalents, wherein said steroid or a pharmaceutically acceptable salt thereof is administered daily. In some embodiments, the amount of said CRF_1 antagonist or a pharmaceutically acceptable salt thereof comprises a range of about 10 mg to about 200 mg, wherein said CRF_1 antagonist or a pharmaceutically acceptable salt thereof is administered daily.

[0014] In some embodiments, the subject is treated for a period of about 4 weeks to about 36 weeks.

[0015] In some embodiments, the subject is treated for a period of about 4 weeks to about 36 weeks, said subject is reassessed to determine if a different method of treating CAH should be administered.

[0016] In some embodiments, the CRF_1 antagonist or pharmaceutically acceptable salt thereof is selected from the group consisting of: Antalarmin hydrochloride, Pfizer CP 154526, CP 376395 hydrochloride, NBI 27914 hydrochloride, NBI 35965 hydrochloride, NGD 98-2 hydrochloride, Pexacerfont, R 121919 hydrochloride, SN003.

[0017] In some embodiments, the CRF_1 antagonist is a compound of Formula (I):

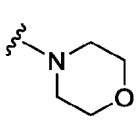
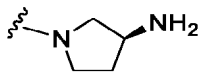
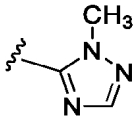
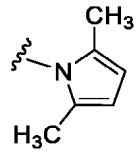


(I),

or a pharmaceutically acceptable salt thereof, wherein:

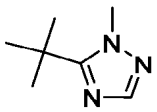
R^1 and R^2 are independently ethyl or *n*-propyl;

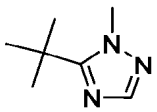
R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy; and

R^4 is hydrogen, Br, R^aR^bN–, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , , , or ; and R^a and R^b are independently hydrogen, C_1-C_3alkyl, $H_2NCH_2CH_2$–, $(CH_3)_3COC(O)NHCH_2CH_2$–, or $CH_3CH_2CH_2NHCH_2CH_2$–.

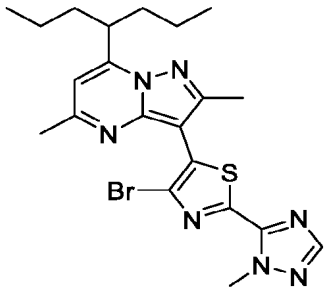
[0018] In some embodiments, R^3 is Cl, Br, methyl, or trifluoromethyl.

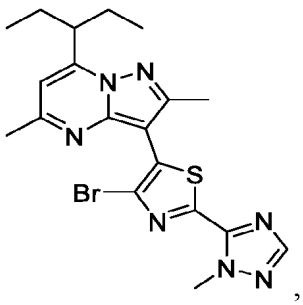
[0019] The method of claims 38 or 39, wherein R^3 is Cl, Br, or methyl.

[0020] In some embodiments, R^4 is Br, R^aR^bN–, pyridin-4-yl, morpholin-4-yl, or .

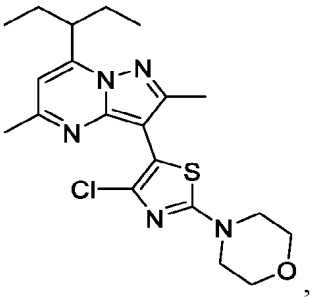
[0021] In some embodiments, R^4 is morpholin-4-yl or .

[0022] In some embodiments, R^4 is hydrogen, Br, R^aR^bN– and R^a and R^b are independently C_1-C_3alkyl.

[0023] In some embodiments the compound is , or a pharmaceutically acceptable salt thereof.

[0024] In some embodiments the compound is , or a pharmaceutically acceptable salt thereof.

[0025] In some embodiments the compound is



, or a pharmaceutically acceptable salt thereof.

[0026] In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 50 mg to about 200 mg total daily dose to the subject.

[0027] In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 200 mg total daily dose to the subject. In some embodiments, wherein the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 150 mg total daily dose to the subject.

[0028] In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 100 mg total daily dose to the subject. In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 50 mg total daily dose to the subject.

[0029] In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is in the form of microparticles. In some embodiments, the average size of the microparticles is between about 1 μm to about 20 μm. In some embodiments, the average size of the microparticles is between about 1 μm to about 20 μm. In some embodiments, the average size of the microparticles is between about 5 μm to about 15 μm. In some embodiments, the average size of the microparticles is less than about 10 μm.

[0030] In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt thereof is administered as a pharmaceutical composition. In some embodiments, the steroid or a pharmaceutically acceptable salt thereof is administered as a pharmaceutical composition.

[0031] In some embodiments, the pharmaceutical composition is in the form of a capsule or a tablet. 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.

[0032] 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. In some embodiments, the steroid or pharmaceutically acceptable salt thereof is a glucocorticoid or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid or a pharmaceutically acceptable salt thereof is prednisone, cortisone, prednisolone, triamcinolone, methylprednisolone, betamethasone, dexamethasone, hydrocortisone or a pharmaceutically acceptable salt thereof. In some embodiments, the levels of steroid hormones in the subject are determined from a biological sample from the subject. In some embodiments, the biological sample is selected from the group of blood, blood fractions, plasma, serum, and saliva. In some embodiments, the biological sample is obtained non-invasively.

[0033] In some embodiments, the subject is tested in the morning. In some embodiments, subject is tested to determine morning levels of A4 and ACTH. In some embodiments, the subject is a pediatric patient. In some embodiments, the subject is from about newborn to about 18 years old.

[0034] In some embodiments, the subject is an adult patient. In some embodiments, the steroid and the CRF1 antagonist are administered concurrently. In some embodiments, the steroid and the CRF1 antagonist are administered in one pharmaceutical composition.

[0035] In some embodiments, the steroid and the CRF1 antagonist are administered concurrently in separate pharmaceutical compositions. In some embodiments, the steroid and the CRF1 antagonist are administered sequentially. In some embodiments, the steroid and the CRF1 antagonist are administered sequentially within 24 hours. In some embodiments, the steroid and the CRF1 antagonist are administered sequentially within 8 hours. In some embodiments, the steroid and the CRF1 antagonist are administered sequentially within 2 hours. In some embodiments, the steroid and the CRF1 antagonist are administered sequentially within 10 minutes.

[0036] In some embodiments, CAH is classic CAH. In some embodiments, CAH is non-classic CAH.

[0037] In another aspects, the present disclosure provides compositions comprising a steroid or a pharmaceutically acceptable salt thereof and a corticotropin-releasing factor type-1 (CRF_1) antagonist or a pharmaceutically acceptable salt thereof, wherein said CRF_1 antagonist or pharmaceutically acceptable salt thereof is selected from the group consisting of: Antalarmin hydrochloride, Pfizer CP 154526, CP 376395 hydrochloride, NBI 27914 hydrochloride, NBI 35965 hydrochloride, NGD 98-2 hydrochloride, Pexacerfont, R 121919 hydrochloride, SN003. In some embodiments, the steroid or a pharmaceutically acceptable salt thereof is exogenous glucocorticoids (GC) or a pharmaceutically acceptable salt thereof.

[0038] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0039] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also "Figure" and "FIG."), of which:

[0041] FIG. 1 shows the baseline upper limit of a normal range (ULN)/Target Multiples for Serum 17-OHP, ACTH and A4 by subgroup;

[0042] FIGS. 2A and 2B illustrate a time matched change of ACTH hormone profile;

[0043] FIGS. 3A and 3B illustrate a time matched change of 17-OHP hormone profile; and

[0044] FIGS. 4A and 4B illustrate a time matched change of ACTH hormone profile.

DETAILED DESCRIPTION

[0045] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

Congenital adrenal hyperplasia

[0046] Congenital adrenal hyperplasia (CAH) is a group of rare inherited autosomal recessive disorders characterized by a deficiency of one of the enzymes needed to make specific hormones. CAH effects the adrenal glands located at the top of each kidney. Normally, the adrenal glands are responsible for producing three different hormones: 1) glucocorticoids, which gauge the body's response to stress, illness or injury; 2) mineralocorticoids, which regulate salt and water levels; and 3) androgens, which are male sex hormones. An enzyme deficiency will make the body unable to produce one or more of these hormones, which in turn will result in the overproduction of another type of hormone precursor in order to compensate for the loss.

[0047] The most common cause of CAH is the absence of the enzyme 21-hydroxylase. Different mutations in the gene responsible for 21-hydroxylase result in different levels of the enzyme, producing a spectrum of effects. CAH due to 21-hydroxylase deficiency is responsible for 95% of all cases of CAH and is broken down further into two subcategories: classic CAH, which can be sub-divided into the salt-losing form or the simple-virilizing form, and non-classic CAH. Classic CAH is by far the more severe form and can result in adrenal crisis and death if not detected and treated. Non-classic CAH is milder and may or may not present symptoms. Since the absence of 21-hydroxylase makes these individuals unable to make the hormone cortisol, a glucocorticoid, and, in the case of salt-losing CAH, aldosterone (a mineralocorticoid), the excess steroid precursors, namely 17-hydroxyprogesterone (17-OHP), build up and are shunted down the androgen pathway resulting in excess androgen production which cause a variety of symptoms such as abnormal genital development in infant girls. There are other much rarer forms of CAH as well, including 11-ß hydroxylase deficiency, 17α-hydroxylase deficiency, 3-ß-hydroxysteroid dehydrogenase deficiency, congenital lipoid adrenal hyperplasia and p450 oxidoreductase deficiency which all present different symptoms. CAH patients need adequate care and treatment in order to lead normal lives.

Corticotropin releasing factor

[0048] 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.

[0049] 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.

[0050] 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.

[0051] Treatment of CAH is based on normalization of hormone and steroid levels using a variety of chronic 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.

[0052] 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 (A4), testosterone, renin activity, and electrolytes.

[0053] Adult patients with CAH have an increased prevalence of risk factors for cardiovascular disease including obesity, hypertension, absence of the nadir drop in systolic

blood pressure overnight, 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 and adults). In some embodiments, there is a need for longer acting and more potent agents with narrow therapeutic indices, such as dexamethasone or prednisolone/prednisone, 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.

[0054] 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 CRF1 receptor, a member of the class B family of G-protein coupled receptors.

[0055] Due to the physiological significance of CRF1, the development of biologically-active small molecules having significant CRF receptor binding activity and which are capable of antagonizing the CRF1 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.

[0056] 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-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 (A4), 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.

Certain Definitions

[0057] 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.

[0058] 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.

[0059] The term "about" is used to indicate that a value includes 10% level of error for the device or method being employed to determine the value.

[0060] 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."

[0061] "Alkyl" refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, containing no unsaturation, and preferably having from one to five carbon atoms (i.e. C1-C5alkyl). In some embodiments, an alkyl comprises one to four carbon atoms (i.e., C1-C4alkyl).). In some embodiments, an alkyl comprises one to three carbon atoms (i.e., C1-C3alkyl).). In some embodiments, an alkyl comprises one to two carbon atoms (i.e., C1-C2alkyl). In some embodiments, an alkyl comprises one carbon atom (i.e., C1alkyl). In certain embodiments, the alkyl group is selected from methyl, ethyl, 1-propyl (n-propyl), 1-methylethyl (iso-propyl), 1-butyl (n-butyl), 1-methylpropyl (sec-butyl), 2-methylpropyl (isobutyl), 1,1-dimethylethyl (tert-butyl), or 1-pentyl (n-pentyl). The alkyl is attached to the rest of the molecule by a single bond. Unless stated otherwise specifically in the specification, an alkyl group is optionally substituted by one or more substituents such as those described herein.

[0062] 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.

[0063] "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.

[0064] 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.

[0065] The term "pharmaceutical composition" means a composition comprising at least one active ingredient, such as a steroid or a pharmaceutically acceptable salt thereof or a CRF1 antagonist or a pharmaceutically acceptable salt thereof, 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.

[0066] The term "supraphysiologic amount" describes glucocorticoid dose levels that are above the daily glucocorticoid requirement (production rate) found in healthy individuals.

[0067] The term "physiologic amount" describes glucocorticoid dose levels that meet the daily glucocorticoid requirement (production rate) found in healthy individuals.

[0068] The term "hydrocortisone equivalents" as used herein is understood by a person skilled in the art to be the conversion calculations needed to be considered when substituting one glucocorticoid for another as the potency and duration of action of various glucocorticoids may vary. Hence, the term "hydrocortisone equivalents" is the standard used for comparison of glucocorticoid potency.

[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.

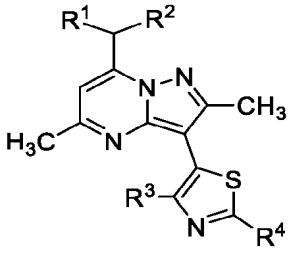
Compounds

[0071] Disclosed herein are CRF1 antagonists or pharmaceutically acceptable salt thereof such as Antalarmin hydrochloride, Pfizer CP 154526, CP 376395 hydrochloride, NBI 27914 hydrochloride, NBI 35965 hydrochloride, NGD 98-2 hydrochloride, Pexacerfont, R 121919 hydrochloride, SN003, and SSR125543.

[0072] In one aspect, the CRF1 antagonist or pharmaceutically acceptable salt thereof is selected from the group consisting of: N-Butyl-N-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine hydrochloride (Antalarmin hydrochloride), N-Butyl-N-ethyl-2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine hydrochloride (Pfizer CP154526), N-(1-Ethylpropyl)-3,6-dimethyl-2-(2,4,6-trimethylphenox y)-4-pyridinamine hydrochloride (Pfizer CP376395 hydrochloride), 5-Chloro-N-(cyclopropylmethyl)-2-methyl-N-propyl-N'-(2,4,6-trichlorophenyl)-4,6-pyrimidinediamine hydrochloride (NBI27914 hydrochloride), (7S)-6-(Cyclopropylmethyl)-2-(2,4-dichlorophenyl)-7-ethyl-7,8-dihydro-4-methyl-6H-1,3,6,8a-tetraazaacenaphthylene hydrochloride (NBI35965 hydrochloride), N-(1-Ethylpropyl)-3-methoxy-5-[2-methoxy-4-

(trifluoromethoxy)phenyl]-6-methyl-2-pyrazinamine hydrochloride (NGD 98-2 hydrochloride), 8-(6-Methoxy-2-methyl-3-pyridinyl)-2,7-dimethyl-N-[(1R)-1-methylpropyl]pyrazolo[1,5-a]-1,3,5-triazin-4-amine (Pexacerfont), 3-[6-(Dimethylamino)-4-methyl-3-pyridinyl]-2,5-dimethyl-N,N-dipropylpyrazolo[1,5-a]pyrimidin-7-amine hydrochloride (R 121919 hydrochloride), and N-(4-Methoxy-2-methylphenyl)-1-[1-(methoxymethyl)propyl]-6-methyl-1H-1,2,3-triazolo[4,5-c]pyridine-4-amine (SN003), (S)-4-(2-chloro-4-methoxy-5-methylphenyl)-N-(2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl)-5-methyl-N-(prop-2-yn-1-yl)thiazol-2-amine (SSR125543).

[0073] Disclosed herein is a compound of Formula (I):

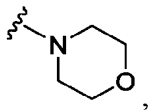
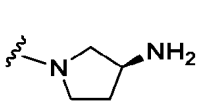
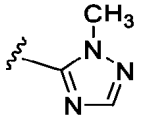
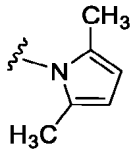
[0074] 

[0075] Formula (I)

[0076] or a pharmaceutically acceptable salt thereof, wherein

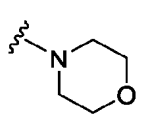
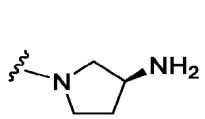
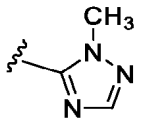
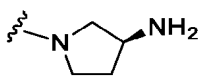
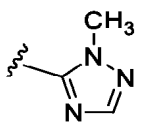
[0077] R1 and R2 are independently ethyl or n-propyl;

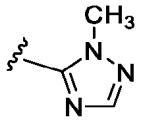
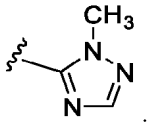
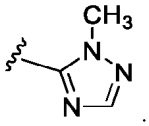
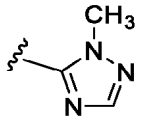
[0078] R3 is H, Cl, Br, methyl, trifluoromethyl or methoxy;

[0079] R4 is H, Br, RaRbN-, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , , , or ; and

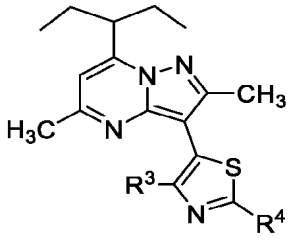
[0080] Ra and Rb are independently hydrogen, C1-C3 alkyl, H2NCH2CH2-, (CH3)3COC(O)NHCH2CH2-, or CH3CH2CH2NHCH2CH2.

[0081] In some embodiments, R3 is hydrogen, Cl, Br, methyl, or trifluoromethyl. In some embodiments, R3 is hydrogen, Cl, Br, or methyl. In some embodiments, R3 is hydrogen, Cl, or Br. In some embodiments, R3 is hydrogen. In some embodiments, R3 is Cl. In some embodiments, R3 is Br.

[0082] In some embodiments, R4 is hydrogen, Br, RaRbN–, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , ,or . In some embodiments, R4 is Br, RaRbN–, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, ,or . In some embodiments, R4 is RaRbN–, methoxymethyl, n-

butyl, acetamido, pyridin-4-yl, morpholin-4-yl, or . In some embodiments, R4 is RaRbN–, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, or . In some embodiments, R4 is RaRbN–,pyridin-4-yl, morpholin-4-yl, or . In some embodiments, R4 is morpholin-4-yl or . In some embodiments, R4 is RaRbN- and Ra and Rb are independently C1-C3alkyl.

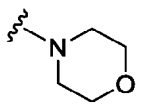
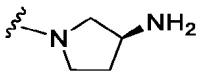
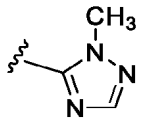
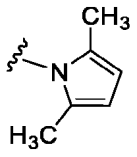
[0083] Disclosed herein is a compound of Formula (II):



[0084] Formula (II)

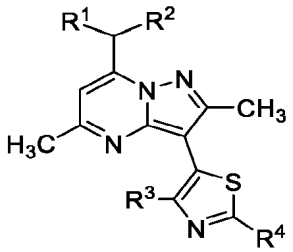
[0085] or a pharmaceutically acceptable salt thereof, wherein

[0086] R3 is H, Cl, Br, methyl, trifluoromethyl or methoxy;

[0087] R4 is H, Br, RaRbN-, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , , , or ; and

[0088] Ra and Rb are independently hydrogen, C1-C3 alkyl, H2NCH2CH2-, (CH3)3COC(O)NHCH2CH2-, or CH3CH2CH2NHCH2CH2.

[0089] Disclosed herein is a compound of Formula (III):

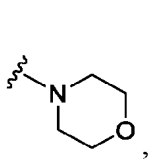
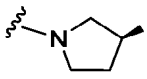
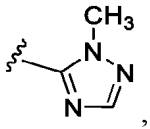
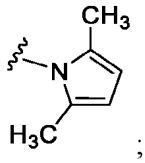


[0090] Formula (III)

[0091] or a pharmaceutically acceptable salt thereof, wherein

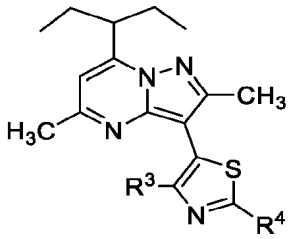
[0092] R1 and R2 are n-propyl;

[0093] R3 is H, Cl, Br, methyl, trifluoromethyl or methoxy;

[0094] R4 is H, Br, RaRbN-, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , , , or ; and

[0095] Ra and Rb are independently hydrogen, C1-C3 alkyl, H2NCH2CH2-, (CH3)3COC(O)NHCH2CH2-, or CH3CH2CH2NHCH2CH2.

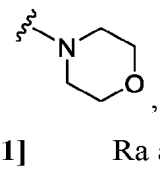
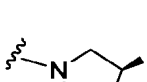
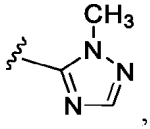
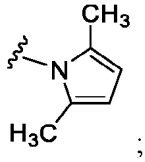
[0096] Disclosed herein is a compound of Formula (IV):



[0097] Formula IV

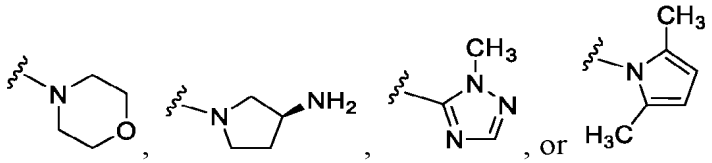
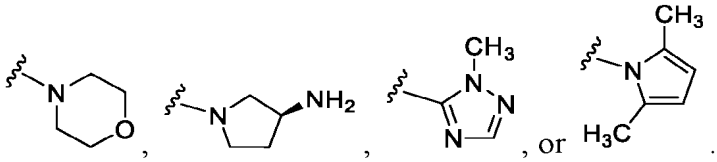
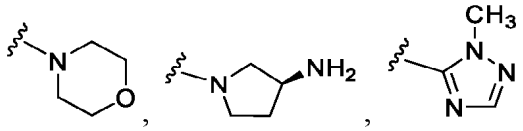
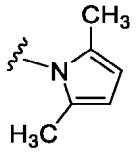
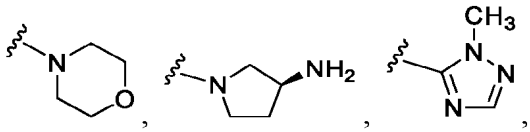
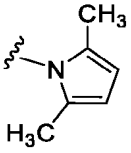
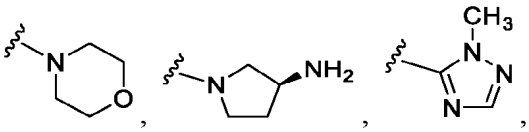
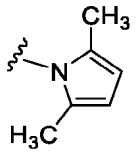
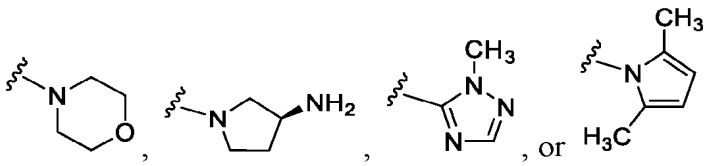
[0098] or a pharmaceutically acceptable salt thereof, wherein

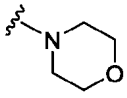
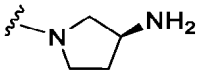
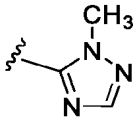
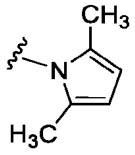
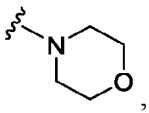
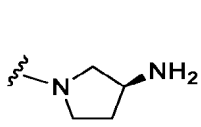
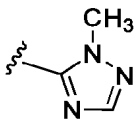
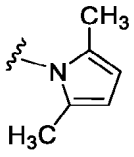
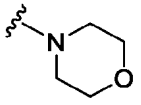
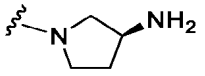
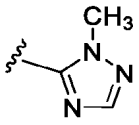
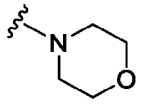
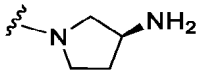
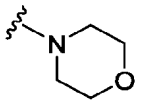
[0099] R3 is Cl, Br, methyl, trifluoromethyl or methoxy;

[0100] R4 is H, Br, RaRbN-, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , , , or ; and

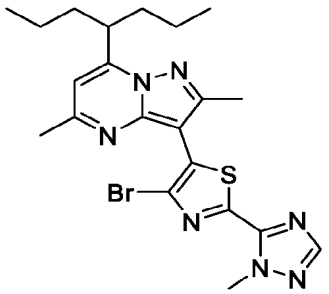
[0101] Ra and Rb are independently hydrogen, C1-C3 alkyl, H2NCH2CH2-, (CH3)3COC(O)NHCH2CH2-, or CH3CH2CH2NHCH2CH2.

[0102] For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R3 may be hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy. For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R3 may be Cl, Br, methyl, trifluoromethyl, or methoxy. For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R3 may be Cl, Br, methyl, or trifluoromethyl. For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R3 may be Cl, Br, or methyl. For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R3 may be Cl. For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R3 may be Br. For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R3 may be methyl.

[0103] For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R4 is Br, RaRbN-, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R4 is Br, methoxymethyl, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R4 is Br, n-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , or . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R4 is Br, acetamido, pyridin-4-yl, morpholin-4-yl, , . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R4 is Br, pyridin-4-yl, morpholin-4-yl, , or . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R4 is pyridin-4-yl, morpholin-4-yl, . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R4 is

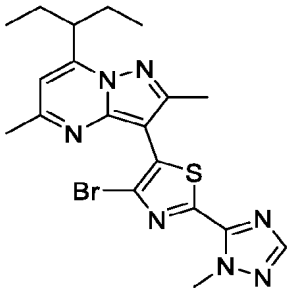
morpholin-4-yl, , , , or . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R4 is , , , or . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R4 is , , or . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R4 is , or . For a compound or pharmaceutically acceptable salt of Formula (I), (II), (III), and (IV), R4 is .

[0104] Disclosed herein is 3-[4-bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl]-2,5-dimethyl-7-(1-propyl-butyl)-pyrazolo[1,5-a]pyrimidine or a pharmaceutically acceptable salt thereof:



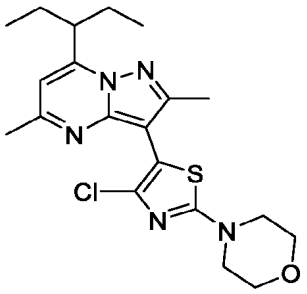
Compound 1

[0105] Disclosed herein is 3-(4-bromo-2-(2-methyl-2H-[1,2,4]triazol-3-yl)-thiazol-5-yl)-7-(1-ethyl-propyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidine or a pharmaceutically acceptable salt thereof:



Compound 2

[0106] 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 thereof:



Compound 3.

[0107] 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 3. 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 3.

[0108] In one aspect, the CRF1 antagonist or pharmaceutically acceptable salt thereof may be an astressin. An astressin generally refers to a nonselective corticotropin releasing hormone antagonist that reduces the synthesis of ACTH and cortisol.

Pharmaceutical Compositions

[0109] Disclosed herein are pharmaceutical compositions comprising a compound or pharmaceutically acceptable salt described herein. In certain embodiments, the composition comprises a steroid or a pharmaceutically acceptable salt thereof and a CRF_1 antagonist or pharmaceutically acceptable salt thereof as disclosed above. In some embodiments, the steroid or a pharmaceutically acceptable salt thereof is exogenous glucocorticoids (GC) or a pharmaceutically acceptable salt thereof.

Dosage Form

[0110] 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 a compound or pharmaceutically acceptable salt described herein 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.

[0111] 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.

[0112] 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

[0113] 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.

[0114] 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.

[0115] In some embodiments, the capsule comprises one or more pharmaceutically acceptable excipients. In some embodiments, the capsule is free of additional excipients.

[0116] 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

[0117] 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.

[0118] In some embodiments, the pharmaceutical composition is formulated as a tablet.

[0119] 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 a compound or pharmaceutically acceptable salt described herein 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 a compound or pharmaceutically acceptable salt described herein, 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 a compound or pharmaceutically acceptable salt described herein, 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 a compound or pharmaceutically acceptable salt described herein. 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.

[0120] 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.

[0121] 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.

[0122] In some embodiments, the tablet is produced at a commercial scale.

[0123] In some embodiments, the tablet comprises one or more pharmaceutically acceptable excipients.

[0124] 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 a compound or pharmaceutically acceptable salt described herein. 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

[0125] 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.

[0126] 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

[0127] In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 500 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 400 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 300 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 200 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 100 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 90 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 80 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 70 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 60 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 50 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 40 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 30 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 20 mg

of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 1 mg and about 10 mg of a compound or pharmaceutically acceptable salt described herein.

[0128] In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 500 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 400 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 300 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 200 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 100 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 90 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 80 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 70 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 60 mg of a compound or pharmaceutically acceptable salt described herein f. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 50 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 40 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 30 mg of a compound or pharmaceutically acceptable salt described herein. In some embodiments, the pharmaceutical composition, in the form of a tablet or capsule, comprises between about 10 mg and about 20 mg of a compound or pharmaceutically acceptable salt described herein.

Particle size

[0129] In some embodiments, the pharmaceutical composition, in the form of a tablet or a capsule, comprises a compound or pharmaceutically acceptable salt described herein, in the form

of microparticles. In some embodiments, the microparticles have an average size from about 1 μm to about 100 μm. In some embodiments, the microparticles have an average size from about 1 μm to about 50 μm. In some embodiments, the microparticles have an average size from about 1 μm to about 30 μm. In some embodiments, the microparticles have an average size from about 1 μm to about 20 μm. In some embodiments, the microparticles have an average size from about 5 μm to about 15 μm. In some embodiments, the microparticles have an average size from about 1 μm to about 10 μm. In some embodiments, the microparticles have an average size from about 3 μm to about 10 μm. In some embodiments, the microparticles have an average size from about 4 μm to about 9 μm.

Methods of Treatment

[0130] Disclosed herein are methods of treating congenital adrenal hyperplasia (CAH) in a subject in need thereof, comprising administering a compound or pharmaceutically acceptable salt described herein. In some embodiments, the methods described herein result in the reduction of a level of a hormone. Such hormones include deoxycorticosterone, 11-deoxycortisol, cortisol, corticosterone, aldosterone, pregnenolone, 17α-hydroxy pregnenolone, progesterone, 17-OHP, dehydroepiandrosterone, androstenediol, A4, testosterone, dihydrotestosterone, estrone, estradiol, estriol, and ACTH. In some embodiments, the methods described herein result in the reduction of 17-OHP levels. In some embodiments, the methods described herein result in the reduction of A4 levels. In some embodiments, the methods described herein result in the reduction of ACTH levels, also known as corticotropin.

[0131] Further, in some embodiments, the methods described herein result in the maintenance of the reduction of 17-OHP, A4, and/or ACTH levels. In some embodiments, the reductions of 17-OHP, A4, and/or ACTH levels may last for at least 24 hours, 36 hours, 48 hours, 60 hours, 72 hours, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, 21 weeks, 22 weeks, 23 weeks, 24 weeks, 25 weeks, 26 weeks, 27 weeks, 28 weeks, 29 weeks, 30 weeks, 31 weeks, 32 weeks, 33 weeks, 34 weeks, 35 weeks, 36 weeks, 37 weeks, 38 weeks, 39 weeks, 40 weeks, 41 weeks, 42 weeks, 43 weeks, 44 weeks, 45 weeks, 46 weeks, 47 weeks, 48 weeks, 49 weeks, 50 weeks, 51 weeks, 52 weeks, 2 years, 5 years, 10 years, 15 years, 20 years, 25 years, 30 years, 35 years, 40 years, 45 years, 50 years, 55 years, 60 years, 65 years, 70 years, 75 years, 80 years, 85 years, 90 years, 95 years, or 100 years.

[0132] Moreover, the present disclosure provides different patient subtype regimens for treating Congenital Adrenal Hyperplasia (CAH) patients. In one aspect, the present disclosure provides a method of treating Congenital Adrenal Hyperplasia (CAH), comprising administering

an effective amount of a steroid or a pharmaceutically acceptable salt thereof and a corticotropin-releasing factor type-1 (CRF1) antagonist or a pharmaceutically acceptable salt thereof to a subject, i.e., a CAH patient/individual, over a 24 hour period, which the effective amount of the steroid is dependent on the levels of steroid hormones, such as ACTH, A4, 17-OHP, or any combinations thereof, of the subject measured at the time of beginning the treatment of CAH, typically in the am. Prior to implementing the method of treatment disclosed herein, a CAH patient may be evaluated to determine the patient's plasma steroid hormone levels.

[0133] Generally, a normal range, a normal laboratory reference range, or a reference range of ACTH level or A4 level in a subject may be broad and dependent on many different factors. For example, ACTH, 17-OHP or A4 is secreted in bursts with a daily rhythm in a subject. Plasma samples collected at 8 am in the morning may show different range of ACTH or A4 levels in a subject compared to plasma samples collected at 2 pm, 4 pm, or midnight during the day. Further, the normal range of ACTH or A4 levels are dependent on a factor such as laboratory setting. Laboratory setting may comprise different types of assays for detecting ACTH or A4 levels, equipment utilized in the laboratory, personnel in the laboratory performing the assays, and different batches of reagents used during the assays. Different ages of a subject may provide different normal ranges of ACTH or A4 levels as well. Moreover, a normal range of ACTH level or A4 level in a subject may be dependent on the sex of the subject. For example, not meant to be limiting, a male subject's normal range of ACTH level may be from 7 to 63.3 pg/mL, as assessed in the am, typically before 10 am, and A4 level, also assessed in the am, typically before 10 am, may be from 50 to 220 ng/dL for males aged 18 to 30 years, from 40 to 190 ng/dL for males aged 31 to 50 years and from 50 to 220 ng/dL for males aged 51 to 60 years. Additionally, a normal range of ACTH level or A4 level in a female subject may be dependent on the female subject's menopausal stage. For example, not meant to be limiting, a pre-menopausal female subject's normal range of ACTH level may be from 7 to 63 pg/mL, as assessed in the am, typically before 10 am, and A4 level may be from 51 to 213 ng/dL for women in the mid-follicular stage, from 73 to 230 ng/dL for women in the surge stage, from 73 to 184 for women in the mid luteal stage. A post-menopausal female subject's normal range of ACTH level may be from 7 to 63.3 pg/mL and A4 level may be from 20 to 75 ng/dL. Normal ranges for PM assessments of ACTH and A4 have not been established.

[0134] In some embodiments, when a subject has levels that are less than two (2) times an upper limit of the normal ranges or the normal laboratory reference ranges of ACTH and A4 level respectively, a percentage of the total daily GC dose, typically done in 5 mg hydrocortisone equivalents, of the effective amount of the steroid or a pharmaceutically acceptable salt thereof is reduced compared to a dose of the effective amount of the steroid or a pharmaceutically

acceptable salt thereof administered to a CAH patient without receiving a CRF1 antagonist (i.e., a compound disclosed herein) or a pharmaceutically acceptable salt thereof. In some embodiments, the subject has a level that is less than 2 times the upper limit of the normal range of ACTH or A4 level. In some embodiments, the subject has a level that is less than 1.75 times the upper limit of the normal range of ACTH or A4 level. In some embodiments, the subject has a level that is less than 1.5 times the upper limit of the normal range of ACTH or A4 level. In some embodiments, the subject has a level that is less than 1.25 times the upper limit of the normal range of ACTH or A4 level. In some embodiments, the subject has a level that is less than 1.0 times the upper limit of the normal range of ACTH or A4 level.

[0135] In certain embodiments, as long as the levels of both the ACTH and A4 are less than 2 times an upper limit of the normal reference ranges of ACTH and A4 respectively, the dosage of the steroid or a pharmaceutically acceptable salt thereof is reduced. For example, a CAH patient's biological sample may be measured to show that the patient's ACTH level is less than 1.75 times the upper limit of the normal range of ACTH and A4 level is less than 1.25 the upper limit of the normal range of A4. Further, a CAH patient's biological sample may be measured to show that the patient's ACTH level is less than 1.25 times the upper limit of the normal range of ACTH and A4 level is less than 1.5 the upper limit of the normal range of A4. Moreover, a CAH patient's biological sample may be measured to show that the patient's ACTH level is less than 1.5 times the upper limit of the normal range of ACTH and A4 level is less than 1.5 the upper limit of the normal range of A4. Additionally, a CAH patient's biological sample may be measured to show that the patient's ACTH level is less than 1.25 times the upper limit of the normal range of ACTH and A4 level is less than 1.25 the upper limit of the normal range of A4.The regimen of treating with reduced amount of the steroid or a pharmaceutically acceptable salt thereof compared to the amount of the steroid or a pharmaceutically acceptable salt thereof administered to a CAH patient without receiving a CRF1 antagonist (i.e., a compound disclosed herein) or a pharmaceutically acceptable salt thereof may be utilized. The reduction of GC daily does may be done in a 5 mg hydrocortisone equivalents, 4 mg hydrocortisone equivalents, 3 mg hydrocortisone equivalents, 2 mg hydrocortisone equivalents, 1 mg hydrocortisone equivalents, or 0.5 mg hydrocortisone equivalents.

[0136] In some embodiments, when a subject has levels that are less than two (2) times an upper limit of the normal ranges or the normal laboratory reference ranges of ACTH and 17-OHP level respectively, a percentage of the total daily GC dose, typically done in 5 mg hydrocortisone equivalents, of the effective amount of the steroid or a pharmaceutically acceptable salt thereof is reduced compared to a dose of the effective amount of the steroid or a pharmaceutically acceptable salt thereof administered to a CAH patient without receiving a CRF1 antagonist (i.e.,

a compound disclosed herein) or a pharmaceutically acceptable salt thereof. In some embodiments, the subject has a level that is less than 2 times the upper limit of the normal range of ACTH or 17-OHP level. In some embodiments, the subject has a level that is less than 1.75 times the upper limit of the normal range of ACTH or 17-OHP level. In some embodiments, the subject has a level that is less than 1.5 times the upper limit of the normal range of ACTH or 17-OHP level. In some embodiments, the subject has a level that is less than 1.25 times the upper limit of the normal range of ACTH or 17-OHP level. In some embodiments, the subject has a level that is less than 1.0 times the upper limit of the normal range of ACTH or 17-OHP level. As long as the levels of both the ACTH and 17-OHP are less than 2 times an upper limit of the normal reference ranges of ACTH and 17-OHP respectively, the dosage of the steroid or a pharmaceutically acceptable salt thereof is reduced. For example, a CAH patient's biological sample may be measured to show that the patient's ACTH level is less than 1.5 times the upper limit of the normal range of ACTH and 17-OHP level is less than 1.75 the upper limit of the normal range of 17-OHP. Further, a CAH patient's biological sample may be measured to show that the patient's ACTH level is less than 1 times the upper limit of the normal range of ACTH and 17-OHP level is less than 1.25 the upper limit of the normal range of 17-OHP. The regimen of treating with reduced amount of the steroid or a pharmaceutically acceptable salt thereof compared to the amount of the steroid or a pharmaceutically acceptable salt thereof administered to a CAH patient without receiving a CRF1 antagonist (i.e., a compound disclosed herein) or a pharmaceutically acceptable salt thereof may be utilized.

[0137] In some embodiments, when a subject has levels that are less than two (2) times an upper limit of the normal ranges or the normal laboratory reference ranges of A4 and 17-OHP level respectively, a percentage of the total daily GC dose, typically done in 5 mg hydrocortisone equivalents, of the effective amount of the steroid or a pharmaceutically acceptable salt thereof is reduced compared to a dose of the effective amount of the steroid or a pharmaceutically acceptable salt thereof administered to a CAH patient without receiving a CRF1 antagonist (i.e., a compound disclosed herein) or a pharmaceutically acceptable salt thereof. In some embodiments, the subject has a level that is less than 2 times the upper limit of the normal range of A4 or 17-OHP level. In some embodiments, the subject has a level that is less than 1.75 times the upper limit of the normal range of A4 or 17-OHP level. In some embodiments, the subject has a level that is less than 1.5 times the upper limit of the normal range of A4 or 17-OHP level. In some embodiments, the subject has a level that is less than 1.25 times the upper limit of the normal range of A4 or 17-OHP level. In some embodiments, the subject has a level that is less than 1.0 times the upper limit of the normal range of A4 or 17-OHP level. As long as the levels of both the A4 and 17-OHP are less than 2 times an upper limit of the normal reference ranges of

A4 and 17-OHP respectively, the dosage of the steroid or a pharmaceutically acceptable salt thereof is reduced. For example, a CAH patient's biological sample may be measured to show that the patient's A4 level is less than 1 times the upper limit of the normal range of A4 and 17-OHP level is less than 1.75 the upper limit of the normal range of 17-OHP. Further, a CAH patient's biological sample may be measured to show that the patient's A4 level is less than 1.5 times the upper limit of the normal range of A4 and 17-OHP level is less than 1.25 the upper limit of the normal range of 17-OHP. The regimen of treating with reduced amount of the steroid or a pharmaceutically acceptable salt thereof compared to the amount of the steroid or a pharmaceutically acceptable salt thereof administered to a CAH patient without receiving a CRF1 antagonist (i.e., a compound disclosed herein) or a pharmaceutically acceptable salt thereof may be utilized.

[0138] When a CRF1 antagonist is not administered, treatment of CAH patients needs to replace the cortisol deficiency and normalize the excess androgen production, which is a sequalae of the enzymatic deficiency in CAH, by administering exogenous glucocorticoids (GC) at a dose that is usually well above the normal physiologic does used for cortisol replacement in patients with other diseases, such as Addison's disease. Reduced levels of exposure to steroids, form supraphysiologic to physiologic levels, may be beneficial to CAH patients. In some embodiments, a dose of about 10 mg to about 80 mg GC is administered to a CAH patient when a CRF1 antagonist is not administered. In some embodiments, the dose of GC is administered to CAH patients one time, two times, three times, or four times daily.

[0139] For example, not meant to be limiting, a dose of about 80mg GC could be administered to a CAH patient when a CRF1 antagonist is not administered. If this CAH patient's ACTH level and A4 level are less than 2, 1.5, or 1.25 times the upper limit of the normal range of ACTH level and A4 levels respectively after controlled by factors such as age, sex, menopausal status, laboratory setting, the dose of 80mg GC may be reduced in a 5 mg hydrocortisone equivalent increment when administered together with the CRF1 antagonist (i.e., a compound disclosed herein). In some embodiments, a corticotropin-releasing factor type-1 (CRF1) antagonist or a pharmaceutically acceptable salt thereof in a dose of about 10 mg to about 200 mg total daily dose is administered to the subject. The CAH patient may be receiving a dose of 75 mg GC during the treatment regimen when the compound is administered together. After a certain period, when the CAH patient's ACTH level and A4 levels remain less than 2, 1.5, or 1.25 times the upper limit of the normal range of the ACTH and A4 levels respectively, the dose of 75 mg GC may be further reduced in a 5 mg increment to 70 mg when administered with the CRF1 antagonist. The dose of the GC may be continuously reduced in a 5 mg increment hydrocortisone equivalent when administered together with the CRF1 antagonist, over a 24 hour

period, until the subject's ACTH or A4 level is not less than 2, 1.5, or 1.25 times the upper limit of the normal range of ACTH or A4 level.

[0140] Moreover, if this CAH patient's ACTH level is less than 1.75 times the upper limit of the normal range of ACTH level and A4 level is less than 1.5 times the upper limit of the normal range of A4 level after controlled by factors such as age, sex, menopausal status, laboratory setting, the dose of 80mg GC may be reduced in a 5 mg increment when administered together the CRF1 antagonist (i.e., a compound disclosed herein). If this CAH patient's ACTH level is less than 1.5 times the upper limit of the normal range of ACTH level and A4 level is less than 1.75 times the upper limit of the normal range of A4 level after controlled by factors such as age, sex, menopausal status, laboratory setting, the dose of 80mg GC may be reduced in a 5 mg increment when administered together the CRF1 antagonist (i.e., a compound disclosed herein). If this CAH patient's ACTH level is less than 1.25 times the upper limit of the normal range of ACTH level and A4 level is less than 1.25 times the upper limit of the normal range of A4 level after controlled by factors such as age, sex, menopausal status, laboratory setting, the dose of 80mg GC may be reduced in a 5 mg increment when administered together the CRF1 antagonist (i.e., a compound disclosed herein). In some embodiments, if this CAH patient's ACTH level is less than 1.0 times the upper limit of the normal range of ACTH level and A4 level is less than 1.0 times the upper limit of the normal range of A4 level after controlled by factors such as age, sex, menopausal status, laboratory setting, the dose of 80mg GC may be reduced in a 5 mg increment when administered together with the CRF1 antagonist (i.e., a compound disclosed herein).

[0141] In some embodiments, the subject will be treated for a period of about 1 week to about 40 weeks. In some embodiments, the subject will be treated for a period of about 2 weeks to about 39 weeks. In some embodiments, the subject will be treated for a period of about 3 weeks to about 38 weeks. In some embodiments, the subject will be treated for a period of about 4 weeks to about 36 weeks. In some embodiments, if a subject has been treated for a period of time described herein and has levels of A4 and ACTH less than the lower limit of the normal laboratory reference range, the subject may continue with steroid reduction until the subject maintain hormone levels consistently within the normal range of A4 and ACTH.

[0142] Further, in some embodiments, when a subject has a level that is more than an upper limit of the normal range or the normal laboratory reference range of ACTH or A4 level, the total daily dose of the effective amount of the steroid or a pharmaceutically acceptable salt thereof is not reduced compared to a dose of the effective amount of the steroid or a pharmaceutically acceptable salt thereof administered to a CAH patient without receiving a CRF1 antagonist (i.e., a compound disclosed herein) or a pharmaceutically acceptable salt thereof. In some

embodiments, the subject has a level that is more than 2 times the upper limit of the normal range of ACTH or A4 level. Some subjects may further be categorized as having severe control issues when the subjects have more than 4 times an upper limit of the normal range of both ACTH and A4 levels. Moreover, some subjects may be further categorized as having moderate control issues when the subjects have more than 2 times the upper limit of normal range of the ACTH level but less than 4 times the upper limit of normal range of the A4 level. In some embodiments, the subject has a level that is more than 1.75 times the upper limit of the normal range of ACTH or A4 level. In some embodiments, the subject has a level that is more than 1.5 times the upper limit of the normal range of ACTH or A4 level. In some embodiments, the subject has a level that is more than 1.25 times the upper limit of the normal range of ACTH or A4 level. In some embodiments, the subject has a level that is more than 1.0 times the upper limit of the normal range of ACTH or A4 level.

[0143] Additionally, in some embodiments, when a subject has an ACTH level that is more than an upper limit of the normal range or the normal laboratory reference range of ACTH level and an A4 level that is more than an upper limit of the normal range or the normal laboratory reference range of A4, the total daily dose of the effective amount of the steroid or a pharmaceutically acceptable salt thereof is not reduced compared to a dose of the effective amount of the steroid or a pharmaceutically acceptable salt thereof administered to a CAH patient without receiving a CRF1 antagonist (i.e., a compound disclosed herein) or a pharmaceutically acceptable salt thereof. In some embodiments, the subject has an ACTH level that is more than 1.75 times the upper limit of the normal range of ACTH and an A4 level that is more than 1.5 times the upper limit of the normal range of A4. In some embodiments, the subject has an ACTH level that is more than 1.5 times the upper limit of the normal range of ACTH and an A4 level that is more than 1.25 times the upper limit of the normal range of A4. In some embodiments, the subject has an ACTH level that is more than 1.25 times the upper limit of the normal range of ACTH and an A4 level that is more than 1 times the upper limit of the normal range of A4. In some embodiments, the subject has an ACTH level that is more than 1 times the upper limit of the normal range of ACTH and an A4 level that is more than 1.25 times the upper limit of the normal range of A4. In some embodiments, the subject has an ACTH level that is more than 1.25 times the upper limit of the normal range of ACTH and an A4 level that is more than 1.5 times the upper limit of the normal range of A4. In some embodiments, the subject has an ACTH level that is more than 1.5 times the upper limit of the normal range of ACTH and an A4 level that is more than 1.75 times the upper limit of the normal range of A4.

[0144] In some embodiments, a dose of about 10 mg to about 80 mg GC is administered to a CAH patient when a CRF1 antagonist is not administered. In some embodiments, the dose of

GC is administered to CAH daily one time, two times, three times, or four times. In some embodiments, a corticotropin-releasing factor type-1 (CRF1) antagonist or a pharmaceutically acceptable salt thereof in a dose of about 10 mg to about 200 mg total daily dose is administered to the subject. For example, not meant to be limiting, a dose of about 80 mg GC administered to a CAH patient when a CRF1 antagonist is not administered. If this CAH patient's ACTH level or A4 level is more than the upper limit of the normal range of ACTH level or A4 level after controlled by factors such as age, sex, menopausal status, laboratory setting, the dose of 80 mg GC may be maintained when administered together the CRF1 antagonist (i.e., a compound disclosed herein). Further, if this CAH patient's ACTH level and A4 levels are more than the upper limit of the normal range of ACTH level and A4 level respectively after controlled by factors such as age, sex, menopausal status, laboratory setting, the dose of 80 mg GC may be maintained when administered together the CRF1 antagonist (i.e., a compound disclosed herein).

[0145] During the treatment of administering the maintained dose of GC together with the CRF1 antagonist, the CAH patient's ACTH and A4 levels are monitored. In some embodiments, as soon as the CAH patient's ACTH and A4 level become less than 2 times the upper limit of the normal range of the ACTH and A4 level respectively, the dose of GC may be reduced in a 5 mg hydrocortisone equivalent increment to the CAH patient when administered together with the CRF1 antagonist. In some embodiments, as soon as the CAH patient's ACTH and A4 levels become less than 1.75 times the upper limit of the normal range of the ACTH and A4 level respectively, the dose of GC may be reduced in a 5 mg hydrocortisone equivalent increment to the CAH patient when administered together with the CRF1 antagonist. In some embodiments, as soon as the CAH patient's ACTH and A4 levels become less than 1.5 times the upper limit of the normal range of the ACTH and A4 level respectively, the dose of GC may be reduced in a 5 mg hydrocortisone equivalent increment to the CAH patient when administered together with the CRF1 antagonist. In some embodiments, as soon as the CAH patient's ACTH and A4 levels become less than 1.25 times the upper limit of the normal range of the ACTH and A4 levels respectively, the dose of GC may be reduced in a 5 mg hydrocortisone equivalent increment to the CAH patient when administered together with the CRF1 antagonist. In some embodiments, as soon as the CAH patient's ACTH and A4 levels become less than 1.0 times the upper limit of the normal range of the ACTH and A4 levels respectively, the dose of GC may be reduced in a 5 mg hydrocortisone equivalent increment to the CAH patient when administered together with the CRF1 antagonist. Moreover, the dose of the GC may be continuously reduced in a 5 mg increment when administered together with the CRF1 antagonist until the subject's ACTH and A4 levels are not less than 2, 1.5, or 1.25 times the upper limit of the normal range of ACTH and A4 levels respectively.

[0146] In some embodiments, the subject will be treated for a period of about 1 week to about 40 weeks. In some embodiments, the subject will be treated for a period of about 2 weeks to about 39 weeks. In some embodiments, the subject will be treated for a period of about 3 weeks to about 38 weeks. In some embodiments, the subject will be treated for a period of about 4 weeks to about 36 weeks. In some embodiments, after the subject has been treated for a period of time described herein, the subject may be reassessed to determine whether a different patient subtype regimen may be applicable.

[0147] In some embodiments, the dose of steroid or a pharmaceutically acceptable salt thereof is in a dose of about 10 mg to about 80 mg of hydrocortisone equivalents. In some embodiments, the steroid is a glucocorticoid or a pharmaceutically acceptable salt thereof. In some embodiments, the subject is tested in the morning to determine morning levels of A4 and ACTH.

[0148] With specificity provided from a particular laboratory setting, in some embodiments, two times the upper limit of the normal laboratory reference range of A4 for a male subject is more than about 440 ng/dl, more than about 430 ng/dl, more than about 420 ng/dl, more than about 410 ng/dl, more than about 400 ng/dl, more than about 390 ng/dl, more than about 380 ng/dl, more than about 370 ng/dl. In some embodiments, the normal laboratory reference range of A4 for a male subject is from about 40 ng/dl to about 190 ng/dl. In some embodiments, the normal laboratory reference range of A4 for a male subject is from about 50 ng/dl to about 220 ng/dl.

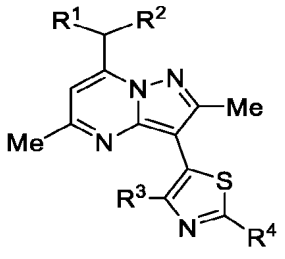
[0149] In some embodiments, two times the upper limit of the normal laboratory reference range of A4 for a female subject is more than about 560 ng/dl, more than about 550 ng/dl, more than about 540 ng/dl, more than about 530 ng/dl, more than about 520 ng/dl, more than about 510 ng/dl, 500 ng/dl, more than about 490 ng/dl, more than about 480 ng/dl, more than about 470 ng/dl, more than about 460 ng/dl, more than about 450 ng/dl, more than about 430 ng/dl, more than about 420 ng/dl, more than about 410 ng/dl, 400 ng/dl, more than about 390 ng/dl, more than about 380 ng/dl, more than about 370 ng/dl, more than about 360 ng/dl, more than about 350 ng/dl, or more than about 340 ng/dl. In some embodiments, the normal laboratory reference range of A4 for a female subject is from about 51 ng/dl to about 213 ng/dl. In some embodiments, the normal laboratory reference range of A4 for a female subject is from about 73 ng/dl to about 230 ng/dl. In some embodiments, the normal laboratory reference range of A4 for a female subject is from about 73 ng/dl to about 184 ng/dl.

[0150] In some embodiments, two time the upper limit of the normal laboratory reference of ACTH is more than about 110 pg/dl, more than about 120 pg/dl, more than about 130 pg/dl,

more than about 140 pg/dl, or more than about 150 pg/dl. In some embodiments, the normal laboratory reference range of ACTH is from about 7 pg/dl to about 70 pg/dl.

[0151] In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt thereof is selected from the group consisting of: Antalarmin hydrochloride, Pfizer CP 154526, CP 376395 hydrochloride, NBI 27914 hydrochloride, NBI 35965 hydrochloride, NGD 98-2 hydrochloride, Pexacerfont, R 121919 hydrochloride, SN003.

[0152] In some embodiments, the CRF_1 antagonist is a compound of Formula (I):

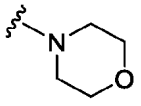
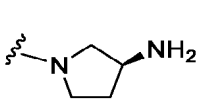
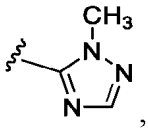
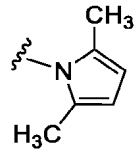


(I),

or a pharmaceutically acceptable salt thereof, wherein:

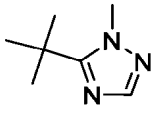
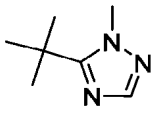
R^1 and R^2 are independently ethyl or *n*-propyl;

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

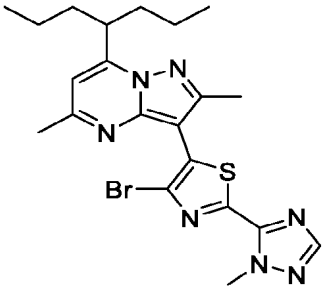
R^4 is hydrogen, Br, R^aR^bN–, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , , , or ; and

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

[0153] In some embodiments, R^3 is Cl, Br, methyl, or trifluoromethyl. In some embodiments, R^3 is Cl, Br, or methyl.

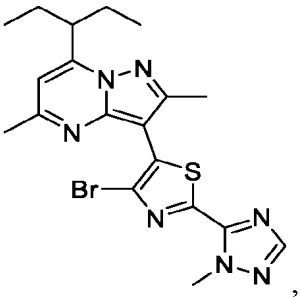
[0154] In some embodiments, R^4 is Br, R^aR^bN–, pyridin-4-yl, morpholin-4-yl, or . In some embodiments, R^4 is morpholin-4-yl or . In some embodiments, R^4 is hydrogen, Br, R^aR^bN– and R^a and R^b are independently C_1-C_3alkyl.

[0155] In some embodiments, the compound is



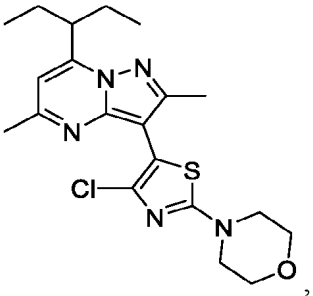
, or a pharmaceutically acceptable salt thereof.

[0156] In some embodiments, the compound is



, or a pharmaceutically acceptable salt thereof.

[0157] In some embodiments, the compound is



, or a pharmaceutically acceptable salt thereof.

[0158] In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 25 mg to about 200 mg total daily dose to the subject. In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 200 mg total daily dose to the subject.

[0159] In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 150 mg total daily dose to the subject. In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 100 mg total daily dose to the subject. In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 50 mg total daily dose to the subject. In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 40 mg total daily dose to the subject. In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 30 mg total daily dose to the subject. In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 25 mg total daily dose to the subject. In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 20 mg

total daily dose to the subject. In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 15 mg total daily dose to the subject. In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 10 mg total daily dose to the subject. In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 5 mg total daily dose to the subject.

[0160] In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt is in the form of microparticles. In some embodiments, the average size of the microparticles is between about 1 μm to about 20 μm. In some embodiments, the average size of the microparticles is between about 5 μm to about 15 μm. In some embodiments, the average size of the microparticles is less than about 10 μ.

[0161] In some embodiments, the CRF1 antagonist or pharmaceutically acceptable salt thereof is administered as a pharmaceutical composition. In some embodiments, the steroid or a pharmaceutically acceptable salt thereof is administered as a pharmaceutical composition.

[0162] In some embodiments, the pharmaceutical composition is in the form of a capsule or a tablet. 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.

[0163] 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.

[0164] In some embodiments, CRF1 antagonist or pharmaceutically acceptable salt 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, CRF1 antagonist or pharmaceutically acceptable salt 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, CRF1 antagonist or pharmaceutically acceptable salt 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, CRF1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 3 to about 5 hours in a subject.

[0165] In some embodiments, CRF1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 8 hours in a subject. In some

embodiments, CRF1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 7 hours in a subject. In some embodiments, CRF1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 6 hours in a subject. In some embodiments, CRF1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 5 hours in a subject. In some embodiments, CRF1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 4 hours in a subject. In some embodiments, CRF1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 3 hours in a subject. In some embodiments, CRF1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 2 hours in a subject. In some embodiments, CRF1 antagonist or pharmaceutically acceptable salt is formulated as a capsule or a tablet as to provide a Tmax of about 1 hour in a subject.

[0166] In some embodiments, the methods described herein include administration of the pharmaceutical composition comprising CRF1 antagonist or pharmaceutically acceptable salt, 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 CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof once a day. In some embodiments, the methods described herein administer CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof twice a day.

[0167] In some embodiments, the methods described herein include administration of about 1 mg to about 2000 mg of CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, per day. In some embodiments, CRF1 antagonist or pharmaceutically acceptable salt 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.

[0168] 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.

[0169] In some embodiments, about 100 mg to about 1600 mg of CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 1600 mg of CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 1200 mg of CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 1000 mg of CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 800 mg of CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 100 mg to about 800 mg of CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 800 mg of CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 100 mg to about 600 mg of CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is

administered per day. In some embodiments, about 200 mg to about 600 mg of CRF1 antagonist or pharmaceutically acceptable salt is administered per day. In some embodiments, about 300 mg to about 600 mg of CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 100 mg to about 400 mg of CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 200 mg to about 400 mg of CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, about 300 mg to about 400 mg of CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered each day.

[0170] In some embodiments, less than about 2000 mg CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 1800 mg CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 1600 mg CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 1400 mg CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 1200 mg CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 1000 mg CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 800 mg CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 600 mg CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 500 mg CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 400 mg CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 300 mg CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day. In some embodiments, less than about 200 mg CRF1 antagonist or pharmaceutically acceptable salt, or a pharmaceutically acceptable salt or solvate thereof, is administered per day.

[0171] 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.

[0172] In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein at bedtime.

[0173] 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.

[0174] In some embodiments, the methods described herein include administration of the pharmaceutical compositions described herein in the evening.

[0175] 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.

[0176] In some embodiments, the steroid or pharmaceutically acceptable salt thereof is a glucocorticoid or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid or a pharmaceutically acceptable salt there is prednisone, cortisone, prednisolone, triamcinolone, methylprednisolone, betamethasone, dexamethasone, hydrocortisone or a pharmaceutically acceptable salt thereof.

[0177] In some embodiments, the levels of A4 and ACTH in the subject are determined from a biological sample from the subject. In some embodiments, the biological sample is selected from the group of blood, blood fractions, plasma, serum, urine, other types of bodily secretions, and saliva. In some embodiments, the biological sample is obtained non-invasively.

[0178] In some embodiments, the subject is a pediatric patient. In some embodiments, the subject is from about 0 years old to about 18 years old. In some embodiments, the subject is an adult patient.

[0179] In some embodiments, the steroid and the CRF1 antagonist are administered concurrently. In some embodiments, the steroid and the CRF1 antagonist are administered in one pharmaceutical composition. In some embodiments, the steroid and the CRF1 antagonist are administered concurrently in separate pharmaceutical compositions. In some embodiments, the steroid and the CRF1 antagonist are administered sequentially.

[0180] In some embodiments, the steroid and the CRF1 antagonist are administered sequentially within 24 hours. In some embodiments, the steroid and the CRF1 antagonist are administered sequentially within 8 hours. In some embodiments, the steroid and the CRF1 antagonist are administered sequentially within 2 hours. In some embodiments, the steroid and the CRF1 antagonist are administered sequentially within 10 minutes.

[0181] In some embodiments, CAH is classic CAH. In some embodiments, CAH is non-classic CAH.

[0182] In some embodiments, the glucocorticoid is beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, or triamcinolone. In some embodiments, the glucocorticoid is hydrocortisone.

[0183] In some embodiments, the glucocorticoid is hydrocortisone and the dose administered is less than the recommended dose of 15-25 mg/day.

[0184] In some embodiments, the glucocorticoid is prednisone and the dose administered is less than the recommended dose of 5-7.5 mg/day.

[0185] In some embodiments, the glucocorticoid is prednisolone and the dose administered is less than the recommended dose of 4-6 mg/day.

[0186] In some embodiments, the glucocorticoid is dexamethasone and the dose administered is less than the recommended dose of 0.25-0.5 mg/day.

EXAMPLES

[0187] 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.

[0188] 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 – Identifying discrete subgroups of patients with CAH based on A4 and ACTH levels in a subject who has CAH

[0189] Study Compound 3, a Phase 2, open-label, first-in-CAH-patients, proof-of-concept, dose ranging study, evaluated the safety and efficacy of repeated doses of SPR001 in adults with classic CAH. The population (n=24; with 2 subjects completing 2 cohorts) consisted of subjects with classic CAH that is not adequately controlled despite a stable glucocorticoid regimen based on the assessment of a single hormone at study screening, 17-OHP > 800 ng/dl, which is 4 times the upper limit of a normal reference range of 17-OHP level. 17-OHP was chosen as the hormone as an inclusion criterion, as at the time, it represented the state-of-art in CAH for assessment of disease severity and adequacy of glucocorticoid therapy. The study comprised 3 cohorts, Cohort A was a 6-week intra-subject dose escalation treatment period with three 2-week treatment periods of 200 mg glucocorticoid (QD) (only daily), 600 mg QD and 1000 mg QD without washout between treatment periods. Cohorts B and C consisted of 2-week treatment periods of either 100 bis in die (BID) (twice daily) (Cohort C) or 200 mg BID (Cohort B). At baseline, the following hormones were assessed, 17-OHP, ACTH and A4. The same hormones were assessed at the end of each 2-week treatment period.

[0190] The inclusion criterion of an elevated 17-OHP (>800 ng/dL) was expected, based on the current state-of-art, to enroll subjects with elevated ACTH, a precursor to 17-OHP, and elevated A4, a downstream androgen of 17-OHP. An elevated 17-OHP corresponded to both elevated and non-elevated pre (ACTH) and post (A4) stream hormones. This is evidenced in Table 1. All cohorts met the criteria of elevated 17-OHP as evidenced in the geometric means. Subjects in Cohort B, while having elevated 17-OHP, did not have elevated ACTH or elevated A4. Cohorts A and C had all 3 hormones elevated corresponding to the state-of-the-art.

Table 1 – Cohorts assessed in a Phase 2, open-label, first-in-CAH-patients, proof-of-concept, dose ranging study

	Cohort A Compound 3 200/600/1000 QD (N=10)	**Cohort B Compound 3 200 BID (N=8)**	**Cohort C Compound 3 100 BID (N=7)**
Screening 17-OHP, ng/dL			
Geometric Mean (CV%)	3887.3 (125.6)	3157.8 (109.1)	6106.8 (113.6)
Baseline (8am) 17-OHP, ng/dL			
Geometric Mean (CV%)	7362.0 (95.2)	1954.2 (344.2)	6753.4 (96.0)
Baseline (8 am)ACTH, pg/mL			

Geometric Mean (CV%)	306.72 (97.1)	27.17 (190.6)	292.45 (100.9)
Baseline (8am) A4, ng/dL			
Geometric Mean (CV%)	286.9 (168.1)	93.0 (194.6)	242.2 (121.8)

[0191] A clustering approach, supported and guided by clinical input, was used to define subgroups based on baseline hormone control of A4 and ACTH. The inclusion of 17-OHP as an input occluded the identification of clear subgroups which was non-obvious.

[0192] Three subgroups of patients were identified (see FIG.1):

- Subgroup One: Subjects with A4 and ACTH levels generally lower than two-fold the upper limit of normal for each laboratory assessment respectively.
- Subgroup Two: Subjects with A4 and ACTH generally higher than two-fold the upper limit of normal for each laboratory assessment respectively.
- Subgroup Three: Subjects with either A4 or ACTH above two fold and the other below two-fold the upper limit of normal for each laboratory assessment respectively.
- Subgroups 2 and 3 can be combined into a single subgroup.

[0193] By classifying the patients into subgroup types based on A4 and ACTH, it provides an alternative method to assessing diseases severity of subjects based on 17-OHP and A4. In addition, these subgroups provide a basis for different, yet sequential, methods of treatment.

Example 2 –Phase 2 Clinical Studies

[0194] A clinical study is conducted involving up to 52 weeks treatment of Compound 3 with concurrent daily glucocorticoid therapy for the treatment of adults with classic CAH. After screening using A4 and ACTH level criteria, eligible patients are enrolled into up to 12 weeks of placebo-controlled treatment followed by 40 weeks of open label treatment and a 4-week washout/safety follow-up period.

[0195] The population are composed of subjects from subgroup 2, approximately n=18 per treatment group and a range of doses between 50 mg to 200 mg and placebo. Each subject receives Compound 3 or placebo and a glucocorticoid daily for up to 12 weeks in a double-blind matter. Following the placebo-controlled period, all subjects receive open label compound 3 as various doses and a glucocorticoid will be administered as an oral daily dose as in the double-blind period. Patients self-administer Compound 3 for up to 52 weeks. The maximum treatment period for placebo is 12 weeks.

[0196] Study Design

- Study Type: Interventional
- Primary Purpose: Treatment
- Study Phase: Phase 2

- Interventional Study Model: double-blind, placebo controlled (12 weeks), open label (up to 40 weeks)
- Allocation: Equal Randomization
- Enrollment: Up to approximately 72

Outcome Measures

Primary Outcome Measures:

1. Assess reduction in key hormones such as ACTH, A4 and 17-OHP.

Secondary Outcome Measures:

2. Assess normalization proportions for key hormones such as ACTH, A4 and 17-OHP.

Eligibility

- Minimum Age: 16 Years
- Maximum Age: 65 Years
- 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 A4 and ACTH more than two times the upper limit of normal laboratory reference range at screening and baseline
- 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.

[0197] The disclosed compound 3 is an inhibitor of the CYP3A4 pathway and dexamethasone (DEX) is primarily metabolized through the CYP3A4 pathway. As part of the study, pre and pose concentrations of DEX and cortisol were measured. In some embodiments, the biomarker reduction analysis comprises all subjects. In some embodiments, the biomarker reduction analysis does not comprise DEX-treated subjects.

[0198] As shown in FIGS. 2A and 2B, time matched changes among different treatment groups of ACTH level measured from 10 pm the day before to 8am the next day are illustrated in

non-dexamethasone subjects. A pooled baseline (dotted) and 2 lines (solid) representing two dose groups (doses = 200 mg and doses > 200 mg) using the percentage change from dose group baseline presented as percentage change from the pooled baseline. The figures are divided by disease control group, poor disease control with highly elevated ACTH and A4 and the good disease control group with ACTH and A4 that is near normal or below normal ranges. Each group has elevated 17-OHP.

[0199] According to FIG. 2A, in the poor disease group the geometric means post 4 am are highly elevated reaching values greater than 400 pg/mL or greater than 6 x upper limit of normal (ULN)(63.3 pg/mL). Treatment with compound 3 reduced the magnitude of the increase in ACTH post 3 am demonstrating an improvement in the serial hormone profile. According to FIG. 2B, in the good disease control group, the baseline serial profile was below the ULN throughout the evaluated time period. Treatment with compound 3 generally maintained the serial profile below the ULN. The observed changes are likely representative of intra-subject daily variation.

[0200] As shown in FIGS. 3A and 3B, the time matched changes among different treatment groups of 17-OHP level measured from 10 pm the day before to 8am the next day are illustrated in non-dexamethasone subjects . A pooled baseline (dotted) and 2 lines (solid) representing two dose groups (doses = 200 mg and doses > 200 mg) using the percentage change from dose group baseline presented as percentage change from the pooled baseline. The figures are divided by disease control group, poor disease control with highly elevated ACTH and A4 and the good disease control group with ACTH and A4 that is near normal or below normal ranges. In both groups, 17-OHP is elevated above its target (1200 ng/dL). The Y-axis is different with the poor disease control group exceeding 8000 ng/dL in FIG. 3A and the good disease group was near an upper bound of approximately 3000 ng/dL in FIG. 3B. In both groups, treatment with compound 3 reduced the magnitude of the increase in 17-OHP post 3 am demonstrating an improvement in the serial hormone profile.

[0201] As shown in FIGS. 4A and 4B, the time matched changes among different treatment groups of A4 level measured from 6 am to 8 am the same day in non-dexamethasone subjects. A pooled baseline (dotted) and 2 lines (solid) representing two dose groups (doses = 200 mg and doses > 200 mg) using the percentage change from dose group baseline presented as percentage change from the pooled baseline. The figures are divided by disease control group, poor disease control with highly elevated ACTH and A4 and the good disease control group with ACTH and A4 that is near normal or below normal ranges. Each group has elevated 17-OHP.

[0202] As shown in FIG. 4A, the baseline profile for the poor disease control group is above the blended gender based ULN of approximately 212 ng/dL. Treatment with compound 3 improves the A4 profile in subjects toward the blended gender based ULN of approximately 212

ng/dL. As shown in FIG. 4B, the baseline profile for the good disease control group is well below the blended gender based ULN of approximately 212 ng/dL. Treatment with compound 3 was able to further reduce A4 level compared to the baseline level. The small variation about the baseline likely representative of intra-subject daily variation.

Example 3 – Phase 2 Clinical Studies.

[0203] A Phase 2 Study is conducted involving a up to 52 weeks treatment multiple-dose study of Compound 3 and a glucocorticoid for the treatment of adults with classic CAH. After screening with the criteria disclosed herein, eligible patients are enrolled into up to 52 weeks of treatment followed by a 4-week washout/safety follow-up period.

[0204] The population are composed of subgroup one subjects, approximately n=30 with a dose of 200 mg and n=30 with placebo. Each subject receives Compound 3 or placebo and a glucocorticoid daily for up to 24 weeks in a double-blind manner. Compound 3 and a glucocorticoid are administered as an oral daily dose. Following the double-blind period, all subject will receive open label compound 3 at 200 mg. Patients self-administer Compound 3 for up to 52 weeks. After a minimum of 2 weeks of concomitant treatment of Compound 3 and a glucocorticoid, subjects are eligible for reductions in their glucocorticoid. Reduction occurs at 5 mg hydrocortisone equivalent increments over a period of 24 weeks with subjects eligible for up to 5 dose reductions prior to study end.

[0205] Study Design

- Study Type: Interventional
- Primary Purpose: Treatment
- Study Phase: Phase 2
- Interventional Study Model: double-blind (24 weeks) open-label (28 weeks)
- Allocation: Equal Randomization
- Enrollment: Up to approximately 60 [n=30 per group]

Outcome Measures

Primary Outcome Measure:

1. Reduction of glucocorticoid dose

Secondary Outcome Measure:

2. Assess normalization proportions for each key hormone
3. Assess impact of reductions of glucocorticoid dose on signs and symptoms of CAH
4. Assess impact of reductions of glucocorticoid dose on overall glucocorticoid toxicity Index.

Eligibility

- Minimum Age: 18 Years
- Maximum Age: 65
- 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 A4 and ACTH <1.5x upper limit of normal at screening and baseline
- 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.

[0206] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of treating Congenital Adrenal Hyperplasia (CAH), comprising administering a steroid or a pharmaceutically acceptable salt thereof and a corticotropin-releasing factor type-1 (CRF_1) antagonist or a pharmaceutically acceptable salt thereof to a subject, wherein when said subject has a level of at least one steroid hormone that is less than two times an upper limit of a reference range of said at least one steroid hormone level , a dose of said steroid or a pharmaceutically acceptable salt thereof is reduced compared to a dose of said steroid or a pharmaceutically acceptable salt thereof administered to a CAH patient that does not receive said CRF_1 antagonist or a pharmaceutically acceptable salt thereof.

2. The method of claim 1, wherein said subject has a level of a second and different steroid hormone that is less than two times an upper limit of a reference range of said second and different steroid hormone level.

3. The method of either claim 1 or claim 2, wherein said steroid hormone is selected from the group consisting of adrenocorticotropic hormone (ACTH), androstenedione (A4), 17-hydroxyprogesterone (17-OHP), deoxycorticosterone, 11-deoxycortisol, cortisol, corticosterone, aldosterone, pregnenolone, 17α-hydroxy pregnenolone, progesterone, dehydroepiandrosterone, androstenediol, testosterone, dihydrotestosterone, estrone, estradiol, and estriol.

4. The method of one of claims 1-3, wherein said subject has a level of 17-OHP that is less than two times an upper limit of a reference range of 17-OHP and a level of ACTH that is less than two times an upper limit of a reference range of ACTH.

5. The method of one of claims 1-3, wherein said subject has a level of 17-OHP that is less than two times an upper limit of a reference range of 17-OHP and a level of A4 that is less than two times an upper limit of a reference range of A4.

6. The method of one of claims 1-3, wherein said subject has a level of A4 that is less than two times an upper limit of a reference range of A4 and a level of ACTH that is less than two times an upper limit of a reference range of ACTH.

7. The method of one of claims 1-3, wherein said subject has a level of 17-OHP that is less than 1.75 times an upper limit of a reference range of 17-OHP and a level of ACTH that is less than 1.75 times an upper limit of a reference range of ACTH.

8. The method of one of claims 1-3, wherein said subject has a level of 17-OHP that is less than 1.75 times an upper limit of a reference range of 17-OHP and a level of A4 that is less than 1.75 times an upper limit of a reference range of A4.

9. The method of one of claims 1-3, wherein said subject has a level of A4 that is less than 1.75 times an upper limit of a reference range of A4 and a level of ACTH that is less than 1.75 times an upper limit of a reference range of ACTH.

10. The method of one of claims 1-3, wherein said subject has a level of 17-OHP that is less than 1.5 times an upper limit of a reference range of 17-OHP and a level of ACTH that is less than 1.5 times an upper limit of a reference range of ACTH.

11. The method of one of claims 1-3, wherein said subject has a level of 17-OHP that is less than 1.5 times an upper limit of a reference range of 17-OHP and a level of A4 that is less than 1.5 times an upper limit of a reference range of A4.

12. The method of one of claims 1-3, wherein said subject has a level of A4 that is less than 1.5 times an upper limit of a reference range of A4 and a level of ACTH that is less than 1.5 times an upper limit of a reference range of ACTH.

13. The method of one of claims 1-3, wherein said subject has a level of 17-OHP that is less than 1.25 times an upper limit of a reference range of 17-OHP and a level of ACTH that is less than 1.25 times an upper limit of a reference range of ACTH.

14. The method of one of claims 1-3, wherein said subject has a level of 17-OHP that is less than 1.25 times an upper limit of a reference range of 17-OHP and a level of A4 that is less than 1.25 times an upper limit of a reference range of A4.

15. The method of one of claims 1-3, wherein said subject has a level of A4 that is less than 1.25 times an upper limit of a reference range of A4 and a level of ACTH that is less than 1.25 times an upper limit of a reference range of ACTH.

16. The method of one of claims 1-3, wherein said subject has a level of 17-OHP that is less than one times an upper limit of a reference range of 17-OHP and a level of ACTH that is less than one times an upper limit of a reference range of ACTH.

17. The method of one of claims 1-3, wherein said subject has a level of 17-OHP that is less than one times an upper limit of a reference range of 17-OHP and a level of A4 that is less than one times an upper limit of a reference range of A4.

18. The method of one of claims 1-3, wherein said subject has a level of A4 that is less than one times an upper limit of a reference range of A4 and a level of ACTH that is less than one times an upper limit of a reference range of ACTH.

19. The method of any of claims 1-18, further comprising reducing said dose of said steroid or a pharmaceutically acceptable salt thereof in a 5 mg increment.

20. A method of treating Congenital Adrenal Hyperplasia (CAH), comprising administering a steroid or a pharmaceutically acceptable salt thereof and a corticotropin-releasing factor type-1 (CRF1) antagonist or a pharmaceutically acceptable salt thereof to a subject, wherein when said

subject has a level of at least one steroid hormone that is more than an upper limit of a reference range of said at least one steroid hormone level, a dose of said steroid or a pharmaceutically acceptable salt thereof is not reduced compared to a dose of said steroid or a pharmaceutically acceptable salt thereof administered to a CAH patient that does not receive said CRF1 antagonist or a pharmaceutically acceptable salt thereof.

21. The method of claim 20, wherein said subject has a level of said at least one steroid hormone that is more than two times an upper limit of a reference range of said at least one steroid hormone level.

22. The method of claim 20, wherein said subject has a level of said at least one steroid hormone that is more than 1.75 times an upper limit of a reference range of said at least one steroid hormone level.

23. The method of claim 20, wherein said subject has a level of said at least one steroid hormone that is more than 1.5 times an upper limit of a reference range of said at least one steroid hormone level.

24. The method of claim 20, wherein said subject has a level of said at least one steroid hormone that is more than 1.25 times an upper limit of a reference range of said at least one steroid hormone level.

25. The method of claim 20, wherein said steroid hormone is selected from the group consisting of adrenocorticotropic hormone (ACTH), androstenedione (A4), 17-hydroxyprogesterone (17-OHP), deoxycorticosterone, 11-deoxycortisol, cortisol, corticosterone, aldosterone, pregnenolone, 17α-hydroxy pregnenolone, progesterone, dehydroepiandrosterone, androstenediol, testosterone, dihydrotestosterone, estrone, estradiol, and estriol.

26. The method of any of claims 20-25, further comprising maintaining said dose of said steroid or a pharmaceutically acceptable salt thereof until said levels of at least two steroid hormones are less than 2 times said upper limit of said reference ranges of said two steroid hormones respectively.

27. The method of any of claims 20-25, further comprising reducing said dose of said steroid or a pharmaceutically acceptable salt thereof in a 5 mg increment when said levels of at least two steroid hormones are less than 2 times said upper limit of said reference ranges of said two steroid hormones respectively.

28. The method of claim 27, wherein said at least two steroid hormones comprise 17-OHP and ACTH.

29. The method of claim 27, wherein said at least two steroid hormones comprise 17-OHP and A4.

30. The method of claim 27, wherein said at least two steroid hormones comprise A4 and ACTH.

31. The method of any of claims 1-30, wherein said reference range of ACTH or A4 level of said subject is controlled by a factor selected from the group consisting of age, sex, menopausal status, laboratory setting, time of a day and combinations thereof.

32. The method of any of claims 1-30, wherein said level of ACTH or A4 is 2 times, 1.5 times, 1.25 times, or 1 times said upper limit of said normal range of ACTH or A4 level of said subject.

33. The method of any of claims 1-30, wherein said amount of said steroid or a pharmaceutically acceptable salt thereof comprises a range of about 10 mg to about 80 mg of hydrocortisone equivalents, wherein said steroid or a pharmaceutically acceptable salt thereof is administered daily.

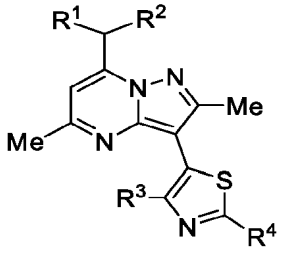
34. The method of any of claims 1-30, wherein said amount of said CRF_1 antagonist or a pharmaceutically acceptable salt thereof comprises a range of about 10 mg to about 200 mg, wherein said CRF_1 antagonist or a pharmaceutically acceptable salt thereof is administered daily.

35. The method of any of claims 1-34, wherein said subject is treated for a period of about 4 weeks to about 36 weeks.

36. The method of any of claims 1-34, wherein after said subject is treated for a period of about 4 weeks to about 36 weeks, said subject is reassessed to determine if a different method of treating CAH should be administered.

37. The method of any one of claims 1-36, wherein said CRF_1 antagonist or pharmaceutically acceptable salt thereof is selected from the group consisting of: Antalarmin hydrochloride, Pfizer CP 154526, CP 376395 hydrochloride, NBI 27914 hydrochloride, NBI 35965 hydrochloride, NGD 98-2 hydrochloride, Pexacerfont, R 121919 hydrochloride, SN003.

38. The method of any one of claims 1-37, wherein said CRF_1 antagonist is a compound of Formula (I):

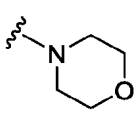
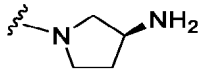
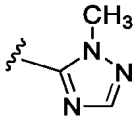
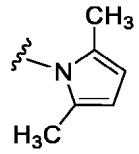


(I),

or a pharmaceutically acceptable salt thereof, wherein:

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

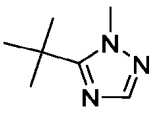
R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy; and

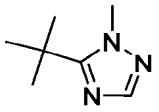
R^4 is hydrogen, Br, R^aR^bN–, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , , , or ; and

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

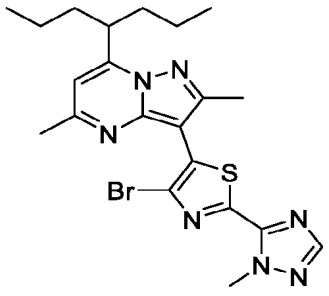
39. The method of claim 38, wherein R^3 is Cl, Br, methyl, or trifluoromethyl.

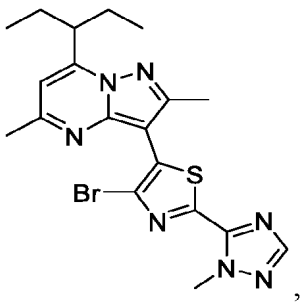
40. The method of claims 38 or 39, wherein R^3 is Cl, Br, or methyl.

41. The method of any one of claims 38-40, wherein R^4 is Br, R^aR^bN–, pyridin-4-yl, morpholin-4-yl, or .

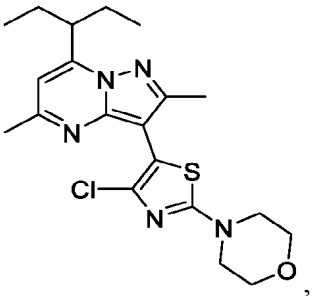
42. The method of any one of claims 38-40, wherein R^4 is morpholin-4-yl or .

43. The method of any one of claims 38-40, wherein R^4 is hydrogen, Br, R^aR^bN– and R^a and R^b are independently C_1-C_3alkyl.

44. The method of claim 38, wherein the compound is , or a pharmaceutically acceptable salt thereof.

45. The method of claim 38, wherein the compound is , or a pharmaceutically acceptable salt thereof.

46. The method of claim 38, wherein the compound is



, or a pharmaceutically acceptable salt thereof.

47. The method of any one of claims 1-46, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 50 mg to about 200 mg total daily dose to the subject.

48. The method of any one of claims 1-46, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 200 mg total daily dose to the subject.

49. The method of any one of claims 1-46, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 150 mg total daily dose to the subject.

50. The method of any one of claims 1-46, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 100 mg total daily dose to the subject.

51. The method of any one of claims 1-46, wherein the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 75 mg total daily dose to the subject.

52. The method of any one of claims 1-46, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 50 mg total daily dose to the subject.

53. The method of any one of claims 1-46, wherein the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 25 mg total daily dose to the subject.

54. The method of any one of claims 1-46, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is in the form of microparticles.

55. The method of claim 54, wherein the average size of the microparticles is between about 1 μm to about 20 μm.

56. The method of claim 54, wherein the average size of the microparticles is between about 5 μm to about 15 μm.

57. The method of claim 54, wherein the average size of the microparticles is less than about 10 μm.

58. The method of any one of claims 1-56, wherein the CRF1 antagonist or pharmaceutically acceptable salt thereof is administered as a pharmaceutical composition.

59. The method of any one of claims 1-56, wherein the steroid or a pharmaceutically acceptable salt thereof is administered as a pharmaceutical composition.

60. The method of claim 58 or 59, wherein the pharmaceutical composition is in the form of a capsule or a tablet.

61. The method of claim 60, wherein the capsule is a hard gelatin capsule.

62. The method of claim 60, wherein the capsule is a soft gelatin capsule.

63. The method of any one of claims 60-62, 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.

64. The method of any one of claims 58-63, wherein the pharmaceutical composition is free of additional excipients.

65. The method of any one of claims 58-63, wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

66. The method of claim 60, wherein the pharmaceutical composition is in the form of a tablet.

67. The method of claim 66, wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

68. The method of any one of claims 1-66, wherein the steroid or pharmaceutically acceptable salt thereof is a glucocorticoid or a pharmaceutically acceptable salt thereof.

69. The method of claim 68, wherein the glucocorticoid or a pharmaceutically acceptable salt thereof is prednisone, cortisone, prednisolone, triamcinolone, methylprednisolone, betamethasone, dexamethasone, hydrocortisone or a pharmaceutically acceptable salt thereof.

70. The method of any one of claims 1-69, wherein levels of steroid hormones in the subject are determined from a biological sample from the subject.

71. The method of claim 70, wherein the biological sample is selected from the group of blood, blood fractions, plasma, serum, and saliva.

72. The method of claim 70, wherein the biological sample is obtained non-invasively.

73. The method of any one of claims 1-72, wherein said subject is tested in the morning.

74. The method of any one of claims 1-72, wherein said subject is tested to determine morning levels of A4 and ACTH.

75. The method of any one of claims 1-72, wherein the subject is a pediatric patient.

76. The method of any one of claims 1-72, wherein the subject is from about 0 years old to about 18 years old.

77. The method of any one of claims 1-72, wherein the subject is an adult patient.

78. The method of any one of claims 1-77, wherein the steroid and the CRF_1 antagonist are administered concurrently.

79. The method of any one of claims 1-77, wherein the steroid and the CRF_1 antagonist are administered in one pharmaceutical composition.

80. The method of any one of claims 1-77, wherein the steroid and the CRF_1 antagonist are administered concurrently in separate pharmaceutical compositions.

81. The method of any one of claims 1-77, wherein the steroid and the CRF_1 antagonist are administered sequentially.

82. The method of claim 81, wherein the steroid and the CRF_1 antagonist are administered sequentially within 24 hours.

83. The method of claim 81, wherein the steroid and the CRF_1 antagonist are administered sequentially within 8 hours.

84. The method of claim 81, wherein the steroid and the CRF_1 antagonist are administered sequentially within 2 hours.

85. The method of claim 81, wherein the steroid and the CRF_1 antagonist are administered sequentially within 10 minutes.

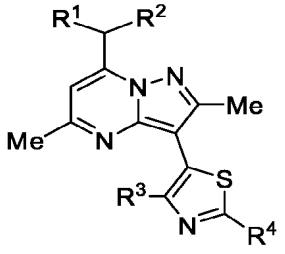
86. The method of any one of claims 1-85, wherein CAH is classic CAH.

87. The method of any one of claims 1-85, wherein CAH is non-classic CAH.

88. A composition comprises a steroid or a pharmaceutically acceptable salt thereof and a corticotropin-releasing factor type-1 (CRF_1) antagonist or a pharmaceutically acceptable salt thereof, wherein said CRF_1 antagonist or pharmaceutically acceptable salt thereof is selected from the group consisting of: Antalarmin hydrochloride, Pfizer CP 154526, CP 376395 hydrochloride, NBI 27914 hydrochloride, NBI 35965 hydrochloride, NGD 98-2 hydrochloride, Pexacerfont, R 121919 hydrochloride, SN003.

89. The composition of claim 88, wherein said a steroid or a pharmaceutically acceptable salt thereof is exogenous glucocorticoids (GC) or a pharmaceutically acceptable salt thereof.

90. The composition of either claim 88 or 89, wherein said CRF_1 antagonist is a compound of Formula (I):

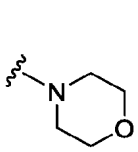
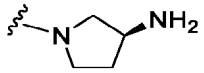
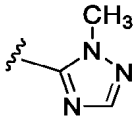
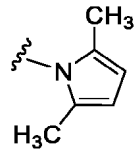


(I),

or a pharmaceutically acceptable salt thereof, wherein:

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

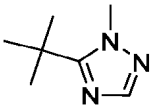
R^3 is hydrogen, Cl, Br, methyl, trifluoromethyl, or methoxy; and

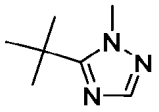
R^4 is hydrogen, Br, R^aR^bN–, methoxymethyl, *n*-butyl, acetamido, pyridin-4-yl, morpholin-4-yl, , , , or ; and

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

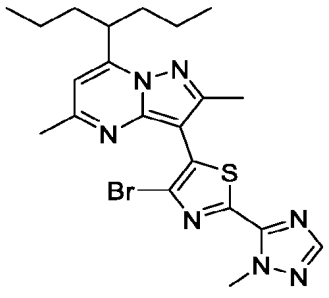
91. The composition of claim 90, wherein R^3 is Cl, Br, methyl, or trifluoromethyl.

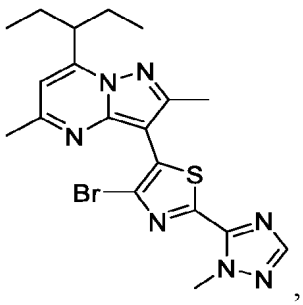
92. The composition of claims 90 or 91, wherein R^3 is Cl, Br, or methyl.

93. The composition of any one of claims 90-92, wherein R^4 is Br, R^aR^bN–, pyridin-4-yl, morpholin-4-yl, or .

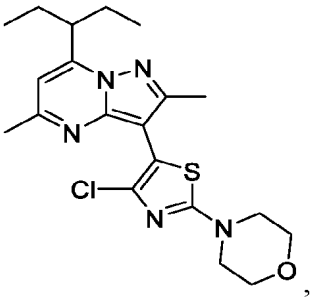
94. The composition of any one of claims 90-92, wherein R^4 is morpholin-4-yl or .

95. The composition of any one of claims 90-92, wherein R^4 is hydrogen, Br, R^aR^bN– and R^a and R^b are independently C_1-C_3alkyl.

96. The composition of claim 90, wherein the compound is , or a pharmaceutically acceptable salt thereof.

97. The composition of claim 90, wherein the compound is , or a pharmaceutically acceptable salt thereof.

98. The composition of claim 90, wherein the compound is



, or a pharmaceutically acceptable salt thereof.

99. The composition of any one of claims 90-98, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 50 mg to about 200 mg total daily dose to the subject.

100. The composition of any one of claims 90-98, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 200 mg total daily dose to the subject.

101. The composition of any one of claims 90-98, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 150 mg total daily dose to the subject.

102. The composition of any one of claims 90-98, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 100 mg total daily dose to the subject.

103. The composition of any one of claims 90-98, wherein the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 75 mg total daily dose to the subject.

104. The composition of any one of claims 90-98, wherein the CRF_1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 50 mg total daily dose to the subject.

105. The composition of any one of claims 90-98, wherein the CRF1 antagonist or pharmaceutically acceptable salt is administered in a dose of about 25 mg total daily dose to the subject.

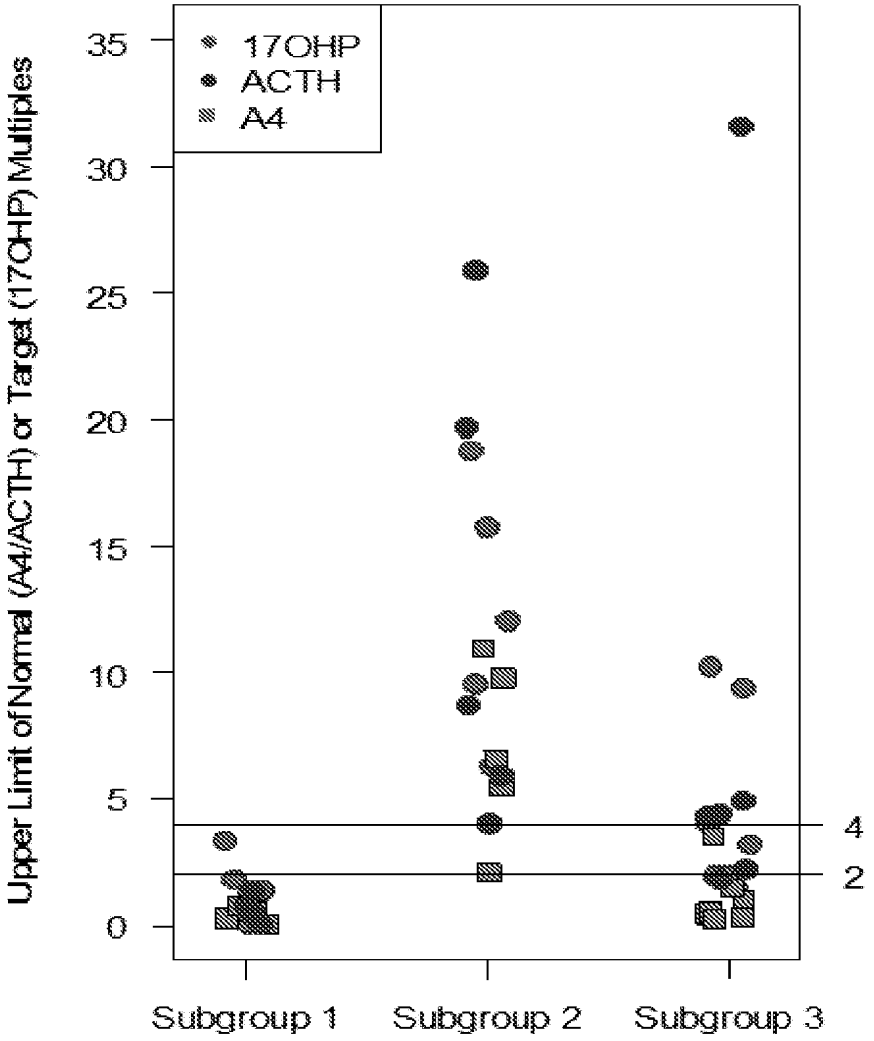

17OHP
ACTH
A4
Upper Limit of Normal (A4/ACTH) or Target (17OHP) Multiples
35
30
25
20
15
10
5
0
4
2
Subgroup 1
Subgroup 2
Subgroup 3

FIG. 1

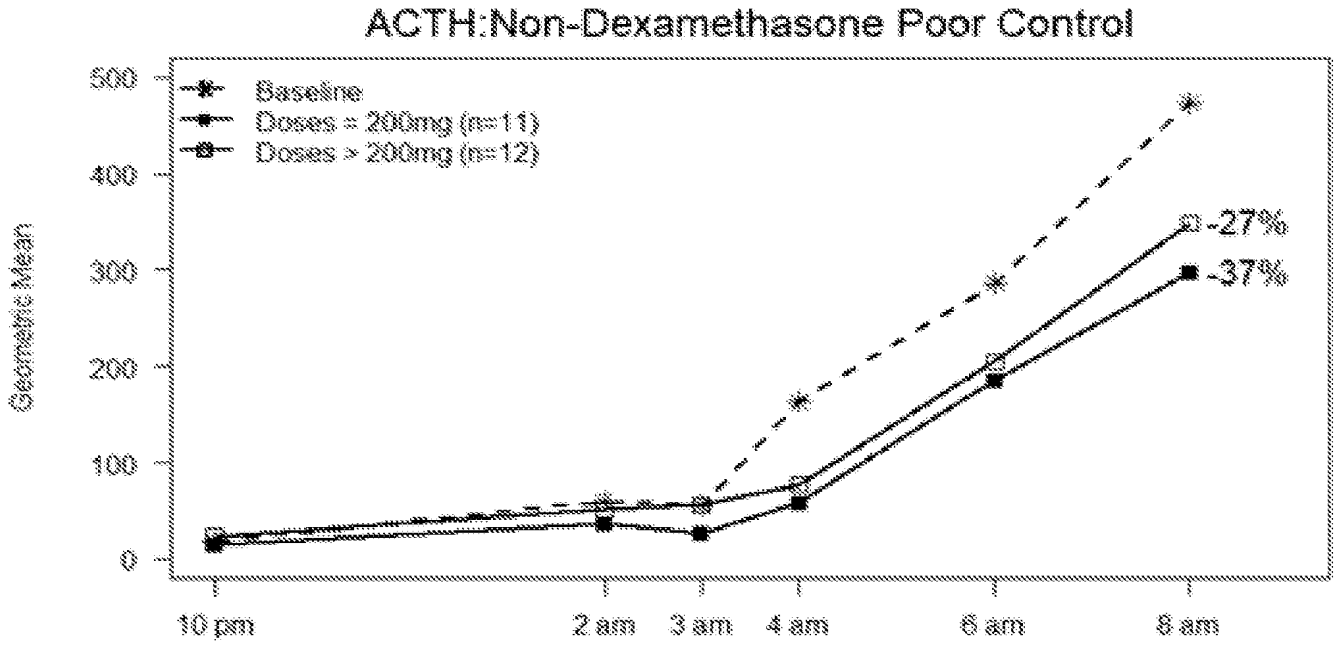

ACTH:Non-Dexamethasone Poor Control
Baseline
Doses = 200mg (n=11)
Doses > 200mg (n=12)
Geometric Mean
500
400
300
200
100
0
-27%
-37%
10 pm
2 am
3 am
4 am
6 am
8 am

FIG. 2A

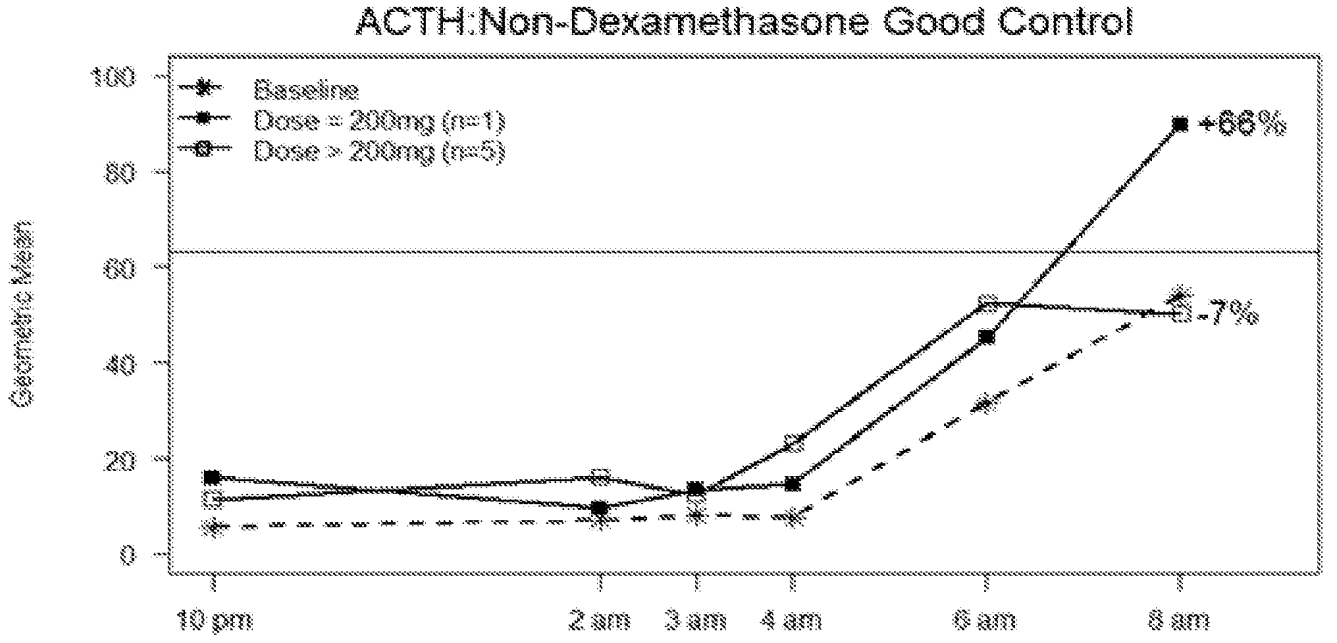

ACTH:Non-Dexamethasone Good Control
Baseline
Dose = 200mg (n=1)
Dose > 200mg (n=5)
Geometric Mean
100
80
60
40
20
0
+66%
-7%
10 pm
2 am
3 am
4 am
6 am
8 am

FIG. 2B

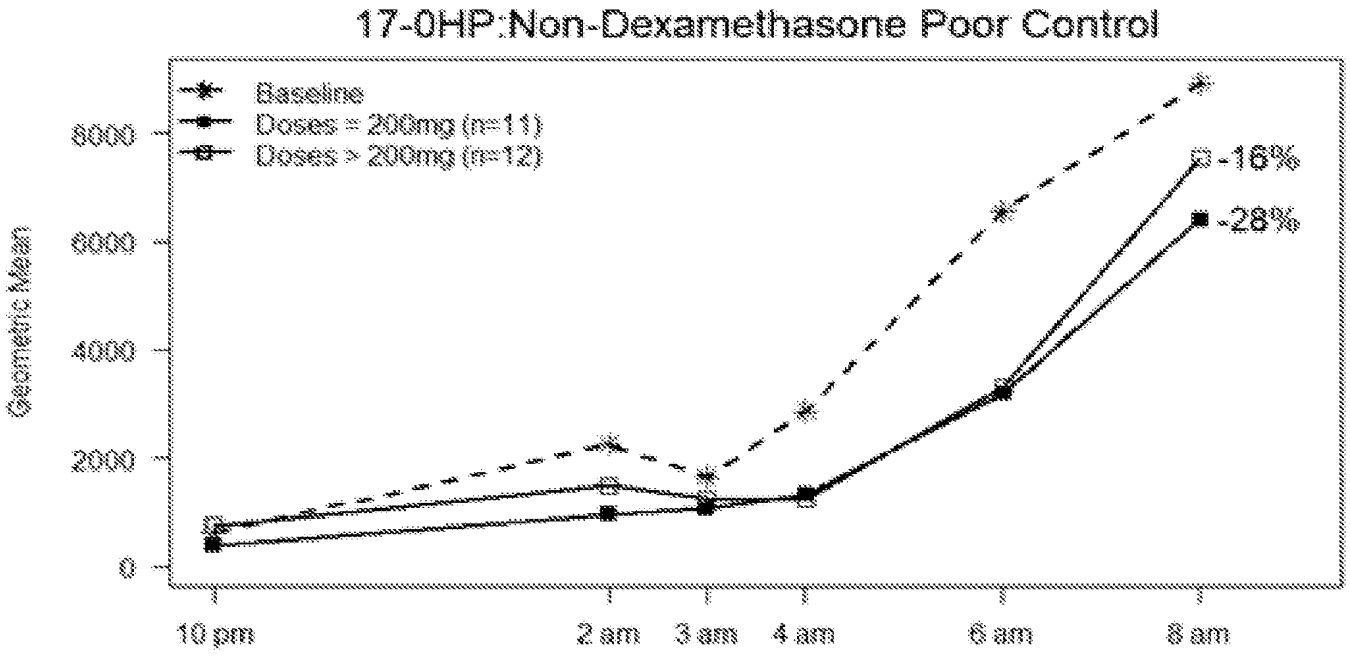

17-0HP:Non-Dexamethasone Poor Control
Baseline
Doses = 200mg (n=11)
Doses > 200mg (n=12)
Geometric Mean
8000
6000
4000
2000
0
-16%
-28%
10 pm
2 am
3 am
4 am
6 am
8 am

FIG. 3A

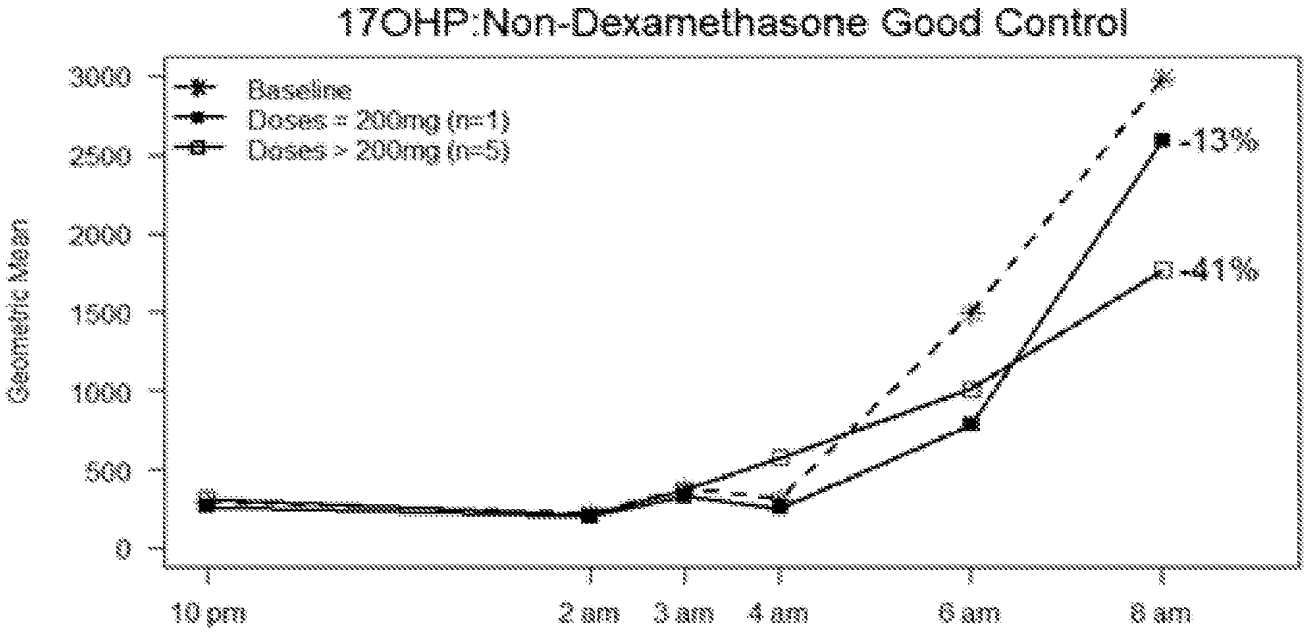

17OHP:Non-Dexamethasone Good Control
Baseline
Doses = 200mg (n=1)
Doses > 200mg (n=5)
Geometric Mean
3000
2500
2000
1500
1000
500
0
-13%
-41%
10 pm
2 am
3 am
4 am
6 am
8 am

FIG. 3B

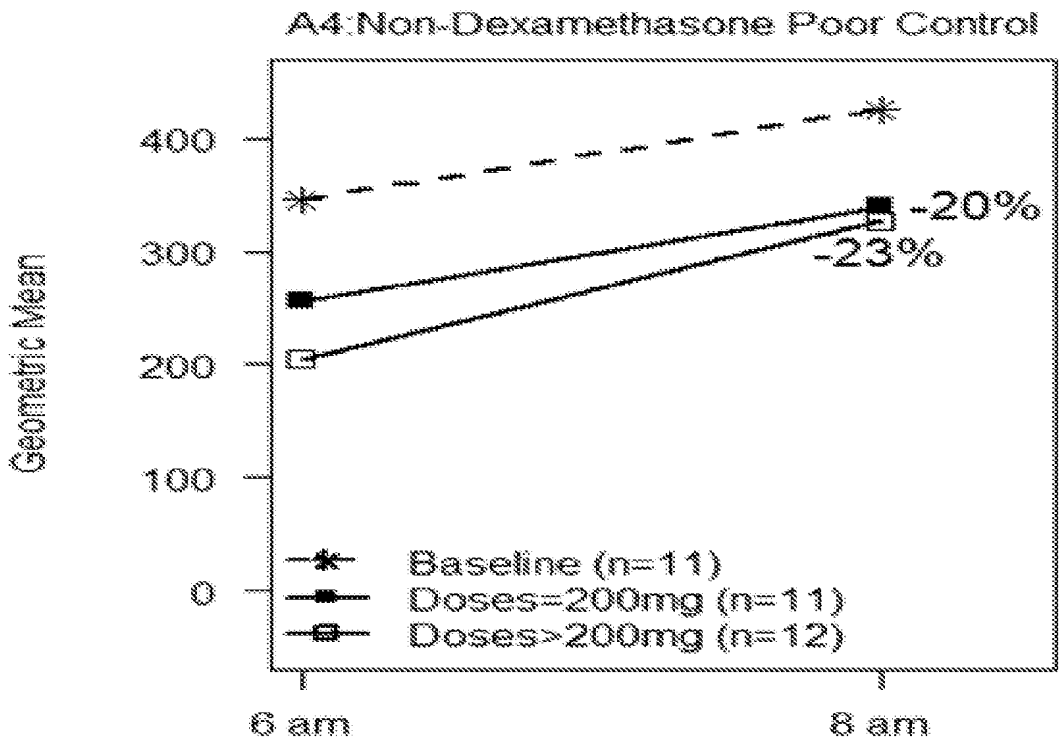

A4:Non-Dexamethasone Poor Control
Geometric Mean
400
300
200
100
0
-20%
-23%
Baseline (n=11)
Doses=200mg (n=11)
Doses>200mg (n=12)
6 am
8 am

FIG. 4A

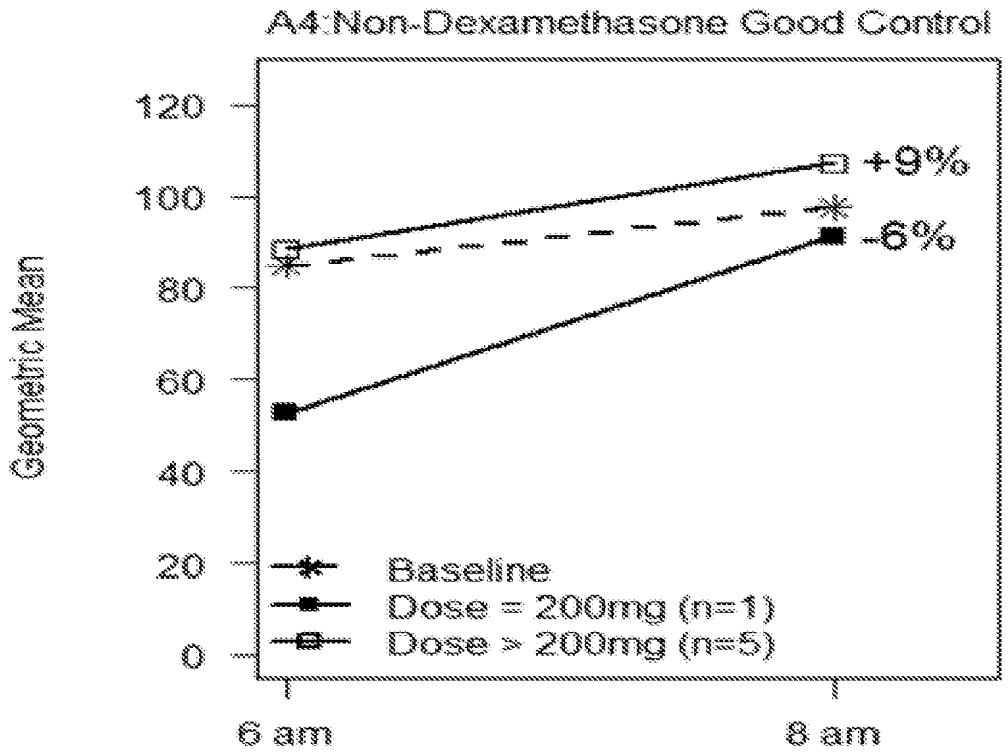

A4:Non-Dexamethasone Good Control
Geometric Mean
120
100
80
60
40
20
0
+9%
-6%
Baseline
Dose = 200mg (n=1)
Dose > 200mg (n=5)
6 am
8 am

FIG. 4B

INTERNATIONAL SEARCII REPORT

International application No.
PCT/US2020/042820

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/56(2006.01)i, A61K 31/57(2006.01)i, A61K 38/22(2006.01)i, A61K 31/519(2006.01)i, A61K 31/5377(2006.01)i, A61P 5/38(2006.01)i, C07D 487/04(2006.01)i

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 31/56; A61K ; A61K 31/4412; A61K 31/4545; A61K 31/519; A61P 25/24; A61K 31/57; A61K 38/22; A61K 31/5377; A61P 5/38; C07D 487/04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & keywords: Congenital Adrenal Hyperplasia (CAH), corticortropin-releasing factor type-1 (CRF1) antagonist

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2017-0020877 A1 (NEUROCRINE BIOSCIENCES, INC.) 26 January 2017 claims 14-18; TABLE 2; paragraphs [0054], [0070], [0086]	88-91,96-98
X	WO 2005-020910 A2 (PHARMACIA CORPORATION) 10 March 2005 claims 1-5	88-91,96-98
A	US 2011-0136865 A1 (BUNTINX, E.) 09 June 2011 the whole document	88-91,96-98
A	WO 2013 160317 A2 (IIOLSBOERMASCIIMEYER NEUROCIIEMIE GMBII) 31 October 2013 the whole document	88 91,96 98

☐ 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
"D" document cited by the applicant in the international application
"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
24 November 2020 (24.11.2020)	**25 November 2020 (25.11.2020)**

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea
Facsimile No. +82-42-481-8578

Authorized officer
Jung, Da Won
Telephone No. +82-42-481-5373

Form PCT/ISA/210 (second sheet) (July 2019)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2020/042820

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2017-0020877 A1	26/01/2017	CN 106102740 A	09/11/2016
		CN 111228274 A	05/06/2020
		EP 3096756 A1	30/11/2016
		JP 2017-503030 A	26/01/2017
		JP 2019-081814 A	30/05/2019
		JP 6585625 B2	02/10/2019
		KR 10-2016-0106176 A	09/09/2016
		US 2019-0231781 A1	01/08/2019
		WO 2015-112642 A1	30/07/2015
WO 2005-020910 A2	10/03/2005	US 2005-0085479 A1	21/04/2005
		WO 2005-020910 A3	09/06/2005
US 2011-0136865 A1	09/06/2011	EP 1708790 A1	11/10/2006
		EP 1708790 B1	21/04/2010
		EP 2272514 A1	12/01/2011
		US 2007-0078162 A1	05/04/2007
		US 2011-0207776 A1	25/08/2011
		US 8304431 B2	06/11/2012
		WO 2005-053796 A1	16/06/2005
WO 2013-160317 A2	31/10/2013	EP 2841068 A2	04/03/2015
		EP 2841068 B1	28/11/2018
		JP 2015-516979 A	18/06/2015
		US 2015-0094310 A1	02/04/2015
		WO 2013-160317 A3	03/01/2014

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/042820

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.: 1-87
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-87 pertain to methods for treatment of the human body and thus relate to a subject-matter which this International Searching Authority is not required to search under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv).

2. ☒ Claims Nos.: 39,44-46,55-57,61,62,66,67,69,71,72,82-85
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:
Claims 39,44-46,55-57,61,62,66,67,69,71,72,82-85 are regarded to be unclear because they refer to claims 38,54,60,66,68,70,81, which does not comply with PCT Rule 6.4(a).

3. ☒ Claims Nos.: 4-19,31-38,40-43,47-54,58-60,63-65,68,70,73-81,86,87,92-95,99-105
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 addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any 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.

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 June 2020 (11.06.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/115555 A2

(51) International Patent Classification:
A61K 9/00 (2006.01) *A61K 31/395* (2006.01)

(21) International Application Number:
PCT/IB2019/001325

(22) International Filing Date:
06 December 2019 (06.12.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/776,763 07 December 2018 (07.12.2018) US
62/816,674 11 March 2019 (11.03.2019) US

(71) Applicants: **NEUROCRINE BIOSCIENCES, INC.** [US/US]; 12780 El Camino Real, San Diego, California 92130 (US). **SANOFI** [FR/FR]; 54 Rue De La Boetie, 75008 Paris (FR).

(72) Inventors: **FARBER, Robert H.**; c/o Neurocrine Biosciences, Inc., 12780 El Camino Real, San Diego, CA 92130 (US). **LOEWEN, Gordon Raphael**; c/o Neurocrine Biosciences, Inc., 12780 El Camino Real, San Diego, California 92130 (US). **ZHANG, Xiaoping**; c/o Neurocrine Biosciences, Inc., 12780 El Camino Real, San Diego, CA 92130 (US). **GIRI, Nagdeep**; c/o Neurocrine Biosciences, Inc., 12780 El Camino Real, San Diego, CA 92130 (US). **CHAN, Jean L.**; c/o Neurocrine Biosciences, Inc., 12780 El Camino Real, San Diego, CA 92130 (US). **STIRN, Scott**; c/o Neurocrine Biosciences, Inc., 12780 El Camino Real, San Diego, California 92130 (US). **SAYERS, Brian**; c/o Neurocrine Biosciences, Inc., 12780 El Camino Real, San Diego, CA 92130 (US). **TAYLOR, Graeme**; c/o Neurocrine Biosciences, Inc., 12780 El Camino Real, San Diego, CA 92130 (US). **COSTA, Christina Marie**; c/o Neurocrine Biosciences, Inc., 12780 El Camino Real, San Diego, CA 92130 (US). **PARKS, Stacy**; c/o Bend Research, Inc., 63045 NE Corporate Pl., Bend, OR 97701 (US). **VICKERY, Anthony D.**; c/o Bend Research, Inc., 63045 NE Corporate Pl., Bend, OR 97701 (US). **DOWNING, Kristie M.**; c/o Bend Research, Inc., 63045 NE Corporate Pl., Bend, OR 97701 (US). **IYOHA, Kingsley**; c/o Quotient Sciences Limited, Trent House, Mere Way, Ruddington, Nottingham, Nottinghamshire NG11 6JS (GB). **NGWENYA-JONES, Ayanda**; c/o Quotient Sciences Limited, Trent House, Mere Way, Ruddington, Nottingham, Nottinghamshire NG11 6JS (GB). **CHARLIER, Anne**; c/o SANOFI, Patent Department, 54 rue de la Boétie, 75008 Paris (FR). **MEHTON, Gurvinder Singh**; Trent House, Mere Way, Ruddington, Nottingham Nottinghamshire NG11 6JS (GB).

(74) Agent: **BOYLE, Craig D.** et al.; Fish & Richardson P.C., P.O. Box 1022, Minneapolis, Minnesota 55440-1022 (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).

Published:
— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

(54) Title: CRF1 RECEPTOR ANTAGONIST, PHARMACEUTICAL FORMULATIONS AND SOLID FORMS THEREOF FOR THE TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA

(57) Abstract: Provided are methods related to treating congenital adrenal hyperplasia in a subject in need thereof comprising administering 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1 S)-2-cyclopropyl- 1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine (Formula 1), or a pharmaceutically acceptable salt thereof. Further provided are pharmaceutical formulations and solid forms of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(lS)-2-cyclopropyl-l-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N -prop-2 -ynyl-l,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, and their use in the treatment of congenital adrenal hyperplasia (CAH).

WO 2020/115555 A2

CRF1 RECEPTOR ANTAGONIST, PHARMACEUTICAL FORMULATIONS AND SOLID FORMS THEREOF FOR THE TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA

TECHNICAL FIELD

The present disclosure relates to 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, for the treatment of congenital adrenal hyperplasia (CAH). The present disclosure further relates to pharmaceutical formulations and solid forms of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, and their use in the treatment of congenital adrenal hyperplasia (CAH).

BACKGROUND

Classic congenital adrenal hyperplasia (CAH) is a disease that includes a group of autosomal recessive disorders that result in an enzyme deficiency that alters the production of adrenal steroids due to 21-hydroxylase deficiency, a condition that results in little or no cortisol biosynthesis. One clinical manifestation of the absence of cortisol is the lack of feedback inhibition of pituitary adrenocorticotropic hormone (ACTH) secretion. Increased ACTH levels cause adrenal hyperplasia and the enzyme mutation causes a shunting of cortisol precursor steroids to alternate pathways. Most notably, the shunting of androgens leads to virilization and other developmental complications in females and the over-accumulation of ACTH is associated with the formation of testicular adrenal rest tumors in males. In addition, since the same enzyme (21-hydroxylase) is used in the pathway for the biosynthesis of the mineralocorticoids, a number of these patients suffer from aldosterone deficiency which can result in dehydration and death due to salt-wasting. The prevalence of classic 21-hydroxylase deficiency CAH in the US general population, based on newborn screening, has been documented as 1:10,000 to 1:20,800 (Trakakis et al., "An update to 21-hydroxylase deficient congenital adrenal hyperplasia," *Gynecol. Endocrinol.* (2010) 26(1):63-71; Hertzberg et al., "Birth prevalence rates of newborn screening disorders in relation to screening practices in the United States," *J. Pediatr.* (2011) 159(4):555-560).

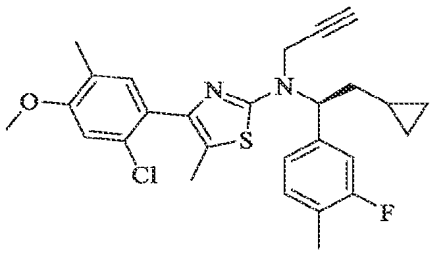
Pediatric patients from birth through adolescence, and females in particular, appear to be the most vulnerable population of CAH sufferers and represent the subgroup of patients with

the greatest unmet medical need (Cheng and Speiser, "Treatment outcomes in congenital adrenal hyperplasia," *Adv. Pediatr.* (2012) 59(1):269-281; Merke and Poppas, "Management of adolescents with congenital adrenal hyperplasia," *Lancet Diabetes Endocrinol.* (2013) 1(4):341-352). Excessive androgen production in these younger patients results in early onset puberty and adrenarche, changes in skeletal maturation patterns, short stature caused by premature growth plate fusion, as well as significant hirsutism and acne problems. While survival is properly ensured through steroid replacement strategies based on physiologic dosing of glucocorticoids (e.g., hydrocortisone) and mineralocorticoids (e.g., fludrocortisone), these doses are often inadequate to suppress the accumulating ACTH and overproduction of progestogens and androgens (e.g., 17-hydroxyprogesterone [17-OHP], androstenedione, and testosterone). The uncontrolled symptoms of androgen excess, indeed, have a substantial impact on the day-to-day functioning and development of these patients.

Currently, exogenous corticosteroids are the standard of care for treating patients with classic CAH. This treatment is used to correct the cortisol deficiency and reduce the excessive ACTH levels and androgen excess. However, the dose and duration of steroid use required to suppress ACTH are typically well above the normal physiological level used for cortisol replacement alone (as in patients with Addison's disease). This increased exposure to glucocorticoids can lead to iatrogenic Cushing's syndrome, increased cardiovascular risk factors, glucose intolerance, reduced growth velocity, and decreased bone mineral density in CAH patients (Elnecave et al., "Bone mineral density in girls with classical congenital adrenal hyperplasia due to CYP21 deficiency," *J. Pediatr. Endocrinol. Metab.* (2008) 21(12):1155-1162; King et al., "Long-term corticosteroid replacement and bone mineral density in adult women with classical congenital adrenal hyperplasia," *J. Clin. Endocrinol. Metab.* (2006) 91(3):865-869; Migeon and Wisniewski, "Congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. Growth, development, and therapeutic considerations," *Endocrinol. Metab. Clin. North Am.* (2001) 30(1):193-206).

Corticotropin releasing factor (CRF) is a hypothalamic hormone released directly into the hypophyseal portal vasculature and acts on specific corticotropin releasing factor 1 (CRF1) receptors on corticotropes in the anterior pituitary to stimulate the release of ACTH. Blockade of these receptors has been shown to decrease the release of ACTH in both animals and humans. Therefore, compounds that block CRF1 receptors have the potential to directly inhibit the excessive ACTH release that occurs in CAH and thereby allow for normalization of androgen production while using lower, more physiologic doses of hydrocortisone.

The compound of Formula (I)



(I)

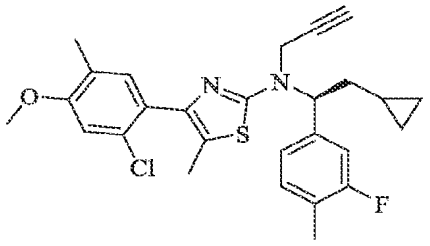
4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, is a selective CRF1 receptor antagonist. The compound of Formula (I) can be prepared according to the methods described in U.S. Patent Nos. 6,586,456 and 8,314,249, each of which is hereby incorporated by reference in its entirety. The compound of Formula (I) is a low solubility compound with low bioavailability. Partially due to its low solubility, attempts at formulating the compound of Formula (I) have proven to be difficult, particularly for formulations suitable for pediatric administration.

Thus, there is a need for a treatment for CAH that avoids the severe complications associated with corticosteroid therapy. There is also a need for a formulation of the compound of Formula (I) with increased bioavailability and a need for a formulation of the compound of Formula (I) that is suitable for pediatric administration. The formulations and methods of the present invention help address these and other needs.

SUMMARY

Provided herein is a pharmaceutical composition comprising:

(a) a compound of Formula (I):



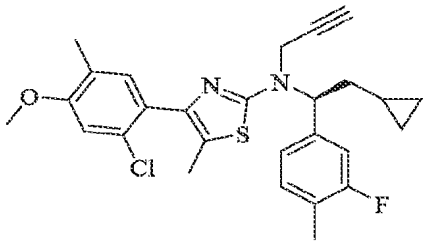
(I)

or a pharmaceutically acceptable salt thereof; and

(b) one or more of an oily phase vehicle, an emulsifying agent, a nonionic surfactant, and a solubilizing agent.

Provided herein is a pharmaceutical composition in oral solution dosage form comprising:

(a) a compound of Formula (I):



(I)

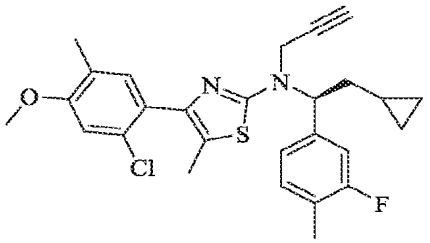
or a pharmaceutically acceptable salt thereof;

(b) one or more of a sweetener, an anti-oxidant, and a flavor; and

(c) a liquid vehicle.

Also provided herein is a pharmaceutical composition of the present disclosure (e.g., a pharmaceutical composition in oral solution dosage form of the present disclosure) for use in therapy, for example, for use in any of the methods disclosed herein.

Provided herein is a method for treating congenital adrenal hyperplasia (CAH) comprising administering 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine having the Formula (I):



(I)

or a pharmaceutically acceptable salt thereof.

Also provided herein is a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, for use in a method of treating congenital adrenal hyperplasia in a subject.

Also provided herein is use of a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in a method of treating congenital adrenal hyperplasia in a subject.

Provided herein is a method of treating congenital adrenal hyperplasia in a subject in need thereof comprising administering 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, in an amount sufficient to reduce the level of one or more biomarkers selected from (a) 17-hydroxyprogesterone (17-OHP); (b) adrenocorticotropic hormone (ACTH); and (c) androstenedione in the subject.

Also provided herein is a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, for use in a method of treating congenital adrenal hyperplasia in a subject, wherein the compound, or pharmaceutically acceptable salt thereof, is administered in an amount sufficient to reduce the level of one or more biomarkers selected from (a) 17-hydroxyprogesterone (17-OHP); (b) adrenocorticotropic hormone (ACTH); and (c) androstenedione in the subject.

Also provided herein is use of a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in a method of treating congenital adrenal hyperplasia in a subject, wherein the compound, or pharmaceutically acceptable salt thereof, is administered in an

amount sufficient to reduce the level of one or more biomarkers selected from (a) 17-hydroxyprogesterone (17-OHP); (b) adrenocorticotropic hormone (ACTH); and (c) androstenedione in the subject.

In some embodiments, the reduction in level of any of biomarkers is determined by comparing the level of the biomarker as measured during the circadian release on a day prior to administering 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof and the level of the biomarker as measured during the circadian release on the day after administering the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof. In some embodiments, the circadian release occurs between the hours of 2 a.m. and 10 a.m.

In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, is administered three to eight hours prior to the circadian release of the biomarker.

In some embodiments, the level of 17-hydroxyprogesterone is reduced by at least 25%. In some embodiments, the level of 17-hydroxyprogesterone is reduced by at least 50%.

In some embodiments, the level of adrenocorticotropic hormone is reduced by at least 25%. In some embodiments, the level of adrenocorticotropic hormone is reduced by at least 40%. In some embodiments, the level of adrenocorticotropic hormone is reduced by at least 50%.

In some embodiments, the level of androstenedione is reduced by at least 25%. In some embodiments, the level of androstenedione is reduced by at least 30%. In some embodiments, the level of androstenedione is reduced by at least 50%.

In some embodiments, the level of 17-hydroxyprogesterone is reduced by at least 50% and the level of androstenedione is reduced by at least 50%.

In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, is administered once daily at an amount equivalent to about 50 mg or about 100 mg of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine free base.

In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine is administered in the free base form.

Provided herein is a method for reducing the severity of one or more symptoms selected from hirsutism, precocious puberty, fertility problems, acne, and growth impairment in a subject having classic congenital adrenal hyperplasia, comprising administering 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, in an amount sufficient to reduce the level of androstenedione in the subject. In some embodiments, the growth impairment is selected from one or more of accelerated height velocity, accelerated weight velocity, or accelerated bone age.

Also provided herein is a compound which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, for use in a method of reducing the severity of one or more symptoms selected from hirsutism, precocious puberty, fertility problems, acne, and growth impairment in a subject having classic congenital adrenal hyperplasia, wherein the compound, or pharmaceutically acceptable salt thereof, is administered in an amount sufficient to reduce the level of androstenedione in the subject.

Also provided herein is use of a compound which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in a method of reducing the severity of one or more symptoms selected from hirsutism, precocious puberty, fertility problems, acne, and growth impairment in a subject having classic congenital adrenal hyperplasia, wherein the compound, or pharmaceutically acceptable salt thereof, is administered in an amount sufficient to reduce the level of androstenedione in the subject.

In some embodiments, the level of androstenedione is reduced by at least 25%. In some embodiments, the level of androstenedione is reduced by at least 30%. In some embodiments, the level of androstenedione is reduced by at least 50%.

Provided herein is a method of reducing the level of one or more biomarkers of congenital adrenal hyperplasia in a subject having congenital adrenal hyperplasia comprising administering to the subject 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-

1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof.

Also provided herein is a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, for use in a method of reducing the level of one or more biomarkers of congenital adrenal hyperplasia in a subject having congenital adrenal hyperplasia.

Also provided herein is use of a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in a method of reducing the level of one or more biomarkers of congenital adrenal hyperplasia in a subject having congenital adrenal hyperplasia.

In some embodiments, the one or more biomarkers of congenital adrenal hyperplasia are selected from (a) 17-hydroxyprogesterone (17-OHP); (b) adrenocorticotropic hormone (ACTH); and (c) androstenedione.

Provided herein is a method of reducing the dosage of corticosteroid administered to a subject having congenital adrenal hyperplasia for controlling congenital adrenal hyperplasia comprising administering to the subject 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof. In some embodiments, the corticosteroid is a glucocorticoid.

Also provided herein is a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, for use in a method of reducing the dosage of corticosteroid administered to a subject having congenital adrenal hyperplasia.

Also provided herein is use of a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in a method of reducing the dosage of corticosteroid administered to a subject having congenital adrenal hyperplasia.

Provided herein is a method of reducing the severity of one or more side effects of glucocorticoid treatment in a subject having congenital adrenal hyperplasia comprising

administering to the subject 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, wherein the side effect is selected from osteoporosis, avascular necrosis of bone, myopathy, hyperglycemia, diabetes mellitus, dyslipidemia, weight gain, Cushing syndrome, Cushingoid features, growth suppression, adrenal suppression, gastritis, peptic ulcer, gastrointestinal bleeding, visceral perforation, hepatic steatosis, pancreatitis, hypertension, coronary heart disease, ischemic heart disease, heart failure, dermatoprosis, skin atrophy, ecchymosis, purpura, erosions, striae, delayed wound healing, easy bruising, acne, hirsutism, hair loss, mood changes, depression, euphoria, mood lability, irritability, akathisia, anxiety, cognitive impairment, psychosis, dementia, delirium, cataract, glaucoma, ptosis, mydriasis, opportunistic ocular infections, central serous chorioretinopathy, suppression of cell-mediated immunity, predisposition to infections, and reactivation of latent infections.

Also provided herein is a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, for use in a method of reducing the severity of one or more side effects of glucocorticoid treatment in a subject having congenital adrenal hyperplasia, wherein the side effect is selected from osteoporosis, avascular necrosis of bone, myopathy, hyperglycemia, diabetes mellitus, dyslipidemia, weight gain, Cushing syndrome, Cushingoid features, growth suppression, adrenal suppression, gastritis, peptic ulcer, gastrointestinal bleeding, visceral perforation, hepatic steatosis, pancreatitis, hypertension, coronary heart disease, ischemic heart disease, heart failure, dermatoprosis, skin atrophy, ecchymosis, purpura, erosions, striae, delayed wound healing, easy bruising, acne, hirsutism, hair loss, mood changes, depression, euphoria, mood lability, irritability, akathisia, anxiety, cognitive impairment, psychosis, dementia, delirium, cataract, glaucoma, ptosis, mydriasis, opportunistic ocular infections, central serous chorioretinopathy, suppression of cell-mediated immunity, predisposition to infections, and reactivation of latent infections.

Also provided herein is use of a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in a method of reducing the severity of one or more side effects of glucocorticoid treatment in a subject having congenital adrenal hyperplasia, wherein the side

effect is selected from osteoporosis, avascular necrosis of bone, myopathy, hyperglycemia, diabetes mellitus, dyslipidemia, weight gain, Cushing syndrome, Cushingoid features, growth suppression, adrenal suppression, gastritis, peptic ulcer, gastrointestinal bleeding, visceral perforation, hepatic steatosis, pancreatitis, hypertension, coronary heart disease, ischemic heart disease, heart failure, dermatoprosis, skin atrophy, ecchymosis, purpura, erosions, striae, delayed wound healing, easy bruising, acne, hirsutism, hair loss, mood changes, depression, euphoria, mood lability, irritability, akathisia, anxiety, cognitive impairment, psychosis, dementia, delirium, cataract, glaucoma, ptosis, mydriasis, opportunistic ocular infections, central serous chorioretinopathy, suppression of cell-mediated immunity, predisposition to infections, and reactivation of latent infections.

In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof is administered at an amount sufficient to reduce the level of 17-hydroxyprogesterone (17-OHP) by at least 50% as compared to the level prior to administration.

In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof is administered at an amount sufficient to reduce the level of androstenedione by at least 30% as compared to the level prior to administration.

In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof is administered at an amount sufficient to (a) reduce the level of 17-hydroxyprogesterone (17-OHP) by at least 50% as compared to the level prior to administration; and (b) reduce the level of androstenedione by at least 30% as compared to the level prior to administration.

In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, is administered once daily at an amount equivalent to from about 25 mg to about 150 mg 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine free base.

In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine,

or a pharmaceutically acceptable salt thereof, is administered once daily at an amount equivalent to about 50 mg or about 100 mg of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine free base.

In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine is administered in the free base form.

Provided herein is a method of treating congenital adrenal hyperplasia in a subject comprising

(i) measuring the level of one or more biomarkers selected from (a) 17-hydroxyprogesterone (17-OHP); (b) adrenocorticotropic hormone (ACTH); and (c) androstenedione in a biological sample obtained from the subject;

(ii) analyzing the level of the one or more biomarkers to determine if the level of the one or more biomarkers is elevated compared to a healthy subject not having congenital adrenal hyperplasia; and

(iii) administering to the subject 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof if the subject is determined to have elevated levels of the one or more biomarkers.

Also provided herein is a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, for use in a method of treating congenital adrenal hyperplasia in a subject comprising

(i) measuring the level of one or more biomarkers selected from (a) 17-hydroxyprogesterone (17-OHP); (b) adrenocorticotropic hormone (ACTH); and (c) androstenedione in a biological sample obtained from the subject;

(ii) analyzing the level of the one or more biomarkers to determine if the level of the one or more biomarkers is elevated compared to a healthy subject not having congenital adrenal hyperplasia; and

(iii) administering to the subject 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof if the subject is determined to have elevated levels of the one or more biomarkers.

Also provided herein is use of a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in a method of treating congenital adrenal hyperplasia in a subject, wherein the method comprises:

(i) measuring the level of one or more biomarkers selected from (a) 17-hydroxyprogesterone (17-OHP); (b) adrenocorticotropic hormone (ACTH); and (c) androstenedione in a biological sample obtained from the subject;

(ii) analyzing the level of the one or more biomarkers to determine if the level of the one or more biomarkers is elevated compared to a healthy subject not having congenital adrenal hyperplasia; and

(iii) administering to the subject 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof if the subject is determined to have elevated levels of the one or more biomarkers.

In some embodiments, the method further comprises (iv) measuring the level of the one or more biomarkers after administering 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, in a biological sample obtained from the subject to determine whether the subject has reduced levels of the one or more biomarkers as compared with the measurement of step (i). In some embodiments, the method further comprises (v) continuing the administration of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof if the subject has reduced levels of the one or more biomarkers.

In some embodiments, steps (i) and (iv) are performed on biological samples taken from the subject in a similar manner and within a same time of day window. In some embodiments, steps (i) and (iv) are performed on biological samples taken from the subject within the time of day window from 2 a.m. to 10 a.m. In some embodiments, steps (i) and (iv) are performed on biological samples taken from the subject within the time of day window from 6 a.m. to 10 a.m.

In some embodiments, steps (i) and (iv) comprise measuring the levels of at least two biomarkers selected from (a) 17-hydroxyprogesterone (17-OHP); (b) adrenocorticotropic hormone (ACTH); and (c) androstenedione.

In some embodiments, steps (i) and (iv) comprise measuring the levels of (a) 17-hydroxyprogesterone (17-OHP); (b) adrenocorticotropic hormone (ACTH); and (c) androstenedione.

In some embodiments, step (i) comprises measuring the level of 17-hydroxyprogesterone (17-OHP), wherein the level of 17-hydroxyprogesterone (17-OHP) is elevated when it is greater than or equal to 1,000 ng/dL.

In some embodiments, step (i) comprises measuring the level of androstenedione, wherein the level of androstenedione is elevated when it is greater than 200 ng/dL.

In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, is administered once daily at an amount equivalent to from about 25 mg to about 150 mg of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine free base. In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, is administered once daily at an amount equivalent to about 50 mg or about 100 mg of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine free base. In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine is administered in the free base form.

Provided herein is a method of treating congenital adrenal hyperplasia (CAH), in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, where in the subject is in a fed state.

Also provided herein is a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, for use in a method

of treating congenital adrenal hyperplasia (CAH) in a subject, wherein the subject is in a fed state.

Also provided herein is use of a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in a method of treating congenital adrenal hyperplasia (CAH) in a subject, wherein the subject is in a fed state.

In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, is administered to the subject with a nutritional composition. In some embodiments, the nutritional composition is a liquid dietary supplement comprising about 1500 calories per liter with a caloric distribution of about 14.7% protein, about 32% fat and about 53.3% carbohydrate. In some embodiments, the nutritional composition is administered in an amount of about 8 fluid ounces. In some embodiments, the nutritional composition is administered within 30 minutes of administration of the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof.

In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, is administered to the subject with a nutritional composition. In some embodiments, the nutritional composition is a liquid dietary supplement comprising 1500 calories per liter with a caloric distribution of 14.7% protein, 32% fat and 53.3% carbohydrate. In some embodiments, the nutritional composition is administered in an amount of about 8 fluid ounces. In some embodiments, the nutritional composition is administered within 30 minutes of administration of the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof.

In some embodiments, administering the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, exhibits a positive food effect. In some embodiments, the positive food effect is measured in terms of C_{max}, AUC, or combinations thereof when comparing oral administration of the 4-(2-chloro-4-methoxy-5-methylphenyl)-

N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, in the fed and fasting states.

In some embodiments, the ratio of the AUC in the fed state to the AUC in the fasted state is about 5 to about 10. In some embodiments, the ratio of the C_{max} in the fed state to the C_{max} in the fasted state is about 5 to about 10.

Provided herein is a method of reducing glucocorticoid burden in a subject as measured after a time period of administration of a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, wherein the decrease in glucocorticoid burden is relative to the glucocorticoid burden prior to administration of the compound, or pharmaceutically acceptable salt thereof.

Also provided herein is a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, for use in a method of reducing glucocorticoid burden in a subject, wherein the decrease in glucocorticoid burden is relative to the glucocorticoid burden prior to administration of the compound, or pharmaceutically acceptable salt thereof.

Also provided herein is use of a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in a method of reducing glucocorticoid burden in a subject, wherein the decrease in glucocorticoid burden is relative to the glucocorticoid burden prior to administration of the compound, or pharmaceutically acceptable salt thereof.

Provided herein is a method of improving one or more symptoms selected from quality of life, fatigue, sleep, insulin resistance, glucose tolerance, glucose control, dyslipidemia, hyperlipidemia, bone mineral density, bone turnover, fat mass, weight, central obesity, blood pressure, hirsutism severity, menstrual cyclicity, control of testicular adrenal rest tumor and fertility in a subject having classic congenital adrenal hyperplasia, comprising administering 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, wherein the symptom is improved after a time period of administration of the compound , or pharmaceutically acceptable salt thereof, wherein the improvement in the

one or more symptoms is relative to the status of the one or more symptoms prior to administration of the compound, or pharmaceutically acceptable salt thereof.

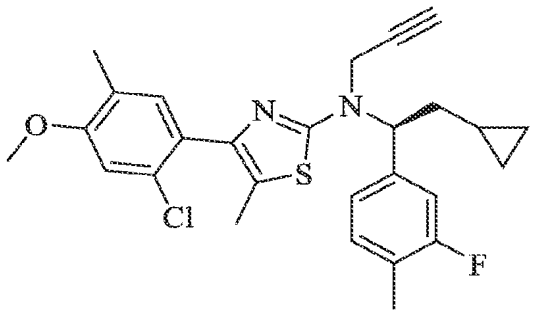
Also provided herein is a compound which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, for use in a method of improving one or more symptoms selected from quality of life, fatigue, sleep, insulin resistance, glucose tolerance, glucose control, dyslipidemia, hyperlipidemia, bone mineral density, bone turnover, fat mass, weight, central obesity, blood pressure, hirsutism severity, menstrual cyclicity, control of testicular adrenal rest tumor and fertility in a subject having classic congenital adrenal hyperplasia, wherein the symptom is improved after a time period of administration of the compound, or pharmaceutically acceptable salt thereof, wherein the improvement in the one or more symptoms is relative to the status of the one or more symptoms prior to administration of the compound, or pharmaceutically acceptable salt thereof.

Also provided herein is use of a compound which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in a method of improving one or more symptoms selected from quality of life, fatigue, sleep, insulin resistance, glucose tolerance, glucose control, dyslipidemia, hyperlipidemia, bone mineral density, bone turnover, fat mass, weight, central obesity, blood pressure, hirsutism severity, menstrual cyclicity, control of testicular adrenal rest tumor and fertility in a subject having classic congenital adrenal hyperplasia, wherein the symptom is improved after a time period of administration of the compound, or pharmaceutically acceptable salt thereof, wherein the improvement in the one or more symptoms is relative to the status of the one or more symptoms prior to administration of the compound, or pharmaceutically acceptable salt thereof.

Also provided herein is a compound, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof for use in therapy, for example, for use in any of the methods disclosed herein.

Also provided herein is use of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, for manufacture of a medicament for use in any of the methods disclosed herein.

Also provided herein is a spray-dried dispersion containing 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine having the Formula (I):



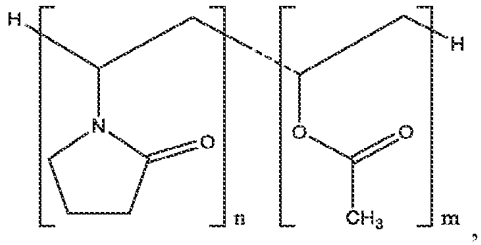
(I)

or a pharmaceutically acceptable salt thereof, and a polymer. In some embodiments, the polymer is selected from a neutral polymer, an enteric polymer, and a pyrrolidone polymer. In some embodiments, the weight ratio of the compound of Formula (I) to the polymer is from about 1:9 to about 1:1.

In some embodiments, the polymer is a neutral polymer. In some embodiment, the neutral polymer is selected from hydroxypropyl methylcellulose (HPMC) and hydroxyethyl cellulose (HEC).

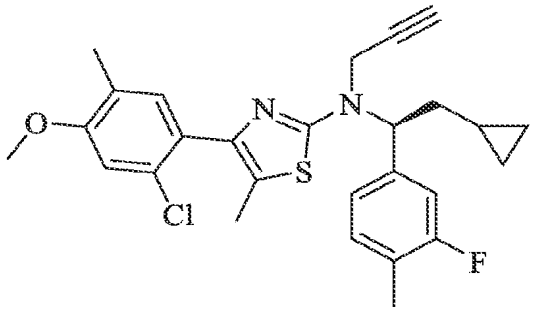
In some embodiments, the polymer is an enteric polymer. In some embodiments, the enteric polymer is selected from hydroxypropyl methyl cellulose acetate succinate (HPMCAS), cellulose acetate phthalate (CAP), hydroxypropyl methyl cellulose phthalate (HPMCP), an amino methacrylate copolymer, an ammonioalkyl methacrylate copolymer, and a methacrylic copolymer.

In some embodiments, the polymer is a pyrrolidone polymer. In some embodiments, the pyrrolidone polymer is selected from polyvinyl pyrrolidone (PVP) and a polyvinyl pyrrolidone vinyl acetate (PVP/VA). In some embodiments, the pyrrolidone polymer is PVP/VA. In some embodiments, the copolymer comprises 1-vinyl-2-pyrrolidone and vinyl acetate at a ratio of about 40:60 to about 60:40 by weight. In some embodiments, the copolymer comprises 1-vinyl-2-pyrrolidone and vinyl acetate at a ratio of about 60:40 by weight. In some embodiments, the copolymer has the structure:



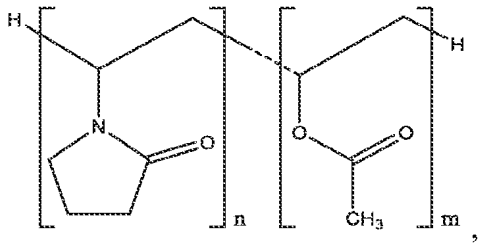
wherein the value of n is about 1 to about 2 times the value of m. In some embodiments, the copolymer is copovidone, wherein the value of n is about 1.16 times the value of m. In some embodiments, the copolymer is copovidone having an average molecular weight of about 45,000 to about 70,000.

Provided herein is a spray-dried dispersion comprising 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine having the Formula (I):



(I)

or a pharmaceutically acceptable salt thereof; and a polymer that is a copolymer of 1-vinyl-2-pyrrolidone and vinyl acetate having the structure:



wherein the value of n is about 1 to about 2 times the value of m and the copolymer comprises 1-vinyl-2-pyrrolidone and vinyl acetate at a ratio of about 60:40 by weight; and wherein the weight ratio of the compound of Formula (I) to the copolymer is from about 1:1 to about 1:9.

In some embodiments of the spray-dried dispersions of the present disclosure, the compound of Formula (I) and the polymer together form homogeneous particles. In some embodiments, the particles have a particle size distribution D_{50} of about 5 μm to about 100 μm. In some embodiments, the particles have a particle size distribution D_{50} of about 10 μm to

about 50 μm. In some embodiments, the particles have a particle size distribution D_{50} of about 15 μm to about 30 μm.

In some embodiments, the weight ratio of the compound of Formula (I) to the polymer is from about 1:1.5 to about 1:9. In some embodiments, the weight ratio of the compound of Formula (I) to the polymer is from about 1:2.5 to about 1:4. In some embodiments, the weight ratio of the compound of Formula (I) to the polymer is about 1:3.

In some embodiments, the particles have a residual solvent content less than about 2 wt %. In some embodiments, the particles have a residual solvent content less than about 1 wt %. In some embodiments, the particles have a residual solvent content of about 0.5 wt % or less.

In some embodiments, the compound of Formula (I) in the dispersion is substantially amorphous.

Also provided herein is a method for preparing a spray-dried dispersion of the present disclosure, comprising: dissolving the compound of Formula (I), or a pharmaceutically acceptable salt thereof, and the polymer in an organic solvent to form a solution; and spray-drying the solution to produce the spray-dried dispersion, wherein the spray-drying forms homogeneous particles of the compound of Formula (I) and the polymer.

In some embodiments, the method comprises removing the organic solvent after formation of the spray-dried dispersion by drying the spray-dried dispersion. In some embodiments, the spray-dried dispersion is dried with a convection tray dryer. In some embodiments, the organic solvent is acetone.

In some embodiments, the spray dryer inlet temperature is about 60°C to about 80°C. In some embodiments, the spray dryer inlet temperature is about 72°C.

In some embodiments, the spray dryer outlet temperature is about 25°C to about 45°C. In some embodiments, the spray dryer outlet temperature is about 35°C.

In some embodiments, the homogeneous particles comprise a bulk density of less than about 0.2 g/mL. In some embodiments, the homogeneous particles comprise a bulk density of less than about 0.15 g/mL.

In some embodiments, the homogeneous particles comprise a tapped density of less than about 0.3 g/mL. In some embodiments, the homogeneous particles comprise a tapped density of less than about 0.25 g/mL.

Also provided herein is a pharmaceutical composition comprising the spray-dried dispersion of the present disclosure and one or more pharmaceutically acceptable excipients. In some embodiments, the spray-dried dispersion is present in an amount of about 20% to about 90% w/w of the composition. In some embodiments, the spray-dried dispersion is present in an amount of about 40% to 80% w/w of the composition.

In some embodiments, the pharmaceutical excipients are selected from the group consisting of a filler, a lubricant, and combinations thereof. In some embodiments, the filler is selected from the group consisting of a binder, a diluent, a disintegrant, a glidant, a surfactant, and combinations thereof.

In some embodiments, the pharmaceutical composition is formulated in unit dosage form, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is present in an amount of about 5 mg to about 200 mg. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is present in an amount of about 75 mg to about 150 mg. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is present in an amount of about 50 mg. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is present in an amount of about 100 mg.

In some embodiments, the pharmaceutical composition is formulated as a tablet, capsule, sachet, powder, granules, coated particle, coated tablet, enterocoated tablet, enterocoated capsule, melting strip, or melting film. In some embodiments, the pharmaceutical composition is in tablet form. In some embodiments, the pharmaceutical composition is in capsule form. In some embodiments, the pharmaceutical composition is coated.

In some embodiments, the spray-dried dispersion is formulated for oral administration and exhibits a positive food effect when administered orally. In some embodiments, the spray-dried dispersion has a ratio of the AUC in the fed state to the AUC in the fasted state of about 5 to about 10. In some embodiments, wherein the spray-dried dispersion has a ratio of the C_{max} in the fed state to the C_{max} in the fasted state of about 5 to about 10.

Also provided herein is a method for preparing a pharmaceutical composition, comprising combining the spray-dried dispersion of the present disclosure with one or more pharmaceutically acceptable excipients.

Also provided herein is a method of treating congenital adrenal hyperplasia (CAH), in a subject in need thereof, comprising administering to the subject a therapeutically effective

amount of a spray-dried dispersion of the present disclosure or a pharmaceutical composition comprising a spray-dried dispersion of the present disclosure.

Also provided herein is a spray-dried dispersion of the present disclosure or a pharmaceutical composition comprising a spray-dried dispersion of the present disclosure for use in a method of treating congenital adrenal hyperplasia (CAH) in a subject.

Also provided herein is use of a spray-dried dispersion of the present disclosure in the manufacture of a medicament for use in a method of treating congenital adrenal hyperplasia (CAH) in a subject.

In some embodiments, the spray-dried dispersion or the pharmaceutical composition is administered to the subject in a fed state. In some embodiments, the spray-dried dispersion or the pharmaceutical composition is administered to the subject with a nutritional composition. In some embodiments, the nutritional composition is a liquid dietary supplement comprising about 1000 to about 2000 calories per liter with a fat content greater than 30%. In some embodiments, the nutritional composition is a liquid dietary supplement comprising 1500 calories per liter with a caloric distribution of 14.7% protein, 32% fat and 53.3% carbohydrate. In some embodiments, the nutritional composition is administered in an amount of about 8 fluid ounces. In some embodiments, the nutritional composition is administered within 30 minutes of administration of the spray-dried dispersion or the pharmaceutical composition.

In some embodiments, administering the spray-dried dispersion or the pharmaceutical composition exhibits a positive food effect. In some embodiments, the positive food effect is measured in terms of C_{max}, AUC, or combinations thereof when comparing oral administration of the spray-dried dispersion or pharmaceutical composition in the fed and fasting states. In some embodiments, the ratio of the AUC in the fed state to the AUC in the fasted state is about 5 to about 10. In some embodiments, the ratio of the C_{max} in the fed state to the C_{max} in the fasted state is about 5 to about 10. In some embodiments, the ratio of the AUC in the fed state to the AUC in the fasted state is about 10 to about 20. In some embodiments, the ratio of the C_{max} in the fed state to the C_{max} in the fasted state is about 10 to about 20.

In some embodiments of the disclosed methods, the subject is a pediatric subject.

Also provided herein is a spray-dried dispersion of the present disclosure or a pharmaceutical composition comprising a spray-dried dispersion of the present disclosure, for use in therapy, for example, for use in any of the methods disclosed herein.

Also provided herein is use of a spray-dried dispersion of the present disclosure for manufacture of a medicament for use in any of the methods disclosed herein.

Also provided herein is a method of treating congenital adrenal hyperplasia (CAH), in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the present disclosure, wherein the pharmaceutical composition comprises a therapeutically effective amount of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Also provided herein is a pharmaceutical composition of the present disclosure, wherein the pharmaceutical composition comprises a therapeutically effective amount of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, for use in a method of treating congenital adrenal hyperplasia (CAH) in a subject.

Also provided herein is use of pharmaceutical composition of the present disclosure, wherein the pharmaceutical composition comprises a therapeutically effective amount of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in a method of treating congenital adrenal hyperplasia (CAH) in a subject.

Also provided herein is pharmaceutical composition of the present disclosure, wherein the pharmaceutical composition comprises a therapeutically effective amount of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, for use in therapy, for example, for use in any of the methods disclosed herein.

Also provided herein is use of pharmaceutical composition of the present disclosure, wherein the pharmaceutical composition comprises a therapeutically effective amount of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, for manufacture of a medicament for use in any of the methods disclosed herein.

Also provided herein is a method of improving gastrointestinal absorption of a compound of Formula (I) in a subject, comprising orally administering to the subject a therapeutically effective amount of a spray-dried dispersion of the present disclosure or a pharmaceutical composition comprising a spray-dried dispersion of the present disclosure, wherein the improvement is relative to oral administration of the compound of Formula (I) which has not been prepared as a spray-dried dispersion.

Also provided herein is a spray-dried dispersion of the present disclosure or a pharmaceutical composition comprising a spray-dried dispersion of the present disclosure, for use in a method of improving gastrointestinal absorption of a compound of Formula (I) in a subject, wherein the improvement is relative to oral administration of the compound of Formula (I) which has not been prepared as a spray-dried dispersion.

Also provided herein is use of a spray-dried dispersion of the present disclosure in the manufacture of a medicament for use in a method of improving gastrointestinal absorption of

a compound of Formula (I) in a subject, wherein the improvement is relative to oral administration of the compound of Formula (I) which has not been prepared as a spray-dried dispersion.

In some embodiments, the subject is a pediatric subject.

Also provided herein is a method of improving oral bioavailability of a compound of Formula (I) in a subject, comprising orally administering to the subject a therapeutically effective amount of a spray-dried dispersion of the present disclosure or a pharmaceutical composition comprising a spray-dried dispersion of the present disclosure, wherein the improvement is relative to oral administration of the compound of Formula (I) which has not been prepared as a spray-dried dispersion.

Also provided herein is a spray-dried dispersion of the present disclosure or a pharmaceutical composition comprising a spray-dried dispersion of the present disclosure, for use in a method of improving oral bioavailability of a compound of Formula (I) in a subject, wherein the improvement is relative to oral administration of the compound of Formula (I) which has not been prepared as a spray-dried dispersion.

Also provided herein is use of a spray-dried dispersion of the present disclosure in the manufacture of a medicament for use in a method of improving oral bioavailability of a compound of Formula (I) in a subject, wherein the improvement is relative to oral administration of the compound of Formula (I) which has not been prepared as a spray-dried dispersion.

In some embodiments, the subject is a pediatric subject.

Also provided herein is a crystalline salt, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, p-toluenesulfonic acid salt.

Also provided herein is a crystalline salt, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, p-toluenesulfonic acid salt, for use in therapy, for example, for use in any of the methods disclosed herein.

Also provided herein is use of a crystalline salt, which is 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, p-toluenesulfonic acid salt, for manufacture of a medicament for use in any of the methods disclosed herein.

Other features and advantages of the methods, processes, formulations, and uses provided herein will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF DRAWINGS

FIG. 1 shows the dissolution performance of several spray-dried dispersion formulations in 0.5 wt% simulated intestinal fluid (SIF) in phosphate buffered saline (PBS), pH 6.5.

FIG. 2 shows the vertical membrane flux cell integrated in the µDiss Profiler™ used for the membrane flux assay.

FIG. 3 shows non-sink dissolution data for several spray-dried dispersion formulations and the compound of Formula (I) in 0.5 wt% SIF in PBS, pH 6.5.

FIG. 4 is a graph showing membrane flux of 1 mg/mL GB/IB 0.5 wt% SIF doses of the compound of Formula (I) and various spray-dried dispersion formulations over time. The solid lines indicate flux ($\mu g\ min^{-1}\ cm^{-2}$) and the broken lines indicate concentration (µg/mL) in 0.5% SIF.

FIG. 5 is a flow diagram of the spray drying manufacturing process used to prepare a 1000 g batch of a SDD containing 25% of the compound of Formula (I) and 75% PVP/VA 64.

FIGS. 6A and 6B are line graphs showing the pharmacokinetic results of a bioavailability and food effect study in dogs. FIG. 6A shows the results from Cohort 1 and FIG. 6B shows the results from Cohort 2.

FIG. 7 is a flow chart showing the study design of a Phase 1 study of the pharmacokinetics and food effect of the compound of Formula (I) in healthy adult subjects.

FIGS. 8A and 8B are line graphs showing the mean plasma concentration versus time profiles for the compound of Formula (I) under fasted and fed conditions, respectively, in healthy adult subjects.

FIGS. 9A-9C are spaghetti plots of the pharmacokinetics of the compound of Formula (I) in healthy adult subjects under fasted and fed conditions. FIG. 9A shows the $AUC_{0\text{-tlast}}$ values. FIG. 9B shows the $AUC_{0\text{-}\infty}$ values. FIG. 9C shows the C_{max} values.

FIG. 10 is a flow chart showing the study design of a Phase 1 study of the bioavailability, pharmacokinetics and food effect of the compound of Formula (I) in healthy adult subjects.

FIG. 11 shows the study design of a Phase 2 study of the compound of Formula (I) in adult subjects with congenital adrenal hyperplasia.

FIGs. 12A and 12B show the arithmetic mean values for adrenocorticotropic hormone (ACTH) (FIG. 12A) and 17-hydroxyprogesterone (17-OHP) (FIG. 12B) for all 8 Cohort 1 subjects plotted at each time point for pre-treatment baseline (circles), day 1 (squares), and day 14 (triangles).

FIGs. 13A and 13B show arithmetic mean values for androstenedione (FIG. 13A) and testosterone (FIG. 13B) for all 8 Cohort 1 subjects were plotted at each timepoint for pre-treatment baseline (circles), day 1 (squares), and day 14 (triangles).

FIGs. 14A and 14B show the reduction of ACTH at timepoints 8-, 10-, and 12-hours postdose. FIG 14A shows the values for each time point as compared to baseline. FIG. 14B shows the mean values across all three timepoints.

FIGs. 15A and 15B show the reduction of 17-OHP at timepoints 8-, 10-, and 12-hours postdose. FIG 15A shows the values for each time point as compared to baseline. FIG. 15B shows the mean values across all three timepoints.

FIGs. 16A and 16B show the reduction of androstenedione at timepoints 8-, 10-, and 12-hours postdose. FIG 16A shows the values for each time point as compared to baseline. FIG. 16B shows the mean values across all three timepoints.

FIG. 17A shows the plasma ACTH Mean Blood Concentrations following the compound of Formula (I) 50 mg Dose qhs (Cohort 1; n=8). Error bars represent the standard error of the mean for each morning window timepoint. ACTH normal ranges: Female 6 to 58 pg/mL; Male 7 to 69 pg/mL.

FIG. 17B shows the serum 17-OHP Mean Blood Concentrations following the compound of Formula (I) 50 mg Dose qhs (Cohort 1; n=8). Error bars represent the standard error of the mean for each morning window timepoint. 17-OHP normal ranges: Female < 207 ng/dL; Male < 139 ng/dL.

FIG. 17C: shows the serum Androstenedione Mean Blood Concentrations following the compound of Formula (I) 50 mg Dose qhs (Cohort 1; n=8). Error bars represent the standard error of the mean for each morning window timepoint. Androstenedione normal ranges: Female 26 to 214 ng/mL; Male 33 to 134 ng/mL.

FIG. 18A shows the plasma ACTH Mean Blood Concentrations following the compound of Formula (I) 100 mg Dose qhs (Cohort 2; n=4). Error bars represent the standard

error of the mean for each morning window timepoint. ACTH normal ranges: Female 6 to 58 pg/mL; Male 7 to 69 pg/mL.

FIG. 18B shows the serum 17-OHP Mean Blood Concentrations following the compound of Formula (I) 100 mg Dose qhs (Cohort 2; n=4). Error bars represent the standard error of the mean for each morning window timepoint. 17-OHP normal ranges: Female < 207 ng/dL; Male < 139 ng/dL.

FIG. 18C shows the serum Androstenedione Mean Blood Concentrations following the compound of Formula (I) 100 mg Dose qhs (Cohort 2; n=4). Error bars represent the standard error of the mean for each morning window timepoint. Androstenedione normal ranges: Female 26 to 214 ng/mL; Male 33 to 134 ng/mL.

FIG. 19A shows the plasma ACTH Mean Blood Concentrations following the compound of Formula (I) 100 mg Dose with Evening Meal (Cohort 3). Error bars represent the standard error of the mean for each morning window timepoint. ACTH normal ranges: Female 6 to 58 pg/mL; Male 7 to 69 pg/mL.

FIG. 19B shows the serum 17-OHP Mean Blood Concentrations following the compound of Formula (I) 100 mg Dose with Evening Meal (Cohort 3). Error bars represent the standard error of the mean for each morning window timepoint. 17-OHP normal ranges: Female < 207 ng/dL; Male < 139 ng/dL.

FIG. 19C shows the serum Androstenedione Mean Blood Concentrations following the compound of Formula (I) 100 mg Dose with Evening Meal (Cohort 3). Error bars represent the standard error of the mean for each morning window timepoint. Androstenedione normal ranges: Female 26 to 214 ng/mL; Male 33 to 134 ng/mL.

FIG. 20 is a scheme showing the manufacturing process for forming 50 mg capsules of the compound of Formula (I).

FIG. 21 is an alternative scheme showing the manufacturing process for forming 50 mg capsules of the compound of Formula (I).

FIGs. 22A and 22B show a scheme showing the manufacturing process for forming SDD granules of the compound of Formula (I).

FIG. 23 is a scheme showing the manufacturing process for forming 50 mg/nL liquid formulation 1 of the compound of Formula (I).

FIG. 24 is a scheme showing the manufacturing process for forming 50 mg/nL liquid formulation 2 of the compound of Formula (I).

FIG. 25 is an XRPD spectrum of the compound of Formula (I) free base crystalline form I.

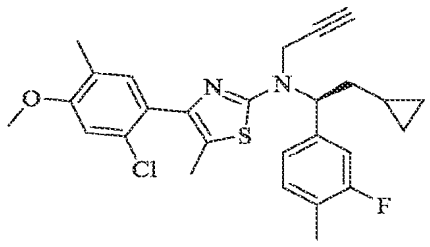
FIG. 26 is a DSC spectrum of the compound of Formula (I) free base crystalline form I.

FIG. 27 is an XRPD spectrum of the compound of Formula (I) tosylate crystalline form 1.

FIG. 28 is a DSC and TGA spectrum of the compound of Formula (I) tosylate crystalline form 1.

DETAILED DESCRIPTION

As described herein, 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine having the Formula (I):



(I)

or a pharmaceutically acceptable salt thereof, is a selective CRF1 receptor antagonist that has been found to be effective treating congenital adrenal hyperplasia. Specifically, the compound of Formula (I) has been found to effectively reduce several biomarkers associated with congenital adrenal hyperplasia.

Newborn screening for CAH is performed by immunoassay to measure 17-OHP levels in heel-stick capillary blood specimens obtained within the first 72 hours of life. The blood sample is analyzed for 17-OHP by commercially available dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA; PerkinElmer, Waltham Massachusetts) (White et al., *J. Pediatr.* 163:10-12 (2013)). Second-tier screening tests utilizing biochemical and molecular genetic testing methods, performed between 8 and 14 days of life, are employed by nine states in the United States and strongly recommended by an additional 5 states. The biochemical method includes immunoassay with organic solvent extraction or liquid chromatography followed by tandem mass spectrometry to measure steroid ratios of 17-OHP, androstenedione, and 21-deoxycortisol to cortisol (*see, e.g.,* Speiser et al., *Int. J. Pediatr. Endocrinol.* 2010:494173,

2010). The genetic screen looks for *CYP21A2* mutations that are associated with CAH. While not widely employed in the U.S., the addition of a second screening could potentially improve the sensitivity of the overall screening process, where sensitivity of the first screen alone is approximately 72%.

In absence of results from the newborn screening, female infants with classical CAH are typically identified due to the presence of ambiguous genitalia. Males have normal genitalia at birth and therefore are not diagnosed unless newborn screening is conducted or other medical complications come to attention. Infants who are not initially diagnosed with CAH and suffer from the salt-wasting form of the disease are later diagnosed in the setting of poor weight gain, vomiting, hyperkalemia and hyponatremia within the first few weeks of life.

Treatment of CAH is based on normalization of hormone and steroid levels using a variety of medications from diagnosis in infancy through adulthood. Glucocorticoids are the current standard treatment in CAH and are used both to correct the endogenous cortisol deficiency and for reducing the elevated ACTH levels from the pituitary, which drives increased androgen production. Unlike the treatment of Addison's disease (adrenal insufficiency), in which cortisol replacement is sufficient, the treatment of CAH must also reduce ACTH production, to control the subsequent androgen excess as well. Thus, the goals of glucocorticoid treatment include cortisol replacement and suppression of ACTH to prevent virilization and menstrual disturbances in women and to inhibit testicular adrenal rest tumors in men. Mineralocorticoid replacement is needed to achieve normal plasma renin activity for maintenance of regular blood pressure, electrolyte balance, and volume status in those patients with the salt-wasting form of CAH.

The regimen of glucocorticoid treatment must support normal physiology and also ensure that sufficient cortisol is available during events that may elicit a strong stress response (*e.g.*, intercurrent illness, exercise, hypotension). Careful monitoring is also necessary to avoid the development of iatrogenic Cushing's syndrome due to glucocorticoid overtreatment in an effort to adequately suppress androgen production, or Addisonian syndrome due to under-treatment.

Overtreatment with mineralocorticoids may cause hypertension while under-treatment may lead to low blood pressure, salt loss, fatigue and increased requirements for glucocorticoids. Typical laboratory tests for monitoring treatment efficacy include measurement of plasma concentrations of 17-OHP, androstenedione, testosterone, renin activity, and electrolytes.

Adult patients with CAH have an increased prevalence of risk factors for cardiovascular disease including obesity, hypertension, and insulin resistance (*see, e.g.,* Kim et al., *Semin. Reprod. Med.* 27(4):316-21 (2009)). A study of a large cohort of pediatric and adult CAH patients (n=244) demonstrated that patients are prescribed a variety of glucocorticoid treatment regimens yet frequently suffer from poor hormonal control and the aforementioned adverse outcomes (*see, e.g.,* Finkielstain et al., *J. Clin. Endocrinol Metab.* 97(12):4429-38 (2012)).

Treatment of CAH includes efforts to normalize the cortisol deficiency with glucocorticoids (usually hydrocortisone in children but often more potent agents with narrow therapeutic indices, such as dexamethasone, in adults) and, if necessary for salt-wasting, mineralocorticoids (usually fludrocortisone). The glucocorticoid doses required to achieve sufficient suppression of excess androgens, however, are usually well above the normal physiologic dose used for cortisol replacement alone as in patients with Addison's disease. This increased exposure to glucocorticoids can lead to iatrogenic Cushing's syndrome, increased cardiovascular risk factors, glucose intolerance, and decreased bone mineral density in CAH patients (*see, e.g.,* Elnecave et al., *J. Pediatr. Endocrinol. Metab.* 21:1155-62 (2008); King et al., *J. Clin. Endocrinol. Metab.* 91(3):8656-59 (2006); Migeon et al., *Endocrinol. Metab. Clin. North Am.* 30:193-206 (2001)). Recently, best practices for the clinical management of congenital adrenal hyperplasia were published in the *Journal of Clinical Endocrinology and Metabolism* (Speiser, P.W., et al. *J. Clin. Endocrinol. Metab.* November 2018, 103(11): 1-46). This article is incorporated by reference in its entirety.

Corticotropin-releasing factor (CRF) was isolated from ovine hypothalami and identified as a 41-amino acid peptide. CRF has been found to produce profound alterations in endocrine, nervous, and immune system function. CRF is believed to be the major physiological regulator of the basal and stress-induced release of adrenocorticotropic hormone ("ACTH"), ß-endorphin, and other pro-opiomelanocortin ("POMC")-derived peptides from the anterior pituitary (*see, e.g.,* Vale et al., *Science 213*:1394-1397, 1981). Secretion of CRF causes release of ACTH from corticotrophs in the anterior pituitary via binding to the CRF_1 receptor, a member of the class B family of G-protein coupled receptors.

Due to the physiological significance of CRF, the development of biologically-active small molecules having significant CRF_1 receptor binding activity and which are capable of antagonizing the CRF_1 receptor remains a desirable goal and has been the subject of ongoing research and development for the treatment of anxiety, depression, irritable bowel syndrome, post-traumatic stress disorder, and substance abuse.

The pituitary hormone ACTH, under the control of hypothalamic corticotropin-releasing factor (CRF), stimulates uptake of cholesterol and drives the synthesis of pregnenolone initiating steroidogenesis in the adrenal gland. 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.

Definitions

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Methods and materials are described herein for use in the present disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

The term "about" preceding a value for DSC, TGA, or T_g, which are reported as degrees Celsius, have an allowable variability of ±5°C. In all other instances, unless otherwise specified, the term "about" preceding a stated value includes the stated value and also includes ±20% of the stated value, and includes more specifically values of ±10%, ±5%, ±2%, and ±1% of the stated value.

To provide a more concise description, some of the quantitative expressions herein are recited as a range from about amount X to about amount Y. It is understood that when a range is recited, the range is not limited to the recited upper and lower bounds, but rather includes the full range from about amount X through about amount Y, or any range therein.

"Room temperature" or "RT" refers to the ambient temperature of a typical laboratory, which is typically around 25°C.

"Spray-drying" refers to the method of producing a dry powder from a solution or slurry. The solution or slurry is atomized or rapidly dried with a hot gas, e.g., air or nitrogen, that causes the solvent to evaporate quickly and uniformly. A "spray-dried dispersion" refers to the powder obtained from the spray-drying process.

The term "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" includes any and all solvents, co-solvents, complexing agents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, which are not biologically or otherwise undesirable. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic formulations is contemplated. Supplementary active ingredients can also be incorporated into the formulations. In addition, various excipients, such as are commonly used in the art, can be included. These and other such compounds are described in the literature, e.g., in the Merck Index, Merck & Company, Rahway, NJ. Considerations for the inclusion of various components in pharmaceutical compositions are described, e.g., in Gilman *et al.* (Eds.) (2010); *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, 12th Ed., The McGraw-Hill Companies.

"Subject," as used herein, means a human or a non-human mammal, e.g., a dog, a cat, a mouse, a rat, a cow, a sheep, a pig, a goat, a non-human primate or a bird, e.g., a chicken, as well as any other vertebrate or invertebrate. In some embodiments, the subject is a human.

In some embodiments, the subject has experienced and/or exhibited at least one symptom of the disease or disorder to be treated and/or prevented. In some embodiments, the subject has been identified or diagnosed as having congenital adrenal hyperplasia (CAH). In some embodiments, the subject is suspected of having CAH. In some embodiments, the subject has a clinical record indicating that the subject has CAH (and optionally the clinical record indicates that the subject should be treated with any of the compositions provided herein). In some embodiments, the subject is a pediatric subject.

The term "pediatric subject" as used herein refers to a subject under the age of 21 years at the time of diagnosis or treatment. The term "pediatric" can be further divided into various subpopulations including: neonates (from birth through the first month of life); infants (1 month up to two years of age); children (two years of age up to 12 years of age); and adolescents (12 years of age through 21 years of age (up to, but not including, the twenty-second birthday)). Berhman et al., *Textbook of Pediatrics*, 15th Ed. Philadelphia: W.B. Saunders Company, 1996; Rudolph et al., *Rudolph's Pediatrics*, 21st Ed. New York: McGraw-Hill, 2002; and Avery et al., *Pediatric Medicine*, 2nd Ed. Baltimore: Williams & Wilkins; 1994. In some embodiments, a pediatric subject is from birth through the first 28 days of life, from 29 days of age to less than two years of age, from two years of age to less than 12 years of age, or 12 years of age through 21 years of age (up to, but not including, the twenty-second birthday). In some embodiments, a pediatric subject is from birth through the first 28 days of life, from 29 days of age to less than 1 year of age, from one month of age to less than four months of age, from three months of age to less than seven months of age, from six months of age to less than 1 year of age, from 1 year of age to less than 2 years of age, from 2 years of age to less than 3 years of age, from 2 years of age to less than seven years of age, from 3 years of age to less than 5 years of age, from 5 years of age to less than 10 years of age, from 6 years of age to less than 13 years of age, from 10 years of age to less than 15 years of age, or from 15 years of age to less than 22 years of age.

As used herein, the terms "treat" or "treatment" refer to therapeutic or palliative measures. Beneficial or desired clinical results include, but are not limited to, alleviation, in whole or in part, of symptoms associated with a disease or disorder or condition, diminishment of the extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state (e.g., one or more symptoms of the disease), and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

The term "preventing," as used herein, means the prevention of the onset, recurrence or spread, in whole or in part, of the disease or condition as described herein, or a symptom thereof.

The term "administration" or "administering" refers to a method of giving a dosage of a compound or pharmaceutical formulation to a vertebrate or invertebrate, including a mammal, a bird, a fish, or an amphibian. The preferred method of administration can vary

depending on various factors, e.g., the components of the pharmaceutical formulation, the site of the disease, and the severity of the disease.

As used herein, "therapeutically effective amount" is an amount of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, or an amount of a pharmaceutical composition comprising the compound of Formula (I), which is sufficient to achieve the desired effect and can vary according to the nature and severity of the disease condition, and the potency of the compound. A therapeutic effect is the relief, to some extent, of one or more of the symptoms of the disease, and can include curing a disease. "Curing" means that the symptoms of active disease are eliminated. However, certain long-term or permanent effects of the disease can exist even after a cure is obtained (such as, e.g., extensive tissue damage).

The term "amorphous" means a solid in a solid state that is a non-crystalline state. Amorphous solids are disordered arrangements of molecules and therefore possess no distinguishable crystal lattice or unit cell and consequently have no definable long range ordering. The solid state form of a solid may be determined by polarized light microscopy, X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), or other standard techniques known to those of skill in the art.

As used herein, "time of day window" refers to a period of time defined by a window start time and a window stop time. These times all refer to local times where a sample was taken. The phrase "same time of day window" when referring to samples taken from the subject mean, e.g., that a sample taken at 8:15 a.m. and a sample taken at 9:15 a.m. are considered to be taken in the same time of day window of, e.g., 2 a.m. to 10 a.m. or 6 a.m. to 10 a.m.

Methods

The present disclosure relates to methods of treating congenital adrenal hyperplasia (CAH). The methods include administering to a subject a therapeutically effective amount of a compound of Formula (I), or pharmaceutically acceptable salt thereof. In some embodiments, the method includes administering to a subject a therapeutically effective amount of a SDD of the present disclosure that includes a polymer and a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the method includes administering to a subject a therapeutically effective amount of pharmaceutical composition of the present disclosure that contains a SDD that includes a polymer and a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Provided herein is a method of treating congenital adrenal hyperplasia (CAH) comprising administering a compound of Formula (I), or a pharmaceutically acceptable salt thereof to normalize or partially normalize levels of biomarkers associated with congenital adrenal hyperplasia. In some embodiments, normalizing or partially normalizing levels of biomarkers comprises reducing levels of elevated biomarkers or increasing levels of depressed biomarkers as compared to subject without CAH.

Provided herein is a method of treating congenital adrenal hyperplasia in a subject in need thereof comprising administering a compound of Formula (I), or a pharmaceutically acceptable salt thereof, in an amount sufficient to reduce the level of one or more biomarkers associated with congenital adrenal hyperplasia. In some embodiments, the biomarkers are selected from (a) 17-hydroxyprogesterone (17-OHP); (b) adrenocorticotropic hormone (ACTH); and (c) androstenedione in the subject.

In some embodiments, the reduction in level of any of the biomarkers (e.g., any of 17-OHP, ACTH, and androstenedione) is determined by comparing the level of the biomarker as measured during the circadian release on a day prior to administering the compound of Formula (I), or a pharmaceutically acceptable salt thereof and the level of the biomarker as measured during the circadian release on the day after administering the compound of Formula (I), or a pharmaceutically acceptable salt thereof. A day prior to administering the compound of Formula (I) applies to a subject that has not previously been administered the compound of Formula (I) within at least the past 24 hours.

In some embodiments, the circadian release of biomarkers associated with CAH occurs between the hours of 2 a.m. and 10 a.m. In other embodiments, the circadian release of biomarkers associated with CAH occurs between the hours of 6 a.m. and 10 a.m.

In some embodiments of any of the methods disclosed herein, the compound of Formula (I), or a pharmaceutically acceptable salt, is administered to the subject at nighttime or administration prior to sleep (i.e., bedtime administration). In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered three to eight hours prior to the circadian release of the biomarker. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered six to eight hours prior to the circadian release of the biomarker. Administration prior to the circadian release may be adapted for shift workers (*e.g.*, those who work at night and sleep during the day), in which case administration will not necessarily occur at nighttime. Administration is

therefore dependent upon the expected circadian release of the biomarker, and can vary depending upon the individual's (*i.e.*, subject, patient) particular work and sleep patterns.

In some embodiments of the methods provided herein, the level of 17-hydroxyprogesterone is reduced by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at lease 35%, at least 40%, at least 50%, at least 55% or at least 60% from pre-administration levels. In some embodiments, the level of 17-hydroxyprogesterone is reduced by at least 25%. In some embodiments, the level of 17-hydroxyprogesterone is reduced by at least 50%. In some embodiments of the methods provided herein, the level of 17-hydroxyprogesterone is reduced by an amount of from about 10% to about 90%, about 15% to about 90%, about 20% to about 90%, about 25% to about 90%, about 30% to about 90%, about 35% to about 90%, about 40% to about 90%, about 50% to about 90%, about 55% to about 90%, or about 60% to about 90% from pre-administration levels.

In some embodiments, the level of 17-hydroxyprogesterone is reduced to a level within the range of 17-hydroxyprogesterone expected for a subject without CAH, i.e., less than 1,000 ng/dL or less than 200 ng/dL.

In some embodiments of the methods provided herein, the level of adrenocorticotropic hormone is reduced by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at lease 35%, at least 40%, at least 50%, at least 55% or at least 60% from pre-administration levels. In some embodiments, the level of adrenocorticotropic hormone is reduced by at least 25%. In some embodiments, the level of adrenocorticotropic hormone is reduced by at least 40%. In some embodiments, the level of adrenocorticotropic hormone is reduced by at least 50%.

In some embodiments of the methods provided herein, the level of adrenocorticotropic hormone is reduced by an amount of from about 10% to about 90%, about 15% to about 90%, about 20% to about 90%, about 25% to about 90%, about 30% to about 90%, about 35% to about 90%, about 40% to about 90%, about 50% to about 90%, about 55% to about 90%, or about 60% to about 90% from pre-administration levels.

In some embodiments, the level of adrenocorticotropic hormone is reduced to a level within the range of adrenocorticotropic hormone expected for a subject without CAH.

In some embodiments of the methods provided herein, the level of androstenedione is reduced by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at lease 35%, at least 40%, at least 50%, at least 55% or at least 60% from pre-administration levels. In some embodiments, the level of androstenedione is reduced by at least 25%. In some embodiments,

the level of androstenedione is reduced by at least 30%. In some embodiments, the level of androstenedione is reduced by at least 50%.

In some embodiments of the methods provided herein, the level of androstenedione is reduced by an amount of from about 10% to about 90%, about 15% to about 90%, about 20% to about 90%, about 25% to about 90%, about 30% to about 90%, about 35% to about 90%, about 40% to about 90%, about 50% to about 90%, about 55% to about 90%, or about 60% to about 90% from pre-administration levels.

In some embodiments, the level of androstenedione is reduced to a level within the range of androstenedione expected for a subject without CAH, i.e., less than 200 ng/dL.

Also provided herein is a method for reducing the severity of one or more symptoms selected from hirsutism, precocious puberty, fertility problems, acne, and growth impairment in a subject having classic congenital adrenal hyperplasia, comprising administering a compound of Formula (I), or a pharmaceutically acceptable salt thereof, in an amount sufficient to reduce one or more biomarker of CAH in a subject, e.g. reduce the androstenedione in the subject. Growth impairment can refer to, e.g., accelerated height velocity, accelerated weight velocity, and/or accelerated bone age.

Provided herein is a method for reducing the level of one or more biomarkers of congenital adrenal hyperplasia in a subject having congenital adrenal hyperplasia comprising administering to the subject a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the one or more biomarkers of congenital adrenal hyperplasia are selected from (a) 17-hydroxyprogesterone (17-OHP); (b) adrenocorticotropic hormone (ACTH); and (c) androstenedione.

Provided herein is a method for reducing the dosage of corticosteroid administered to a subject having congenital adrenal hyperplasia for controlling congenital adrenal hyperplasia comprising administering to the subject a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the corticosteroid is a glucocorticoid.

Also provided herein is a method of reducing the severity of one or more side effects of glucocorticoid treatment in a subject having congenital adrenal hyperplasia comprising administering to the subject a compound of Formula (I), or a pharmaceutically acceptable salt thereof. The long-term effects of glucocorticoid treatment are well documented in the art (see, e.g., Oray, M. et al. (2016): Long-term effect of glucocorticoids, *Expert Opinion on Drug Safety*. DOI: 10.1517/14740338.2016.1140743). Such side effects are associated with every biological system, e.g., musculoskeletal (e.g., osteoporosis, avascular necrosis of bone, and

myopathy), endocrine and metabolic (e.g., hyperglycemia, diabetes mellitus, dyslipidemia, weight gain, Cushing syndrome, Cushingoid features, growth suppression, adrenal suppression), gastrointestinal (e.g., gastritis, peptic ulcer, gastrointestinal bleeding, visceral perforation, hepatic steatosis, pancreatitis), cardiovascular (e.g., hypertension, coronary heart disease, ischemic heart disease, heart failure), dermatologic (e.g., dermatoprosis, skin atrophy, ecchymosis, purpura, erosions, striae, delayed wound healing, easy bruising, acne, hirsutism, and hair loss), neuropsychiatric (e.g., mood changes, depression, euphoria, mood lability, irritability, akathisia, anxiety, cognitive impairment, psychosis, dementia, and delirium), ophthalmologic (e.g., cataract, glaucoma, ptosis, mydriasis, opportunistic ocular infections, and central serous chorioretinopathy), and immunologic (e.g., suppression of cell-mediated immunity, predisposition to infections, and reactivation of latent infections).

Accordingly, in some embodiments, the side effects of glucocorticoid treatment are selected from osteoporosis, avascular necrosis of bone, myopathy, hyperglycemia, diabetes mellitus, dyslipidemia, weight gain, Cushing syndrome, Cushingoid features, growth suppression, adrenal suppression, gastritis, peptic ulcer, gastrointestinal bleeding, visceral perforation, hepatic steatosis, pancreatitis, hypertension, coronary heart disease, ischemic heart disease, heart failure, dermatoprosis, skin atrophy, ecchymosis, purpura, erosions, striae, delayed wound healing, easy bruising, acne, hirsutism, hair loss, mood changes, depression, euphoria, mood lability, irritability, akathisia, anxiety, cognitive impairment, psychosis, dementia, delirium, cataract, glaucoma, ptosis, mydriasis, opportunistic ocular infections, central serous chorioretinopathy, suppression of cell-mediated immunity, predisposition to infections, reactivation of latent infections, and any combination thereof.

Provided herein is a method of treating congenital adrenal hyperplasia in a subject comprising

(i)measuring the level of one or more biomarkers selected from (a) 17-hydroxyprogesterone (17-OHP); (b) adrenocorticotropic hormone (ACTH); and (c) androstenedione in a biological sample obtained from the subject;

(ii)analyzing the level of the one or more biomarkers to determine if the level of the one or more biomarkers is elevated compared to a healthy subject not having congenital adrenal hyperplasia; and

(iii)administering to the subject a compound of Formula (I), or a pharmaceutically acceptable salt thereof if the subject is determined to have elevated levels of the one or more biomarkers.

In some embodiments, the method further comprises (iv) measuring the level of the one or more biomarkers after administering a compound of Formula (I), or a pharmaceutically acceptable salt thereof, in a biological sample obtained from the subject to determine whether the subject has reduced levels of the one or more biomarkers as compared with the measurement of step (i). In some embodiments, the method further comprises (v) continuing the administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof if the subject has reduced levels of the one or more biomarkers.

In some embodiments, steps (i) and (iv) are performed on biological samples taken from the subject in a similar manner and within a same time of day window. In some embodiments, steps (i) and (iv) are performed on biological samples taken from the subject within the time of day window from 2 a.m. to 10 a.m. In some embodiments, steps (i) and (iv) are performed on biological samples taken from the subject within the time of day window from 6 a.m. to 10 a.m.

In some embodiments, steps (i) and (iv) comprise measuring the levels of at least two biomarkers selected from (a) 17-hydroxyprogesterone (17-OHP); (b) adrenocorticotropic hormone (ACTH); and (c) androstenedione.

In some embodiments, steps (i) and (iv) comprise measuring the levels of (a) 17-hydroxyprogesterone (17-OHP); (b) adrenocorticotropic hormone (ACTH); and (c) androstenedione.

In some embodiments, step (i) comprises measuring the level of 17-hydroxyprogesterone (17-OHP), wherein the level of 17-hydroxyprogesterone (17-OHP) is elevated when it is greater than or equal to 1,000 ng/dL.

In some embodiments, step (i) comprises measuring the level of androstenedione, wherein the level of androstenedione is elevated when it is greater than 200 ng/dL.

In some embodiments of the methods of the present disclosure, the compound of Formula (I) is administered at an amount equivalent to from about 25 mg to about 150 mg of the compound of Formula (I) free base. In some embodiments, compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered at an amount equivalent to about 50 mg or about 100 mg of the compound of Formula (I) free base.

In some embodiments of the methods disclosed herein, compound of Formula (I) is administered in the free base form.

In some embodiments of the methods disclosed herein, the compound of Formula (I) is administered once daily.

Also provided herein is a method of treating CAH in a pediatric subject. The methods include administering to a pediatric subject a therapeutically effective amount of a compound of Formula (I), or pharmaceutically acceptable salt thereof. In some embodiments, the method includes administering to a pediatric subject a therapeutically effective amount of a SDD of the present disclosure that includes a polymer and a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the method includes administering to a pediatric subject a therapeutically effective amount of pharmaceutical composition of the present disclosure that contains a SDD that includes a polymer and a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the pediatric subject is a neonate. In some embodiments, the pediatric subject is an infant. In some embodiments, the pediatric subject is a child. In some embodiments, the pediatric subject is an adolescent.

In some embodiments of the methods of the present disclosure, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject in a fed state. The term "fed state," as used herein, refers to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof from about 1 hour before consumption of food or a nutritional composition to about 1 hour after consumption of food or a nutritional composition. The term "fasted state," as used herein, refers to a gap of at least two hours between consumption of food or a nutritional composition and administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof is administered to the subject with food or a nutritional composition, such as a nutritional supplement or formula, a meal replacement beverage, a liquid dietary supplement, or a high caloric liquid meal. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof is administered to the subject within about 1 hour before the subject has consumed food or a nutritional composition. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof is administered to the subject within about 1 hour after the subject has consumed food or a nutritional composition. Examples of suitable nutritional compositions include, but are not limited to, infant formulas, dietary supplements, dietary substitutes, and rehydration compositions. In some embodiments, the food is a product containing concentrated calories and protein. In some embodiments, the nutritional composition is a composition utilized for enteral and parenteral supplementation for infants, specialty infant formulas, supplements for the elderly, and supplements for those with

gastrointestinal difficulties and/or malabsorption. Adult and pediatric nutritional formulas are well known in the art and are commercially available (e.g., Similac®, Ensure®, Jevity® and Alimentum® from Ross Products Division, Abbott Laboratories, Columbus, Ohio).

In some embodiments, the nutritional composition is in liquid form. The energy density of the nutritional compositions, when in liquid form, can range from about 0.6 Kcal to about 3 Kcal per mL. In some embodiments, the nutritional composition is in solid or powdered form. When in solid or powdered form, the nutritional supplements can contain from about 1.2 to more than 9 Kcals per gram, such as about 3 to 7 Kcals per gram.

In some embodiments, the nutritional composition is a meal replacement bar. Examples include PowerBar®, Glucerna® bars, Choice DM® bars, Ensure® bars, and Boost® bars. In some embodiments, the nutritional composition is a nutrition shake or meal replacement beverage. Commercially available examples include the Ensure® branded adult products (such as Ensure® Original, Ensure® Plus, Ensure® Enlive, Ensure® High Protein, Ensure® Clear, and Ensure® Light), Glucerna®, Choice DM®, Slim Fast®, Pediasure®, Glytrol®, and Resource®. In some embodiments, the nutritional composition is Ensure® Plus. In some embodiments, the nutritional composition is vanilla-flavored Ensure® Plus. Ensure Plus® is a high calorie liquid dietary supplement that contains 1500 calories per liter with a caloric distribution of 14.7% protein, 32% fat and 53.3% carbohydrate.

In some embodiments of the disclosed methods, the compound of Formula (I), or a pharmaceutically acceptable salt thereof is administered to the subject with 8 fluid ounces (237 mL) of Ensure® Plus. In some embodiments, the Ensure® Plus is vanilla-flavored.

In some embodiments of the methods, the compound of Formula (I), or a pharmaceutically acceptable salt thereof is administered to the subject after administration of the nutritional composition. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof is administered to the subject before administration of the nutritional composition. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject at the same time as administration of the nutritional composition.

In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof is administered to the subject, followed by administration of the nutritional composition. In some embodiments, the nutritional composition is administered about 1 minute, about 5 minutes, about 10 minutes, about 15 minutes, about 20 minutes, about 25 minutes, about 30 minutes, about 35 minutes, about 40 minutes, about 45 minutes, or about 60

minutes, or within a range defined by any of the preceding values after administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the nutritional composition is administered 1 minute, 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, or 60 minutes, or within a range defined by any of the preceding values after administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the nutritional composition is administered within 30 minutes of administering the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments of the disclosed methods, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject with 8 fluid ounces (237 mL) of Ensure® Plus. In some embodiments, the Ensure® Plus is vanilla-flavored.

In some embodiments, the nutritional composition is administered to the subject, followed by administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, compound of Formula (I), or a pharmaceutically acceptable salt thereof is administered about 1 minute, about 5 minutes, about 10 minutes, about 15 minutes, about 20 minutes, about 25 minutes, about 30 minutes, about 35 minutes, about 40 minutes, about 45 minutes, or about 60 minutes, or within a range defined by any of the preceding values after administration of the nutritional composition. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered 1 minute, 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, or 60 minutes, or within a range defined by any of the preceding values after administration of the nutritional composition. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered within 30 minutes of administering the nutritional composition. In some embodiments of the disclosed methods, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered to the subject with 8 fluid ounces (237 mL) of Ensure® Plus. In some embodiments, the Ensure® Plus is vanilla-flavored.

In some embodiments of the methods, a food effect is observed between administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof in a fed state versus a fasted state. The term "food effect," as used herein, refers to the relative difference in AUC (area under the curve $AUC_{(0-t)}$ and/or $AUC_{(0-\infty)}$) or C_{max} (maximum plasma concentration or peak plasma concentration) of an active substance, when the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered orally to a subject, concomitantly with

food or in a fed state as compared to the same values when the same compound of Formula (I), or a pharmaceutically acceptable salt thereof is administered in a fasted state. The food effect (F) is calculated as:

$$F\% = [(X_{fasted} - X_{fed}) / X_{fasted}] \times 100$$

where X_{fed} and X_{fasted} are the values of AUC ($AUC_{(0-t)}$ and/or $AUC_{(0-\infty)}$) or C_{max} in the fed and fasted state, respectively. In some embodiments, an increased, or positive, food effect is observed when the compound of Formula (I), or a pharmaceutically acceptable salt thereof is administered to a subject in a fed state. In some embodiments, administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, results in an increased, or positive, food effect, whereby an increased C_{max} and/or AUC are observed when administered orally in the fed state as compared to the fasting state.

In some embodiments of the methods, the ratio of the AUC in the fed state to the AUC in the fasted state is about 5 to about 10, such as about 5 to about 9, about 5 to about 8, about 5 to about 7, about 5 to about 6, about 6 to about 10, about 6 to about 9, about 6 to about 8, about 6 to about 7, about 7 to about 10, about 7 to about 9, about 7 to about 8, about 8 to about 10, about 8 to about 9, or about 8 to about 10. In some embodiments, the ratio of the AUC in the fed state to the AUC in the fasted state is about 5, about 6, about 7, about 8, about 9, or about 10, or within a range defined by any of the preceding values. In some embodiments of the methods, the ratio of the AUC in the fed state to the AUC in the fasted state is about 10 to about 20.

In some embodiments of the methods, the ratio of the AUC in the fed state to the AUC in the fasted state is 5 to 10, such as 5 to 9, 5 to 8, 5 to 7, 5 to 6, 6 to 10, 6 to 9, 6 to 8, 6 to 7, 7 to 10, 7 to 9, 7 to 8, 8 to 10, 8 to 9, or 8 to 10. In some embodiments, the ratio of the AUC in the fed state to the AUC in the fasted state is 5, 6, 7, 8, 9, or 10, or within a range defined by any of the preceding values.

In some embodiments of the methods, the ratio of the C_{max} in the fed state to the C_{max} in the fasted state is about 5 to about 10, such as about 5 to about 9, about 5 to about 8, about 5 to about 7, about 5 to about 6, about 6 to about 10, about 6 to about 9, about 6 to about 8, about 6 to about 7, about 7 to about 10, about 7 to about 9, about 7 to about 8, about 8 to about 10, about 8 to about 9, or about 8 to about 10. In some embodiments, the ratio of the C_{max} in the fed state to the C_{max} in the fasted state is about 5, about 6, about 7, about 8, about 9, or about 10, or within a range defined by any of the preceding values. In some embodiments, the mean C_{max} of the compound of Formula (I), or pharmaceutically acceptable salt thereof, is about 1.5

to about 3 times higher in the fed stated compared to the fasted state. In some embodiments of the methods, the ratio of the C_{max} in the fed state to the C_{max} in the fasted state is 5 to 10, such as 5 to 9, 5 to 8, 5 to 7, 5 to 6, 6 to 10, 6 to 9, 6 to 8, 6 to 7, 7 to 10, 7 to 9, 7 to 8, 8 to 10, 8 to 9, or 8 to 10. In some embodiments, the ratio of the C_{max} in the fed state to the C_{max} in the fasted state is 5, 6, 7, 8, 9, or 10, or within a range defined by any of the preceding values. In some embodiments, the mean C_{max} of the compound of Formula (I), or pharmaceutically acceptable salt thereof, is 1.5 to 3 times higher in the fed stated compared to the fasted state. In some embodiments, the mean C_{max} of the compound of Formula (I), or pharmaceutically acceptable salt thereof, is about 2 times higher in the fed stated compared to the fasted state. In some embodiments of the methods, the ratio of the C_{max} in the fed state to the C_{max} in the fasted state is about 10 to about 20.

In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, is administered to the subject with a meal. In some embodiments, the meal is a high fat, high caloric meal. In some embodiments, the meal is a low fat, low caloric meal. In some embodiments, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, is administered within approximately 5 minutes after the start of the meal. In some embodiments, the meal is an evening meal. In some embodiments, the meal is a morning meal.

In some embodiments, the fed state is with a high fat meal. In some embodiments, the fed state is with a low fat meal. The FDA has provided draft guidelines regarding high fat and low fat meals ("Assessing the Effects of Food on Drugs in INDs and NDAs – Clinical Pharmacology Considerations Guidance for Industry," U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), February 2019, Clinical Pharmacology). Table 1 shows test meal definitions provided by the FDA guidance.

Table 1

Meal Type	Total Kcal	Fat		
		Kcal	Grams	Percent
High-Fat	800-1000	500-600	55-65	50
Low-Fat	400-500	100-125	11-14	25

The composition of a high fat meal provided by the FDA guidance is depicted in Table 2.

Table 2. Composition of a High Fat Meal*

Total Calories	800-1000
Calories from Protein	150
Calories from Carbohydrates	250
Calories from Fat	500-600
An Example of a High Fat Breakfast	• Two eggs fried in butter • Two strips of bacon • Two slices of toast with butter • Four ounces of hash brown potatoes • Eight ounces of whole milk

*50 percent of calories are derived from fat. Substitutions can be made to this meal, if the content, volume, and viscosity are maintained.

The composition of a low fat meal provided by the FDA guidance is depicted in Table 3.

Table 3. Composition of a Low Fat Meal

Total Calories	400-500
Fat (g)	250
Percent Calories from Fat	25
An Example of a Low Fat Breakfast*	• Eight ounces milk (1 percent fat) • One boiled egg • One packet flavored instant oatmeal made with water

*This low-fat breakfast contains 387 calories and has 10 grams of fat.

In some embodiments, a high fat meal contains 800-1000 total Kcal and 500-600 fat Kcal. In some embodiments, a low fat meal contains 400-500 total Kcal and 100-125 fat Kcal.

Also provided herein is a method of improving gastrointestinal absorption of a compound of Formula (I), or pharmaceutically acceptable salt thereof, in a subject. The method includes orally administering to the subject a pharmaceutical composition of the present disclosure, wherein the improvement is relative to oral administration of the compound of Formula (I), or pharmaceutically acceptable salt thereof, which has not been prepared as a spray-dried dispersion. In some embodiments, the subject is a pediatric subject.

Also provided herein is a method of improving oral bioavailability of a compound of Formula (I), or pharmaceutically acceptable salt thereof, in a subject. The method includes orally administering to the subject a pharmaceutical composition of the present disclosure, wherein the improvement is relative to oral administration of the compound of Formula (I), or pharmaceutically acceptable salt thereof, which has not been prepared as a spray-dried dispersion.

In some embodiments of the methods provided herein, the subject is a pediatric subject.

Also provided herein is a method of treating congenital adrenal hyperplasia (CAH), in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the present disclosure, wherein the pharmaceutical composition comprises a therapeutically effective amount of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the pharmaceutical composition is a lipidic semi-solid formulation. In some embodiments, the pharmaceutical composition is a liquid formulation. In some embodiments, the pharmaceutical composition is administered to the subject in a fed state.

Also provided herein is a pharmaceutical composition of the present disclosure for use in a method of treating congenital adrenal hyperplasia (CAH) in a subject. In some embodiments, the subject is in a fed state.

In some embodiments, the pharmaceutical composition is administered to the subject with a nutritional composition. In some embodiments, the nutritional composition is a liquid dietary supplement comprising about 1000 to about 2000 calories per liter with a fat content greater than about 30%. In some embodiments, the nutritional composition is a liquid dietary supplement comprising 1500 calories per liter with a caloric distribution of 14.7% protein, 32% fat and 53.3% carbohydrate. In some embodiments, the nutritional composition is administered in an amount of about 6 to about 12 fluid ounces. In some embodiments, the nutritional composition is administered in an amount of about 8 fluid ounces. In some embodiments, the nutritional composition is administered within 30 minutes of administration of the pharmaceutical composition.

In some embodiments, the pharmaceutical composition exhibits a positive food effect. In some embodiments, the positive food effect is measured in terms of C_{max}, AUC, or a combination thereof of a compound of Formula (I) when comparing oral administration of the pharmaceutical composition in the fed and fasting states. In some embodiments, the ratio of the AUC of the compound of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is about 5 to about 10. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is about 5 to about 10. In some embodiments, the ratio of the AUC of the compound of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is about 10 to about 20. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is about 10 to about 20. In some embodiments, the ratio of the AUC of the compound of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is about 1 to about 4 or about 5 to about 10. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is about 1 to about 4 or about 5 to about 10. In some embodiments, the ratio of the AUC of the compound of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is about 1 to about 4. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is about 1 to about 4. In some embodiments, the ratio of the AUC of the compound of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is about 1.5 to about 3. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is about 1.5 to about 3. In some embodiments, the ratio of the AUC of the compound of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is 1 to 4 or 5 to 10. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is 1 to 4 or 5 to 10. In some embodiments, the ratio of the AUC of the compound of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is 1 to 4. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is 1 to 4. In some embodiments, the ratio of the AUC of the compound of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is

1.5 to 3. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is 1.5 to 3.

In some embodiments, the subject is a pediatric subject.

In some embodiments, the pharmaceutical composition is formulated for oral administration and exhibits a positive food effect when administered orally. In some embodiments, the compound of Formula (I) has a ratio of the AUC in the fed state to the AUC in the fasted state of about 5 to about 10. In some embodiments, the compound of Formula (I) has a ratio of the C_{max} in the fed state to the C_{max} in the fasted state of about 5 to about 10. In some embodiments, the compound of Formula (I) has a ratio of the AUC in the fed state to the AUC in the fasted state of about 10 to about 20. In some embodiments, the compound of Formula (I) has a ratio of the C_{max} in the fed state to the C_{max} in the fasted state of about 10 to about 20. In some embodiments, the compound of Formula (I) has a ratio of the AUC in the fed state to the AUC in the fasted state of about 1 to about 4 or about 5 to about 10. In some embodiments, the compound of Formula (I) has a ratio of the C_{max} in the fed state to the C_{max} in the fasted state of about 1 to about 4 or about 5 to about 10. In some embodiments, the compound of Formula (I) has a ratio of the AUC in the fed state to the AUC in the fasted state of about 1 to about 4. In some embodiments, the compound of Formula (I) has a ratio of the C_{max} in the fed state to the C_{max} in the fasted state of about 1 to about 4. In some embodiments, the compound of Formula (I) has a ratio of the AUC in the fed state to the AUC in the fasted state of about 1.5 to about 3. In some embodiments, the compound of Formula (I) has a ratio of the C_{max} in the fed state to the C_{max} in the fasted state of about 1.5 to about 3. In some embodiments, the compound of Formula (I) has a ratio of the AUC in the fed state to the AUC in the fasted state of 1 to 4 or 5 to 10. In some embodiments, the compound of Formula (I) has a ratio of the C_{max} in the fed state to the C_{max} in the fasted state of 1 to 4 or 5 to 10. In some embodiments, the compound of Formula (I) has a ratio of the AUC in the fed state to the AUC in the fasted state of 1 to 4. In some embodiments, the compound of Formula (I) has a ratio of the C_{max} in the fed state to the C_{max} in the fasted state of 1 to 4. In some embodiments, the compound of Formula (I) has a ratio of the AUC in the fed state to the AUC in the fasted state of 1.5 to 3. In some embodiments, the compound of Formula (I) has a ratio of the C_{max} in the fed state to the C_{max} in the fasted state of 1.5 to 3.

In some embodiments, the pharmaceutical composition is administered to the subject with a meal. In some embodiments, the meal is a high fat meal. In some embodiments, the meal is a low fat meal. In some embodiments, the pharmaceutical composition is administered

within about 5 minutes after the start of the meal. In some embodiments, the meal is an evening meal. In some embodiments, the meal is a morning meal.

In some embodiments, administering the pharmaceutical composition exhibits a positive food effect. In some embodiments, the positive food effect is measured in terms of C_{max}, AUC, or combinations thereof of the compound of Formula (I) when comparing oral administration of the pharmaceutical composition in the fed and fasting states. In some embodiments, the ratio of the AUC of the compound of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is about 5 to about 10. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is about 5 to about 10. In some embodiments, the ratio of the AUC of the compound of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is about 10 to about 20. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is about 10 to about 20. In some embodiments, the ratio of the AUC of the compound of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is about 1 to about 4 or about 5 to about 10. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is about 1 to about 4 or about 5 to about 10. In some embodiments, the ratio of the AUC of the compound of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is about 1 to about 4. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is about 1 to about 4. In some embodiments, the ratio of the AUC of the compound of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is about 1.5 to about 3. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is about 1.5 to about 3. In some embodiments, the ratio of the AUC of the compound of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is 1 to 4 or 5 to 10. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is 1 to 4 or 5 to 10. In some embodiments, the ratio of the AUC of the compound of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is 1 to 4. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is 1 to 4. In some embodiments, the ratio of the AUC of the compound

of Formula (I) in the fed state to the AUC of the compound of Formula (I) in the fasted state is 1.5 to 3. In some embodiments, the ratio of the C_{max} of the compound of Formula (I) in the fed state to the C_{max} of the compound of Formula (I) in the fasted state is 1.5 to 3.

For the avoidance of doubt, also provided herein is the corresponding compound of Formula (I), or a pharmaceutically acceptable salt thereof, or corresponding pharmaceutical composition comprising a compound of Formula (I), or a pharmaceutically acceptable salt thereof, for use in the corresponding methods, as described herein.

For the avoidance of doubt, also provided herein is use of the corresponding compound of Formula (I), or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in the corresponding methods, as described herein.

For the avoidance of doubt, also provided herein is use of the corresponding pharmaceutical composition comprising a compound of Formula (I), or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in the corresponding methods, as described herein.

Reduction in glucocorticoid burden, adrenal androgens and precursors

Glucocorticoids are a class of corticosteroids, which are a class of steroid hormones. Glucocorticoids are corticosteroids that bind to the glucocorticoid receptor that is present in almost every vertebrate animal cell. In some embodiments, the subject is concurrently receiving a dose of a glucocorticoid. In some embodiments, the glucocorticoid is selected from cortisol (hydrocortisone), cortisone, prednisone, prednisolone, methylprednisolone, dexamethasone, betamethasone, triamcinolone, fludrocortisone acetate, and deoxycorticosterone acetate. In some embodiments, the glucocorticoid is cortisol (hydrocortisone). In some embodiments, the glucocorticoid is cortisone. In some embodiments, the glucocorticoid is prednisone. In some embodiments, the glucocorticoid is dexamethasone.

In some embodiments, the glucocorticoid dose is measured in hydrocortisone equivalents. In some embodiments, the glucocorticoid dose is measured as a multiple of the upper limit of normal of physiologic dosing in hydrocortisone equivalents. Any glucocorticoid can be given in a dose that provides approximately the same glucocorticoid effects as normal cortisol production; this is referred to as physiologic, replacement, or maintenance dosing.

In some embodiments, the glucocorticoid dose is a physiologic dose as measured after a time period of administration of the compound of Formula (I), or a pharmaceutically

acceptable salt thereof. In some embodiments, the glucocorticoid dose is a physiologic dose of about 4 to about 12 mg/m^2/day as measured after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose is a physiologic dose of about 4 to about 9 mg/m^2/day as measured after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose is a physiologic dose that is less than about 8 mg/m^2/day as measured after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the glucocorticoid dose is a physiologic dose as measured after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose is a physiologic dose of about 4 to about 12 mg/m^2/day as measured after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose is a physiologic dose of about 4 to about 9 mg/m^2/day as measured after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose is a physiologic dose that is less than about 8 mg/m^2/day as measured after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the glucocorticoid dose concurrently given to the subject is a normal physiological dose of hydrocortisone equivalents. In some embodiments, the glucocorticoid dose concurrently given to the subject is determined after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose concurrently given to the subject is determined after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, a normal physiological dose of hydrocortisone equivalents is about 2 to about 16 mg/m^2/day. In some embodiments, a normal physiological dose of hydrocortisone equivalents is about 4 to about 12 mg/m^2/day. In some embodiments, a normal physiological dose of hydrocortisone equivalents is about 5 to about 11 mg/m^2/day. In some embodiments, a normal physiological dose of hydrocortisone equivalents is about 6 to about 10 mg/m^2/day. In some embodiments, a normal physiological dose of hydrocortisone equivalents is about 7 to about 9 mg/m^2/day. In

some embodiments, a normal physiological dose of hydrocortisone equivalents is about 4 to about 9 mg/m^2/day. In some embodiments, a normal physiological dose of hydrocortisone equivalents is about 8 mg/m^2/day. In some embodiments, a normal physiological dose of hydrocortisone equivalents is about 12 mg/m^2/day. In some embodiments, a normal physiological dose of hydrocortisone equivalents is less than about 8 mg/m^2/day. In some embodiments, a normal physiological dose of hydrocortisone equivalents is about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15 or about 16 mg/m^2/day, or within a range defined by any of the preceding values.

In some embodiments, the glucocorticoid dose concurrently given to the subject is at the upper limit of normal of a normal physiological dose of hydrocortisone equivalents. In some embodiments, the glucocorticoid dose concurrently given to the subject is determined after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose concurrently given to the subject is determined after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the upper limit of normal is 1.5 times the normal physiological dose. In some embodiments, the upper limit of normal is about 1.5 times the normal physiological dose. In some embodiments, the upper limit of normal is about 1.5 times the normal physiological dose. In some embodiments, the upper limit of normal is about 2 times the normal physiological dose. In some embodiments, the upper limit of normal is about 2.5 times the normal physiological dose. In some embodiments, the upper limit of normal is about 1.0, about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, about 2.0, about 2.1, about 2.2, about 2.3, about 2.4, about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, or about 3.0 times the normal physiological dose, or within a range defined by any of the preceding values.

In some embodiments, the glucocorticoid dose of the subject is reduced by about 10% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by about 20% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the

glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by about 30% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by about 40% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by about 50% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by about 60% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by about 70% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by less than about 20% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by about 20% to about 50% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by greater than about 50% after a time period

of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the glucocorticoid dose of the subject is reduced by about 10% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by about 20% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by about 30% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by about 40% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by about 50% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by about 60% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof,

wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by about 70% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by less than about 20% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by about 20% to about 50% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the glucocorticoid dose of the subject is reduced by greater than about 50% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the glucocorticoid dose is relative to the glucocorticoid dose prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the glucocorticoid dose of the subject is reduced within a range defined by any of the preceding values.

In some embodiments, the level of 17-hydroxyprogesterone is reduced by at least 25% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of 17-hydroxyprogesterone is relative to the level of 17-hydroxyprogesterone prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of 17-hydroxyprogesterone is reduced by at least 50% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction

of the level of 17-hydroxyprogesterone is relative to the level of 17-hydroxyprogesterone prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of 17-hydroxyprogesterone is less than 1.5 times the upper limit of normal after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of 17-hydroxyprogesterone is within normal limits after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the level of 17-hydroxyprogesterone is reduced by at least about 25% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of 17-hydroxyprogesterone is relative to the level of 17-hydroxyprogesterone prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of 17-hydroxyprogesterone is reduced by at least about 50% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of 17-hydroxyprogesterone is relative to the level of 17-hydroxyprogesterone prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of 17-hydroxyprogesterone is less than about 1.5 times the upper limit of normal after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of 17-hydroxyprogesterone is within normal limits after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the level of 17-hydroxyprogesterone of the subject is reduced within a range defined by any of the preceding values.

In some embodiments, the level of adrenocorticotropic hormone is reduced by at least 25% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of adrenocorticotropic hormone is relative to the level of adrenocorticotropic hormone prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of adrenocorticotropic hormone is reduced by at least 40%

after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of adrenocorticotropic hormone is relative to the level of adrenocorticotropic hormone prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of adrenocorticotropic hormone is reduced by at least 50% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of adrenocorticotropic hormone is relative to the level of adrenocorticotropic hormone prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of adrenocorticotropic hormone is less than 1.5 times the upper limit of normal after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of adrenocorticotropic hormone is within normal limits after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the level of adrenocorticotropic hormone is reduced by at least about 25% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of adrenocorticotropic hormone is relative to the level of adrenocorticotropic hormone prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of adrenocorticotropic hormone is reduced by at least about 40% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of adrenocorticotropic hormone is relative to the level of adrenocorticotropic hormone prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of adrenocorticotropic hormone is reduced by at least about 50% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of adrenocorticotropic hormone is relative to the level of adrenocorticotropic hormone prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of adrenocorticotropic hormone is less than about 1.5 times the upper limit of normal after a time

period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of adrenocorticotropic hormone is within normal limits after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the level of adrenocorticotropic hormone of the subject is reduced within a range defined by any of the preceding values.

In some embodiments, the level of androstenedione is reduced by at least 25% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of androstenedione is relative to the level of androstenedione prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of androstenedione is reduced by at least 30% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of androstenedione is relative to the level of androstenedione prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of androstenedione is reduced by at least 50% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of androstenedione is relative to the level of androstenedione prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of androstenedione is less than 1.5 times the upper limit of normal after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of androstenedione is within normal limits after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the level of androstenedione is reduced by at least about 25% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of androstenedione is relative to the level of androstenedione prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of androstenedione is reduced by at least about 30% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt

thereof, wherein the reduction of the level of androstenedione is relative to the level of androstenedione prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of androstenedione is reduced by at least about 50% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of androstenedione is relative to the level of androstenedione prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of androstenedione is less than about 1.5 times the upper limit of normal after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of androstenedione is within normal limits after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the level of androstenedione of the subject is reduced within a range defined by any of the preceding values.

In some embodiments, the level of testosterone is reduced by at least 25% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of testosterone is relative to the level of testosterone prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of testosterone is reduced by at least 30% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of testosterone is relative to the level of testosterone prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of testosterone is reduced by at least 50% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of testosterone is relative to the level of testosterone prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of testosterone is less than 1.5 times the upper limit of normal after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of testosterone is within normal limits after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the level of testosterone is reduced by at least about 25% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of testosterone is relative to the level of testosterone prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of testosterone is reduced by at least about 30% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of testosterone is relative to the level of testosterone prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of testosterone is reduced by at least about 50% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of testosterone is relative to the level of testosterone prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of testosterone is less than about 1.5 times the upper limit of normal after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of testosterone is within normal limits after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the level of testosterone of the subject is reduced within a range defined by any of the preceding values.

In some embodiments, the level of 17-hydroxyprogesterone is reduced by at least 50% and the level of androstenedione is reduced by at least 50% after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of 17-hydroxyprogesterone and the level of androstenedione is relative to the level of 17-hydroxyprogesterone and the level of androstenedione prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of 17-hydroxyprogesterone is less than 1.5 times the upper limit of normal and the level of androstenedione is less than 1.5 times the upper limit of normal after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of 17-hydroxyprogesterone is within normal

limits and the level of androstenedione is within normal limits after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the level of 17-hydroxyprogesterone is reduced by at least about 50% and the level of androstenedione is reduced by at least about 50% after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of the level of 17-hydroxyprogesterone and the level of androstenedione is relative to the level of 17-hydroxyprogesterone and the level of androstenedione prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of 17-hydroxyprogesterone is less than about 1.5 times the upper limit of normal and the level of androstenedione is less than about 1.5 times the upper limit of normal after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the level of 17-hydroxyprogesterone is within normal limits and the level of androstenedione is within normal limits after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the level of 17-hydroxyprogesterone and androstenedione of the subject is reduced within a range defined by any of the preceding values.

In some embodiments, the subject exhibits a decrease in glucocorticoid burden after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the decrease in glucocorticoid burden is relative to the glucocorticoid burden prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, one or more symptoms selected from quality of life, fatigue, sleep, insulin resistance, glucose tolerance, glucose control, dyslipidemia, hyperlipidemia, bone mineral density, bone turnover, fat mass, weight, central obesity, blood pressure, hirsutism severity, menstrual cyclicity, control of testicular adrenal rest tumor and fertility, is improved after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the improvement in the one or more symptoms is relative to the status of the one or more symptoms prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the subject exhibits a decrease in glucocorticoid burden after a time period of administration of the pharmaceutical composition comprising the compound of

Formula (I), or a pharmaceutically acceptable salt thereof, wherein the decrease in glucocorticoid burden is relative to the glucocorticoid burden prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, one or more symptoms of glucocorticoid burden selected from quality of life, fatigue, sleep, insulin resistance, glucose tolerance, glucose control, dyslipidemia, hyperlipidemia, bone mineral density, bone turnover, fat mass, weight, central obesity, blood pressure, hirsutism severity, menstrual cyclicity, control of testicular adrenal rest tumor and fertility, is improved after a time period of administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the improvement in the one or more symptoms is relative to the status of the one or more symptoms prior to administration of the pharmaceutical composition comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the quality of life as measured by the EuroQol 5 Dimensions 5 Levels (EQ-5D-5L) in the subject is improved after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the improvement in the EuroQol 5 Dimensions 5 Levels (EQ-5D-5L) is relative to the EuroQol 5 Dimensions 5 Levels (EQ-5D-5L) results prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, fatigue is reduced in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction in fatigue is relative to the fatigue prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, sleep is increased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the increase in sleep is relative to the sleep prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, insulin resistance is reduced in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction of insulin resistance is relative to the insulin resistance prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, glucose tolerance is reduced in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof,

wherein the reduction in glucose tolerance is relative to the glucose tolerance prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, glucose control is increased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the increase in glucose control is relative to the glucose control prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, lipid levels reflecting dyslipidemia are reduced in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction in lipid levels is relative to the lipid levels prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, lipid levels reflecting hyperlipidemia are reduced in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the reduction in lipid levels is relative to the lipid levels prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, bone mineral density is increased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the increase in bone mineral density is relative to the bone mineral density prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, bone turnover is increased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the increase in bone turnover is relative to the bone turnover prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, fat mass is decreased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the decrease in fat mass is relative to the fat mass prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, body weight is decreased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the decrease in body weight is relative to the body weight prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, central obesity is decreased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof,

wherein the decrease in central obesity is relative to the central obesity prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, blood pressure is increased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the increase in blood pressure is relative to the blood pressure prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the severity of hirsutism is decreased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the decrease in the severity of hirsutism is relative to the severity of hirsutism prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, menstrual cyclicity is increased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the increase in menstrual cyclicity is relative to the menstrual cyclicity prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, control of testicular adrenal rest tumor is increased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the increase in control of testicular adrenal rest tumor is relative to the control of testicular adrenal rest tumor prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, fertility is increased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the increase in fertility is relative to the fertility prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, gonadotropin levels are increased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the increase in gonadotropin levels is relative to the gonadotropin levels prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, progesterone levels are increased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the increase in progesterone levels is relative to the progesterone levels prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, semen levels are increased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the increase in semen levels is relative to the semen levels prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, LH (luteinizing hormone) levels are increased in the subject after a time period of administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein the increase in LH levels are relative to the LH levels prior to administration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the time period of administration is at least about 4 weeks. In some embodiments, the time period of administration is at least about 24 weeks. In some embodiments, the time period of administration is at least about one year. In some embodiments, the time period of administration is at least 4 weeks. In some embodiments, the time period of administration is at least 24 weeks. In some embodiments, the time period of administration is at least one year. In some embodiments, the time period of administration is less than about 1 day. In some embodiments, the time period of administration is about 1, 2, 3, 4, 5, 6 or 7 days, or within a range of any of the preceding values. In some embodiments, the time period of administration is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 weeks, or within a range of any of the preceding values. In some embodiments, the time period of administration is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or within a range of any of the preceding values. It is understood that comparative measurements occur preferably during the morning.

In some embodiments, the subject is a pediatric subject. In some embodiments, the pediatric subject is less than or equal to six years old. In some embodiments, the pediatric subject is greater than six years old and less than eleven years old. In some embodiments, the pediatric subject is greater than ten years old and less than fifteen years old. In some embodiments, the pediatric subject is greater than fourteen years old and less than nineteen years old.

In some embodiments, the subject is an adult subject. In some embodiments, the subject is over eighteen years old. In some embodiments, the subject is female. In some embodiments, the subject is male.

In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered as a pharmaceutical composition described herein. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is

administered as a pharmaceutical composition described in Example 9. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered as a pharmaceutical composition described in Example 11. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered as a pharmaceutical composition described in Example 12. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered as a pharmaceutical composition described in Example 13. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered as a hydrochloric acid salt or p-toluenesulfonic acid salt.

In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered as a p-toluenesulfonic acid salt described herein.

For the avoidance of doubt, also provided herein is the corresponding compound of Formula (I), or a pharmaceutically acceptable salt thereof, or corresponding pharmaceutical composition comprising a compound of Formula (I), or a pharmaceutically acceptable salt thereof, for use in the corresponding methods, as described herein.

For the avoidance of doubt, also provided herein is use of the corresponding compound of Formula (I), or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in the corresponding methods, as described herein.

For the avoidance of doubt, also provided herein is use of the corresponding pharmaceutical composition comprising a compound of Formula (I), or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in the corresponding methods, as described herein.

p-Toluenesulfonic Acid Salt

In some embodiments, the compound of Formula (I) or a pharmaceutically acceptable salt thereof is 4-(2-chloro-4-methoxy -5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, p-toluenesulfonic acid salt.

In some embodiments, the 4-(2-chloro-4-methoxy -5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, p-toluenesulfonic acid salt is a crystalline salt. In some embodiments, the p-toluenesulfonic acid crystalline salt has Form 1.

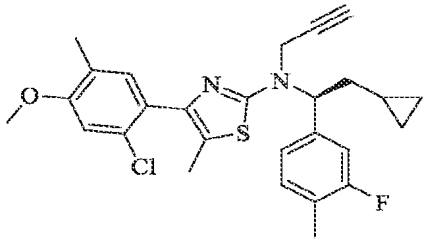
In some embodiments, the p-toluenesulfonic acid crystalline salt has an X-ray powder diffraction pattern as substantially shown in Figure 27. In some embodiments, the p-toluenesulfonic acid crystalline salt has a DSC thermogram substantially as depicted in Figure 28. In some embodiments, the p-toluenesulfonic acid crystalline salt has a thermogravimetric analysis (TGA) thermogram substantially as depicted in Figure 28.

In some embodiments, the p-toluenesulfonic acid crystalline salt has at least one X-ray powder diffraction (XRPD) peak, in terms of 2-theta (± 0.2 degrees), selected 9.1, 11.3, 13.2, 16.3 and 21.1 degrees. In some embodiments, the p-toluenesulfonic acid crystalline salt has at least two X-ray powder diffraction (XRPD) peaks, in terms of 2-theta (± 0.2 degrees), selected from 9.1, 11.3, 13.2, 16.3 and 21.1 degrees. In some embodiments, the p-toluenesulfonic acid crystalline salt has at least three X-ray powder diffraction (XRPD) peaks, in terms of 2-theta (± 0.2 degrees), selected from 9.1, 11.3, 13.2, 16.3 and 21.1 degrees. In some embodiments, the p-toluenesulfonic acid crystalline salt has at least four X-ray powder diffraction (XRPD) peaks, in terms of 2-theta (± 0.2 degrees), selected from 9.1, 11.3, 13.2, 16.3 and 21.1 degrees. In some embodiments, the p-toluenesulfonic acid crystalline salt has characteristic X-ray powder diffraction (XRPD) peaks, in terms of 2-theta (± 0.2 degrees), at 9.1, 11.3, 13.2, 16.3 and 21.1 degrees. In some embodiments, the p-toluenesulfonic acid crystalline salt has an endothermic peak having an onset of melt at about 156 °C (22.2 J/g) in a differential scanning calorimetry (DSC) thermogram.

Lipidic Semi-Solid Formulation

Provided herein is a lipidic semi-solid formulation, which is a pharmaceutical composition comprising:

(a) a compound of Formula (I):



(I)

or a pharmaceutically acceptable salt thereof; and

(b) one or more of an oily phase vehicle, an emulsifying agent, a nonionic surfactant, and a solubilizing agent.

In some embodiments, the pharmaceutical composition comprises about 1 wt% to about 20 wt% of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the pharmaceutical composition comprises about 5 wt% to about 15 wt% of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the pharmaceutical composition comprises about 10 wt% of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the pharmaceutical composition comprises about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 wt% of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base, or within a range of any of the preceding values.

In some embodiments, the pharmaceutical composition comprises an oily phase vehicle. An oily phase vehicle is a solvent that is poorly miscible with water. In some embodiments, the pharmaceutical composition comprises about 1 wt% to about 50 wt% of the oily phase vehicle. In some embodiments, the pharmaceutical composition comprises about 20 wt% to about 50 wt% of the oily phase vehicle. In some embodiments, the pharmaceutical composition comprises about 35 wt% to about 45 wt% of the oily phase vehicle. In some embodiments, the pharmaceutical composition comprises about 39 wt% of the oily phase vehicle. In some embodiments, the pharmaceutical composition comprises about 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, or 45 wt% of the oily phase vehicle, or within a range of any of the preceding values.

In some embodiments, the oily phase vehicle is selected from medium-chain triglycerides, glycerin, propylene glycol, polyethylene glycol, olive oil, soybean oil, corn oil, and transcutol. In some embodiments, the oily phase vehicle is medium-chain triglycerides. In some embodiments, the medium-chain triglycerides are Labrafac TM Lipophile WL1349. In some embodiments, the medium-chain triglycerides are Miglyol 812N.

In some embodiments, the pharmaceutical composition comprises an emulsifying agent. An emulsifying agent is a compound or substance that acts as a stabilizer for emulsions. In some embodiments, the pharmaceutical composition comprises about 5 wt% to about 50 wt% of the emulsifying agent. In some embodiments, the pharmaceutical composition comprises about 10 wt% to about 30 wt% of the emulsifying agent. In some embodiments, the pharmaceutical composition comprises about 15 wt% to about 25 wt% of the emulsifying agent. In some embodiments, the pharmaceutical composition comprises

about 20 wt% of the emulsifying agent. In some embodiments, the pharmaceutical composition comprises about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 wt% of the emulsifying agent, or within a range of any of the preceding values.

In some embodiments, the emulsifying agent is selected from medium-chain triglycerides, propylene glycol dicaprylate/dicaprate, glycerin, propylene glycol, polyethylene glycol, olive oil, soybean oil, corn oil, and transcutol. In some embodiments, the emulsifying agent is propylene glycol dicaprylate/dicaprate. In some embodiments, the propylene glycol dicaprylate/dicaprate is Labrafac TM PG.

In some embodiments, the pharmaceutical composition comprises a nonionic surfactant. A nonionic surfactant is a substance with a hydrophilic head and a hydrophobic tail that has no charge that is a formulation component added to improve solubility or emulsion properties. In some embodiments, the pharmaceutical composition comprises about 5 wt% to about 50 wt% of the nonionic surfactant. In some embodiments, the pharmaceutical composition comprises about 10 wt% to about 30 wt% of the nonionic surfactant. In some embodiments, the pharmaceutical composition comprises about 15 wt% to about 25 wt% of the nonionic surfactant. In some embodiments, the pharmaceutical composition comprises about 19 wt% of the nonionic surfactant. In some embodiments, the pharmaceutical composition comprises about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 wt% of the nonionic surfactant, or within a range of any of the preceding values.

In some embodiments, the nonionic surfactant is selected from oleoyl polyoxyl-6 glycerides, linoleoyl polyoxyl-6 glycerides, Polysorbate 80, Polysorbate 20, Gelucire, lauroyl polyoxyl-32 glycerides, Poloxamer, PEG-32 stearate, and PEG-32 hydrogenated palm glycerides. In some embodiments, the nonionic surfactant is lauroyl polyoxyl-32 glycerides. In some embodiments, the lauroyl polyoxyl-32 glycerides are Gelucire® 44/14.

In some embodiments, the pharmaceutical composition comprises a solubilizing agent. A solubilizing agent is a solvent that assists with solubilizing the compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the pharmaceutical composition comprises about 1 wt% to about 50 wt% of the solubilizing agent. In some embodiments, the pharmaceutical composition comprises about 1 wt% to about 20 wt% of the solubilizing agent. In some embodiments, the pharmaceutical composition comprises about 5 wt% to about 15 wt% of the solubilizing agent. In some

embodiments, the pharmaceutical composition comprises about 11 wt% of the solubilizing agent. In some embodiments, the pharmaceutical composition comprises about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 wt% of the solubilizing agent, or within a range of any of the preceding values.

In some embodiments, the solubilizing agent is selected from oleoyl polyoxyl-6 glycerides, linolcoyl polyoxyl-6 glycerides, Polysorbate 80, Polysorbate 20, vitamin E polyethylene glycol succinate, Gelucire, lauroyl polyoxyl-32 glycerides, and Poloxamer. In some embodiments, the solubilizing agent is vitamin E polyethylene glycol succinate. In some embodiments, the vitamin E polyethylene glycol succinate is Kolliphor® TPGS. In some embodiments, the vitamin E polyethylene glycol succinate is Vitamin E/TPGS 260.

In some embodiments, the pharmaceutical composition comprises:

(a) 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof;

(b) an oily phase vehicle;

(c) an emulsifying agent;

(d) a nonionic surfactant; and

(e) a solubilizing agent.

In some embodiments, the pharmaceutical composition comprises:

(a) about 5 wt% to about 15 wt% of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, based on the weight of the free base;

(b) about 35 wt% to about 45 wt% of an oily phase vehicle;

(c) about 15 wt% to about 25 wt% of an emulsifying agent;

(d) about 15 wt% to about 25 wt% of a nonionic surfactant; and

(e) about 5 wt% to about 15 wt% of a solubilizing agent.

In some embodiments, the pharmaceutical composition comprises:

(a) about 10 wt% of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, based on the weight of the free base;

(b) about 39 wt% of an oily phase vehicle;

(c) about 20 wt% of an emulsifying agent;

(d) about 19 wt% of a nonionic surfactant; and

(e) about 11 wt% of a solubilizing agent.

In some embodiments, the pharmaceutical composition comprises:

(a) 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine;

(b) a medium-chain triglycerides component;

(c) a propylene glycol dicaprylate/dicaprate component;

(d) a lauroyl polyoxyl-32 glycerides component; and

(e) a vitamin E polyethylene glycol succinate component.

In some embodiments, the pharmaceutical composition comprises:

(a) about 5 wt% to about 15 wt% of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine;

(b) about 35 wt% to about 45 wt% of medium-chain triglycerides;

(c) about 15 wt% to about 25 wt% of propylene glycol dicaprylate/dicaprate;

(d) about 15 wt% to about 25 wt% of lauroyl polyoxyl-32 glycerides; and

(e) about 5 wt% to about 15 wt% of vitamin E polyethylene glycol succinate.

In some embodiments, the pharmaceutical composition comprises:

(a) about 10 wt% of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine;

(b) about 39 wt% of medium-chain triglycerides;

(c) about 20 wt% of propylene glycol dicaprylate/dicaprate;

(d) about 19 wt% of lauroyl polyoxyl-32 glycerides; and

(e) about 11 wt% of vitamin E polyethylene glycol succinate.

In some embodiments, the lipidic semi-solid pharmaceutical composition has a viscosity between about 15 to about 40 centipoise at about 45 °C. In some embodiments, the lipidic semi-solid pharmaceutical composition has a viscosity between about 26 to about 30 centipoise at about 45 °C. In some embodiments, the lipidic semi-solid pharmaceutical

composition has a viscosity between about 5 to about 25 centipoise at about 60 °C. In some embodiments, the lipidic semi-solid pharmaceutical composition has a viscosity between about 14 to about 18 centipoise at about 60 °C.

In some embodiments, the pharmaceutical composition does not comprise a combination of mannitol, croscarmellose sodium, maize starch, hydroxypropyl methylcellulose, and magnesium stearate.

In some embodiments, the pharmaceutical composition does not comprise at least one of mannitol, croscarmellose sodium, maize starch, hydroxypropyl methylcellulose, and magnesium stearate.

In some embodiments, the pharmaceutical composition comprises a compound of Formula (I), or pharmaceutically acceptable salt thereof, in crystalline form. In some embodiments, the pharmaceutical composition comprises a compound of Formula (I), or pharmaceutically acceptable salt thereof, in amorphous form. In some embodiments, the pharmaceutical composition comprises a compound of Formula (I) as a free base. In some embodiments, the crystalline form of the compound of Formula (I) is of Form I.

In some embodiments, the pharmaceutical composition is formulated in unit dosage form, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is present in an amount of about 5 mg to about 200 mg, based on the weight of the free base. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is present in the unit dosage form in an amount of about 75 mg to about 150 mg, based on the weight of the free base. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is present in the unit dosage form in an amount of about 50 mg, based on the weight of the free base. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is present in the unit dosage form in an amount of about 100 mg, based on the weight of the free base. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is present in the unit dosage form in an amount of about 25 mg, based on the weight of the free base.

In some embodiments, the pharmaceutical composition is in the form of a tablet, capsule, sachet, powder, granules, coated particle, coated tablet, enterocoated tablet, enterocoated capsule, melting strip, or melting film. In some embodiments, the pharmaceutical composition is in tablet form. In some embodiments, the pharmaceutical composition is in capsule form. In some embodiments, the dosage form is coated.

Some embodiments provide a method for preparing the pharmaceutical composition, comprising:

(a) heating a mixture of an oily phase vehicle, an emulsifying agent, a nonionic surfactant, and a solubilizing agent;

(b) mixing the mixture of step (a) until a homogeneous mixture is achieved; and

(c) mixing the compound of Formula (I), or a pharmaceutically acceptable salt thereof, with the homogeneous mixture of step (b) until the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is dissolved, forming a composition.

In some embodiments, the method further comprises:

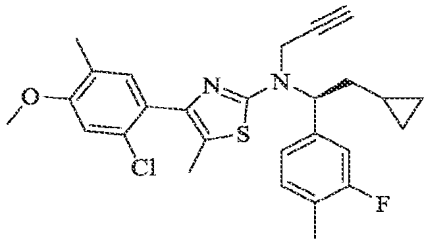
(d) encapsulating the composition of step (c) in a capsule shell to form a capsule; and

(e) banding the capsule of step (d) in a mixture of banding agent and banding solvent.

Liquid Formulations

Provided herein is a pharmaceutical composition in oral solution dosage form comprising:

(a) a compound of Formula (I):



(I)

or a pharmaceutically acceptable salt thereof;

(b) one or more of a sweetener, an anti-oxidant, and a flavor; and

(c) a liquid vehicle.

In some embodiments, the pharmaceutical composition comprises about 1 w/v% to about 50 w/v% of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the pharmaceutical composition comprises about 1 w/v% to about 10 w/v% of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the pharmaceutical composition comprises about 5 w/v% of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free

base. In some embodiments, the pharmaceutical composition comprises about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 w/v% of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base, or within a range of any of the preceding values.

In some embodiments, the pharmaceutical composition comprises a sweetener. A sweetener is a formulation component added to improve taste. In some embodiments, the pharmaceutical composition comprises about 0.01 w/v% to about 1.5 w/v% of the sweetener. In some embodiments, the pharmaceutical composition comprises about 0.1 w/v% to about 0.5 w/v% of the sweetener. In some embodiments, the pharmaceutical composition comprises about 0.15 w/v% of the sweetener. In some embodiments, the pharmaceutical composition comprises about 0.1, 0.2, 0.3, 0.4, or 0.5 w/v% of the sweetener, or within a range of any of the preceding values.

In some embodiments, the sweetener is selected from saccharin, sucrose, sucralose, aspartame, dextrose, fructose, maltitol, mannitol, sorbitol, and avantame. In some embodiments, the sweetener is saccharin.

In some embodiments, the pharmaceutical composition comprises an anti-oxidant. An anti-oxidant is a formulation component included to improve stability by preventing oxidation. In some embodiments, the pharmaceutical composition comprises about 0.01 w/v% to about 1.5 w/v% of the anti-oxidant. In some embodiments, the pharmaceutical composition comprises about 0.1 w/v% to about 0.5 w/v% of the anti-oxidant. In some embodiments, the pharmaceutical composition comprises about 0.17 w/v% of the anti-oxidant. In some embodiments, the pharmaceutical composition comprises about 0.1, 0.2, 0.3, 0.4, or 0.5 w/v% of the anti-oxidant, or within a range of any of the preceding values.

In some embodiments, the anti-oxidant is selected from butylated hydroxytoluene, vitamin E TPGS, butylated hydroxyanisole, ascorbic acid, lecithin, tert-butylhydroquinone, and citric acid. In some embodiments, the anti-oxidant is butylated hydroxytoluene.

In some embodiments, the pharmaceutical composition comprises a flavor. A flavor is a formulation component added to mask taste through aromatics. In some embodiments, the pharmaceutical composition comprises about 0.01 w/v% to about 0.5 w/v% of the flavor. In some embodiments, the pharmaceutical composition comprises about 0.05 w/v% to about 0.2 w/v% of the flavor. In some embodiments, the pharmaceutical composition comprises about 0.10 w/v% of the flavor. In some embodiments, the pharmaceutical composition comprises about 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19 or 0.2 w/v% of the flavor, or within a range of any of the preceding values.

In some embodiments, the flavor is selected from FONA orange flavor, FONA Juicy Flavor, FONA Grape Flavor, Firmenich SA Lemon Flavor, Firmenich Tetrarome Orange Flavor, IFF Cherry Flavor, and IFF Grape Flavor. In some embodiments, the flavor is FONA orange flavor.

A liquid vehicle is a solvent capable of dissolving or partially dissolving the compound of Formula (I), or a pharmaceutically acceptable salt thereof, for the purposes of delivery as an oral dosing solution. In some embodiments, the pharmaceutical composition comprises about 50 w/v% to about 99.9 w/v% of the liquid vehicle. In some embodiments, the pharmaceutical composition comprises about 90 w/v% to about 99 w/v% of the liquid vehicle. In some embodiments, the pharmaceutical composition comprises about 92 w/v% to about 97 w/v% of the liquid vehicle. In some embodiments, the pharmaceutical composition comprises about 94.6 w/v% of the liquid vehicle. In some embodiments, the pharmaceutical composition comprises about 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 w/v% of the liquid vehicle, or within a range of any of the preceding values.

In some embodiments, the liquid vehicle is selected from medium-chain triglycerides, propylene glycol dicaprylate/dicaprate, glycerin, propylene glycol, polyethylene glycol, olive oil, soybean oil, corn oil, and transcutol. In some embodiments, the liquid vehicle is medium-chain triglycerides. In some embodiments, the medium-chain triglycerides is Labrafac Lipophile WL1349.

In some embodiments, the pharmaceutical composition further comprises a surfactant. A surfactant is a formulation component added to improve solubility or emulsion properties. In some embodiments, the pharmaceutical composition comprises about 1 w/v% to about 50 w/v% of the surfactant. In some embodiments, the pharmaceutical composition comprises about 10 w/v% to about 30 w/v% of the surfactant. In some embodiments, the pharmaceutical composition comprises about 20 w/v% of the surfactant. In some embodiments, the pharmaceutical composition comprises about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 w/v% of the surfactant, or within a range of any of the preceding values.

In some embodiments, the surfactant is selected from oleoyl polyoxyl-6 glycerides, linoleoyl polyoxyl-6 glycerides, Polysorbate 80, Polysorbate 20, vitamin E polyethylene glycol succinate, Gelucire, lauroyl polyoxyl-32 glycerides, sodium lauryl sulfate, Poloxamer, corn oil PEG-6 esters, and hydrogenated palm/palm kernel oil PEG-6 esters. In some

embodiments, the surfactant is oleoyl polyoxyl-6 glycerides. In some embodiments, the oleoyl polyoxyl-6 glycerides is LABRAFIL M 1944 CS.

In some embodiments, the pharmaceutical composition comprises about 50 w/v% to about 90 w/v% of the liquid vehicle. In some embodiments, the pharmaceutical composition comprises about 70 w/v% to about 80 w/v% of the liquid vehicle. In some embodiments, the pharmaceutical composition comprises about 75 w/v% of the liquid vehicle. In some embodiments, the pharmaceutical composition comprises about 74.6 w/v% of the liquid vehicle. In some embodiments, the pharmaceutical composition comprises about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 w/v% of the liquid vehicle, or within a range of any of the preceding values.

In some embodiments, the liquid vehicle is selected from medium-chain triglycerides, propylene glycol dicaprylate/dicaprate, glycerin, propylene glycol, polyethylene glycol, olive oil, soybean oil, corn oil, and transcutol. In some embodiments, the liquid vehicle is medium-chain triglycerides. In some embodiments, the medium-chain triglycerides is Labrafac Lipophile WL1349.

In some embodiments, the pharmaceutical composition comprises:

(a) 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof;

(b) a sweetener;

(c) an anti-oxidant;

(d) a flavor; and

(e) a liquid vehicle.

In some embodiments, the pharmaceutical composition further comprises a surfactant.

In some embodiments, the pharmaceutical composition comprises:

(a) about 4 w/v% to about 6 w/v% of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, based on the weight of the free base;

(b) about 0.1 w/v% to about 0.2 w/v% of a sweetener;

(c) about 0.1 w/v% to about 0.2 w/v% of an anti-oxidant;

(d) about 0.05 w/v% to about 0.2 w/v% of a flavor; and

(e) about 92 w/v% to about 97 w/v% of a liquid vehicle.

In some embodiments, the pharmaceutical composition comprises:

(a) about 5 w/v% of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, based on the weight of the free base;

(b) about 0.15 w/v% of a sweetener;

(c) about 0.17 w/v% of an anti-oxidant;

(d) about 0.1 w/v% of a flavor; and

(e) about 94.6 w/v% of a liquid vehicle.

In some embodiments, the pharmaceutical composition comprises:

(a) about 4 w/v% to about 6 w/v% of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, based on the weight of the free base;

(b) about 0.1 w/v% to about 0.2 w/v% of a sweetener;

(c) about 0.1 w/v% to about 0.2 w/v% of an anti-oxidant;

(d) about 0.05 w/v% to about 0.2 w/v% of a flavor;

(e) about 15 w/v% to about 25 w/v% of a surfactant; and

(f) about 70 w/v% to about 80 w/v% of a liquid vehicle.

In some embodiments, the pharmaceutical composition comprises:

(a) about 5 w/v% of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, based on the weight of the free base;

(b) about 0.15 w/v% of a sweetener;

(c) about 0.17 w/v% of an anti-oxidant;

(d) about 0.1 w/v% of a flavor;

(e) about 20 w/v% of a surfactant; and

(f) about 75 w/v% of a liquid vehicle.

In some embodiments, the pharmaceutical composition comprises:

(a) 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof;

(b) saccharin;

(c) butylated hydroxytoluene;

(d) FONA orange flavor; and

(e) medium-chain triglycerides.

In some embodiments, the pharmaceutical composition further comprises oleoyl polyoxyl-6 glycerides.

In some embodiments, the pharmaceutical composition comprises:

(a) about 4 w/v% to about 6 w/v% of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, based on the weight of the free base;

(b) about 0.1 w/v% to about 0.2 w/v% of saccharin;

(c) about 0.1 w/v% to about 0.2 w/v% of butylated hydroxytoluene;

(d) about 0.05 w/v% to about 0.2 w/v% of FONA orange flavor; and

(e) about 92 w/v% to about 97 w/v% of medium-chain triglycerides.

In some embodiments, the pharmaceutical composition comprises:

(a) about 5 w/v% of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, based on the weight of the free base;

(b) about 0.15 w/v% of saccharin;

(c) about 0.17 w/v% of butylated hydroxytoluene;

(d) about 0.1 w/v% of FONA orange flavor; and

(e) about 94.6 w/v% of medium-chain triglycerides.

In some embodiments, the pharmaceutical composition comprises:

(a) about 4 w/v% to about 6 w/v% of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, based on the weight of the free base;

(b) about 0.1 w/v% to about 0.2 w/v% of saccharin;

(c) about 0.1 w/v% to about 0.2 w/v% of butylated hydroxytoluene;

(d) about 0.05 w/v% to about 0.2 w/v% of FONA orange flavor;

(e) about 15 w/v% to about 25 w/v% of oleoyl polyoxyl-6 glycerides; and

(f) about 70 w/v% to about 80 w/v% of medium-chain triglycerides.

In some embodiments, the pharmaceutical composition comprises:

(a) about 5 w/v% of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, based on the weight of the free base;

(b) about 0.15 w/v% of saccharin;

(c) about 0.17 w/v% of butylated hydroxytoluene;

(d) about 0.1 w/v% of FONA orange flavor;

(e) about 20 w/v% of oleoyl polyoxyl-6 glycerides; and

(f) about 75 w/v% of medium-chain triglycerides.

In some embodiments, the pharmaceutical composition comprises the compound of Formula (I) as a free base.

In some embodiments, the pharmaceutical composition is formulated in unit dosage form, wherein the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is present in an amount of about 5 mg/mL to about 200 mg/mL, based on the weight of the free base. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is present in the unit dosage form in an amount of about 75 mg/mL to about 150 mg/mL, based on the weight of the free base. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is present in the unit dosage form in an amount of about 50 mg/mL, based on the weight of the free base. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is present in the unit dosage form in an amount of about 100 mg/mL, based on the weight of the free base.

In some embodiments, the liquid pharmaceutical composition has a viscosity between about 1 to about 50 centipoise at about 25 °C.

Some embodiments provide a method for preparing a pharmaceutical composition comprising:

(a) mixing a liquid vehicle with a sweetener;

(b) mixing the mixture of step (a) with an anti-oxidant and a flavor;

(c) mixing the compound of Formula (I), or a pharmaceutically acceptable salt thereof, with the mixture of step (b); and

(d) mixing the mixture of step (c) with an additional portion of the liquid vehicle.

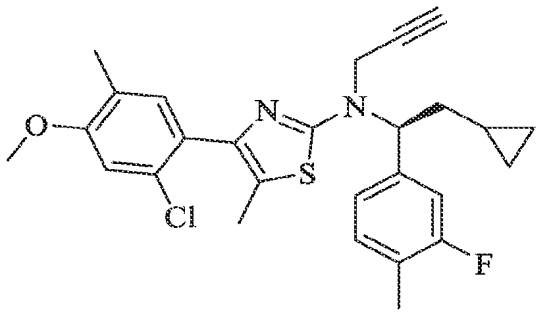
In some embodiments, step (a) of the method comprises mixing a liquid vehicle with a sweetener and a surfactant.

Spray-dried dispersions

The methods and uses of the present disclosure may comprise administering a spray-dried dispersion (SDDs) of 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, and to the use of the SDDs in the treatment of congenital adrenal hyperplasia (CAH).

In some embodiments, concentration and bioavailability enhancement in an aqueous environment of a low-solubility drug in a spray-dried dispersion is achieved if the SDD exhibits one or more properties, including, for example: (1) the solid dispersion is substantially homogeneous; (2) the drug is substantially amorphous; (3) the SDD has a relatively high drug loading; and (4) the SDD has a low residual solvent content. In some embodiments, the dispersion, when administered to an aqueous environment, provides at least a temporary dissolved drug concentration in the aqueous environment that is greater than the solubility of the crystalline form of the drug in the same environment. The aqueous environment can be, for example, an *in vitro* environment, such as a dissolution test media (e.g., phosphate buffered saline (PBS) solution), or an *in vivo* environment, such as the gastrointestinal (GI) tract of an animal, for example, a human. In some embodiments, the aqueous environment is the lower GI tract, such as the small intestine and large intestine.

Provided in the present disclosure is a spray-dried dispersion that contains a polymer and a compound having the structure of Formula (I):



(I)

or a pharmaceutically acceptable salt thereof. In some embodiments, the SDD includes a polymer selected from a neutral polymer, an enteric polymer, and a pyrrolidone polymer. In some embodiments, the weight ratio of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to the polymer is from about 1:1 to about 1:9.

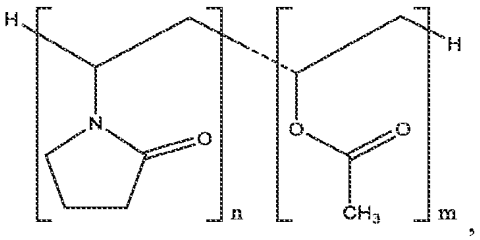
In some embodiments, the polymer is a neutral polymer. For example, the polymer does not contain any charged functional groups. In some embodiments, the neutral polymer is a cellulosic polymer. For example, the cellulosic polymer can be a polymer with at least one ester- and/or ether-linked substituent in which the polymer has a degree of substitution of at least 0.05 for each substituent. Examples of suitable neutral polymers include, but are not limited to, hydroxypropyl methylcellulose (HPMC) and hydroxyethyl cellulose (HEC).

In some embodiments, the polymer is an enteric polymer. An "enteric polymer," as used herein, is a polymeric substance that is substantially insoluble and/or substantially stable under acidic conditions exhibiting a pH of less than about 7 and which is substantially soluble or can decompose under conditions exhibiting a pH of about 7 or more. Examples of suitable enteric polymers include, but are not limited to, carboxymethyl ethyl cellulose (CMEC), cellulose acetate phthalate (CAP), cellulose acetate succinate (CAS), methylcellulose phthalate, hydroxymethyl ethyl cellulose phthalate, hydroxypropyl methyl cellulose phthalate (HPMCP), hydroxypropyl methyl cellulose acetate succinate (HPMCAS), polyvinyl alcohol phthalate, polyvinyl butyrate phthalate, polyvinyl acetal phthalate, a copolymer of vinyl acetate/maleic anhydride, a copolymer of vinylbutylether/maleic anhydride, a copolymer of styrene/maleic acid monoester, a copolymer of methyl acrylate/methacrylic acid, a copolymer of styrene/acrylic acid, a copolymer of methyl acrylate/methacrylic acid/octyl acrylate, a copolymer of methacrylic acid/methyl methacrylate, an amino methacrylate copolymer, an ammonioalkyl methacrylate copolymer, a methacrylic copolymer, and mixtures thereof. In some embodiments, the enteric polymer is selected from hydroxypropyl methyl cellulose acetate succinate (HPMCAS), cellulose acetate phthalate (CAP), hydroxypropyl methyl

cellulose phthalate (HPMCP), an amino methacrylate copolymer, an ammonioalkyl methacrylate copolymer, and a methacrylic copolymer. In some embodiments, the enteric polymer is a Eudragit® polymer sold by Evonik Industries (Essen, Germany). In some embodiments, the enteric polymer is an amino methacrylate copolymer. In some embodiments, the amino methacrylate copolymer is Eudragit® E PO/100. In some embodiments, the enteric polymer is an ammonioalkyl methacrylate copolymer. In some embodiments, the ammonioalkyl methacrylate copolymer is Eudragit® RLPO. In some embodiments, the enteric polymer is a methacrylic copolymer. In some embodiments, the methacrylic copolymer is Eudragit® L100 or Eudragit® S100.

In some embodiments, the polymer is a pyrrolidone polymer. For example, the pyrrolidone polymer can be a vinyl pyrrolidone polymer, such as polyvinyl pyrrolidone (PVP) or polyvinyl pyrrolidone vinyl acetate (PVP/VA), including homopolymers and copolymers of PVP and homopolymers and copolymers of N-vinyl pyrrolidone. In some embodiments, the pyrrolidone polymer is PVP/VA. In some embodiments, the PVP/VA is a copolymer of 1-vinyl-2-pyrrolidone and vinyl acetate. In some embodiments, the copolymer contains 1-vinyl-2-pyrrolidone and vinyl acetate at a ratio of about 30:70 to about 70:30 by weight, such as about 40:60 to about 60:40 by weight, or about 45:55 to about 55:45 by weight. In some embodiments, the copolymer contains 1-vinyl-2-pyrrolidone and vinyl acetate at a ratio of about 30:70, about 35:65, about 40:60, about 45:55, about 50:50, about 55:45, about 60:40, about 65:35, or about 70:30 by weight. In some embodiments, the copolymer contains 1-vinyl-2-pyrrolidone and vinyl acetate at a ratio of about 60:40 by weight.

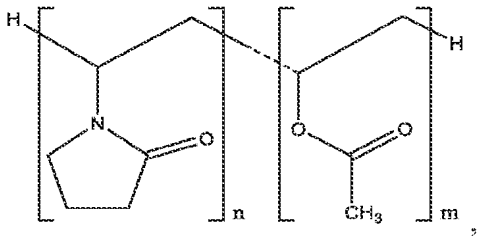
In some embodiments, the pyrrolidone polymer has the structure:



,

where the value of n is about 1 to about 2 times the value of m. For example, the value of n can be about 1, about 1.05, about 1.1, about 1.15, about 1.16, about 1.2, about 1.25, about 1.3, about 1.35, about 1.4, about 1.45, about 1.5, about 1.55, about 1.6, about 1.65, about 1.7, about 1.75, about 1.8, about 1.85, about 1.9, about 1.95, or about 2 times the value of m, or a value within a range defined by any of the preceding values. In some embodiments, the value of n is about 1.16 times the value of m. In some embodiments, the copolymer is copovidone, and the value of n is about 1.16 times the value of m.

In some embodiments, the pyrrolidone polymer has the structure:



where the value of n is 1 to 2 times the value of m. For example, the value of n can be 1, 1.05, 1.1, 1.15, 1.16, 1.2, 1.25, 1.3, 1.35, 1.4, 1.45, 1.5, 1.55, 1.6, 1.65, 1.7, 1.75, 1.8, 1.85, 1.9, 1.95, or 2 times the value of m, or a value within a range defined by any of the preceding values. In some embodiments, the value of n is 1.16 times the value of m. In some embodiments, the copolymer is copovidone, and the value of n is 1.16 times the value of m.

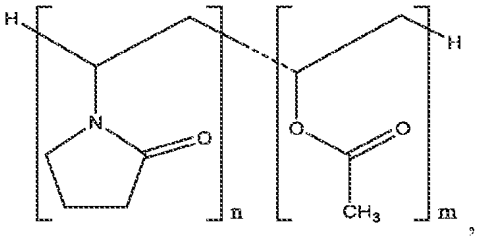
In some embodiments, the polymer used in the disclosed SDDs is present in an amount sufficient to increase the maximum drug concentration of the amorphous form of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, in the environment of use (e.g., aqueous environment) relative to a control composition that contains an equivalent amount of a crystalline form of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, but without polymer. In some embodiments, once the SDD is introduced into an environment of use (e.g., aqueous environment), the polymer increases the aqueous concentration of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, relative to the control composition. It is to be understood that the control composition is free from solubilizers or other components that would materially affect the solubility of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, and that the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is in solid form in the control composition.

In some embodiments of the SDDs, the weight ratio of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to the polymer is from about 1:1 to about 1:9. For example, the weight ratio of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to the polymer can be about 1:1 to about 1:8, about 1:1 to about 1:7, about 1:1 to about 1:6, about 1:1 to about 1:5, about 1:1 to about 1:4, about 1:1 to about 1:3, about 1:1 to about 1:2, about 1:1 to about 1:1.5, about 1:1.5 to about 1:9, or about 1:2.5 to about 1:4. In some embodiments, the weight ratio of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to the polymer is about 1:9, about 1:8, about 1:7.5, about 1:7, about 1:6, about 1:5, about 1:4, about 1:3, about 1:2.5, about 1:2, about 1:1.5, or about 1:1, or a weight ratio within a range defined by any of the preceding values. In some embodiments, the weight

ratio of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to the polymer is about 1:9. In some embodiments, the weight ratio of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to the polymer is about 1:1.5. In some embodiments, the weight ratio of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to the polymer is about 1:1.

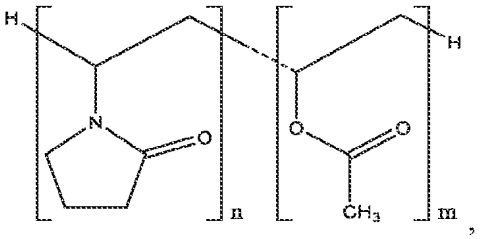
In some embodiments of the SDDs, the weight ratio of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to the polymer is from 1:1 to 1:9. For example, the weight ratio of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to the polymer can be 1:1 to 1:8, 1:1 to 1:7, 1:1 to 1:6, 1:1 to 1:5, 1:1 to 1:4, 1:1 to 1:3, 1:1 to 1:2, 1:1 to 1:1.5, 1:1.5 to 1:9, or 1:2.5 to 1:4. In some embodiments, the weight ratio of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to the polymer is 1:9, 1:8, 1:7.5, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2.5, 1:2, 1:1.5, or 1:1, or a weight ratio within a range defined by any of the preceding values. In some embodiments, the weight ratio of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to the polymer is 1:9. In some embodiments, the weight ratio of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to the polymer is 1:1.5. In some embodiments, the weight ratio of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to the polymer is 1:1.

In some embodiments, the spray dried dispersion includes a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and a polymer that is a copolymer of 1-vinyl-2-pyrrolidone and vinyl acetate having the structure:



where the value of n is about 1 to about 2 times the value of m and the copolymer contains 1-vinyl-2-pyrrolidone and vinyl acetate at a ratio of about 60:40 by weight, and the weight ratio of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to the copolymer is from about 1:1 to about 1:9.

In some embodiments, the spray dried dispersion includes a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and a polymer that is a copolymer of 1-vinyl-2-pyrrolidone and vinyl acetate having the structure:



where the value of n is 1 to 2 times the value of m and the copolymer contains 1-vinyl-2-pyrrolidone and vinyl acetate at a ratio of 60:40 by weight, and the weight ratio of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, to the copolymer is from 1:1 to 1:9.

In some embodiments of the SDDs, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, and the polymer together form homogeneous particles. In some embodiments, the particles are a substantially homogeneous composition that includes the compound of Formula (I), or a pharmaceutically acceptable salt thereof, and the polymer. As used herein, "substantially homogeneous" means that the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is dispersed as homogeneously as possible throughout the polymer and can be thought of as a solid solution of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, dispersed in the polymer. While the dispersion can have some concentrated domains of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, the dispersion itself has a single glass transition temperature (T_g) which demonstrates that the dispersion is substantially homogeneous. This contrasts with a simple physical mixture of pure amorphous particles of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, and pure amorphous polymer particles, which generally displays two distinct T_gs, one of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, and one of the polymer. "T_g," as used herein, is the characteristic temperature where a glassy material, upon gradual heating, undergoes a relatively rapid (e.g., 10 to 100 seconds) physical change from a glass state to a rubber state.

In some embodiments, the particles have a particle size distribution (D-value, D_{50}), of about 5 μm to about 100 μm, such as about 5 μm to about 95 μm, about 5 μm to about 90 μm, about 5 μm to about 85 μm, about 5 μm to about 80 μm, about 5 μm to about 75 μm, about 5 μm to about 70 μm, about 5 μm to about 65 μm, about 5 μm to about 60 μm, about 5 μm to about 55 μm, about 5 μm to about 50 μm, about 5 μm to about 45 μm, about 5 μm to about 40 μm, about 5 μm to about 35 μm, about 5 μm to about 30 μm, about 5 μm to about 25 μm, about 5 μm to about 20 μm, about 5 μm to about 15 μm, about 5 μm to about 10 μm, about 10 μm to about 100 μm, about 10 μm to about 95 μm, about 10 μm to about 90 μm, about 10 μm to

about 85 μm, about 10 μm to about 80 μm, about 10 μm to about 75 μm, about 10 μm to about 70 μm, about 10 μm to about 65 μm, about 10 μm to about 60 μm, about 10 μm to about 55 μm, about 10 μm to about 50 μm, about 10 μm to about 45 μm, about 10 μm to about 40 μm, about 10 μm to about 35 μm, about 10 μm to about 30 μm, about 10 μm to about 25 μm, about 10 μm to about 20 μm, about 10 μm to about 15 μm, about 15 μm to about 100 μm, about 15 μm to about 95 μm, about 15 μm to about 90 μm, about 15 μm to about 85 μm, about 15 μm to about 80 μm, about 15 μm to about 75 μm, about 15 μm to about 70 μm, about 15 μm to about 65 μm, about 15 μm to about 60 μm, about 15 μm to about 55 μm, about 15 μm to about 50 μm, about 15 μm to about 45 μm, about 15 μm to about 40 μm, about 15 μm to about 35 μm, about 15 μm to about 30 μm, about 15 μm to about 25 μm, about 15 μm to about 20 μm, about 20 μm to about 100 μm, about 20 μm to about 95 μm, about 20 μm to about 90 μm, about 20 μm to about 85 μm, about 20 μm to about 80 μm, about 20 μm to about 75 μm, about 20 μm to about 70 μm, about 20 μm to about 65 μm, about 20 μm to about 60 μm, about 20 μm to about 55 μm, about 20 μm to about 50 μm, about 20 μm to about 45 μm, about 20 μm to about 40 μm, about 20 μm to about 35 μm, about 20 μm to about 30 μm, about 20 μm to about 25 μm, about 25 μm to about 100 μm, about 25 μm to about 95 μm, about 25 μm to about 90 μm, about 25 μm to about 85 μm, about 25 μm to about 80 μm, about 25 μm to about 75 μm, about 25 μm to about 70 μm, about 25 μm to about 65 μm, about 25 μm to about 60 μm, about 25 μm to about 55 μm, about 25 μm to about 50 μm, about 25 μm to about 45 μm, about 25 μm to about 40 μm, about 25 μm to about 35 μm, about 25 μm to about 30 μm, about 30 μm to about 100 μm, about 30 μm to about 95 μm, about 30 μm to about 90 μm, about 30 μm to about 85 μm, about 30 μm to about 80 μm, about 30 μm to about 75 μm, about 30 μm to about 70 μm, about 30 μm to about 65 μm, about 30 μm to about 60 μm, about 30 μm to about 55 μm, about 30 μm to about 50 μm, about 30 μm to about 45 μm, about 30 μm to about 40 μm, about 30 μm to about 35 μm, about 35 μm to about 100 μm, about 35 μm to about 95 μm, about 35 μm to about 90 μm, about 35 μm to about 85 μm, about 35 μm to about 80 μm, about 35 μm to about 75 μm, about 35 μm to about 70 μm, about 35 μm to about 65 μm, about 35 μm to about 60 μm, about 35 μm to about 55 μm, about 35 μm to about 50 μm, about 35 μm to about 45 μm, about 35 μm to about 40 μm, about 40 μm to about 100 μm, about 40 μm to about 95 μm, about 40 μm to about 90 μm, about 40 μm to about 85 μm, about 40 μm to about 80 μm, about 40 μm to about 75 μm, about 40 μm to about 70 μm, about 40 μm to about 65 μm, about 40 μm to about 60 μm, about 40 μm to about 55 μm, about 40 μm to about 50 μm, about 40 μm to about 45 μm, about 45 μm to about 100 μm, about 45 μm to about 95 μm,

about 45 μm to about 90 μm, about 45 μm to about 85 μm, about 45 μm to about 80 μm, about 45 μm to about 75 μm, about 45 μm to about 70 μm, about 45 μm to about 65 μm, about 45 μm to about 60 μm, about 45 μm to about 55 μm, about 45 μm to about 50 μm, about 50 μm to about 100 μm, about 50 μm to about 95 μm, about 50 μm to about 90 μm, about 50 μm to about 85 μm, about 50 μm to about 80 μm, about 50 μm to about 75 μm, about 50 μm to about 70 μm, about 50 μm to about 65 μm, about 50 μm to about 60 μm, about 50 μm to about 55 μm, about 55 μm to about 100 μm, about 55 μm to about 95 μm, about 55 μm to about 90 μm, about 55 μm to about 85 μm, about 55 μm to about 80 μm, about 55 μm to about 75 μm, about 55 μm to about 70 μm, about 55 μm to about 65 μm, about 55 μm to about 60 μm, about 60 μm to about 100 μm, about 60 μm to about 95 μm, about 60 μm to about 90 μm, about 60 μm to about 85 μm, about 60 μm to about 80 μm, about 60 μm to about 75 μm, about 60 μm to about 70 μm, about 60 μm to about 65 μm, about 65 μm to about 100 μm, about 65 μm to about 95 μm, about 65 μm to about 90 μm, about 65 μm to about 85 μm, about 65 μm to about 80 μm, about 65 μm to about 75 μm, about 65 μm to about 70 μm, about 70 μm to about 100 μm, about 70 μm to about 95 μm, about 70 μm to about 90 μm, about 70 μm to about 85 μm, about 70 μm to about 80 μm, about 70 μm to about 75 μm, about 75 μm to about 100 μm, about 75 μm to about 95 μm, about 75 μm to about 90 μm, about 75 μm to about 85 μm, about 75 μm to about 80 μm, about 80 μm to about 100 μm, about 80 μm to about 95 μm, about 80 μm to about 90 μm, about 80 μm to about 85 μm, about 85 μm to about 100 μm, about 85 μm to about 95 μm, about 85 μm to about 90 μm, about 90 μm to about 100 μm, about 90 μm to about 95 μm, or about 95 μm to about 100 μm. In some embodiments, the D_{50} is about 5 μm, about 10 μm, about 15 μm, about 16 μm, about 20 μm, about 25 μm, about 30 μm, about 35 μm, about 40 μm, about 45 μm, about 50 μm, about 55 μm, about 60 μm, about 65 μm, about 70 μm, about 75 μm, about 80 μm, about 85 μm, about 90 μm, about 95 μm, or about 100 μm. In some embodiments, the D_{50} is about 10 μm, about 11 μm, about 12 μm, about 13 μm, about 14 μm, about 15 μm, about 16 μm, about 17 μm, about 18 μm, about 19 μm, or about 20 μm, or a value within a range defined by any of the preceding values. In some embodiments, the D_{50} value is about 16 μm. The D_{50} value can be measured by conventional particle size measuring techniques well known to those skilled in the art. Such techniques include, for example, sedimentation field flow fractionation, photon correlation spectroscopy, light scattering, laser diffraction and disc centrifugation.

In some embodiments, the particles have a particle size distribution (D-value, D_{50}), of 5 μm to 100 μm, such as 5 μm to 95 μm, 5 μm to 90 μm, 5 μm to 85 μm, 5 μm to 80 μm, 5

μm to 75 μm, 5 μm to 70 μm, 5 μm to 65 μm, 5 μm to 60 μm, 5 μm to 55 μm, 5 μm to 50 μm, 5 μm to 45 μm, 5 μm to 40 μm, 5 μm to 35 μm, 5 μm to 30 μm, 5 μm to 25 μm, 5 μm to 20 μm, 5 μm to 15 μm, 5 μm to 10 μm, 10 μm to 100 μm, 10 μm to 95 μm, 10 μm to 90 μm, 10 μm to 85 μm, 10 μm to 80 μm, 10 μm to 75 μm, 10 μm to 70 μm, 10 μm to 65 μm, 10 μm to 60 μm, 10 μm to 55 μm, 10 μm to 50 μm, 10 μm to 45 μm, 10 μm to 40 μm, 10 μm to 35 μm, 10 μm to 30 μm, 10 μm to 25 μm, 10 μm to 20 μm, 10 μm to 15 μm, 15 μm to 100 μm, 15 μm to 95 μm, 15 μm to 90 μm, 15 μm to 85 μm, 15 μm to 80 μm, 15 μm to 75 μm, 15 μm to 70 μm, 15 μm to 65 μm, 15 μm to 60 μm, 15 μm to 55 μm, 15 μm to 50 μm, 15 μm to 45 μm, 15 μm to 40 μm, 15 μm to 35 μm, 15 μm to 30 μm, 15 μm to 25 μm, 15 μm to 20 μm, 20 μm to 100 μm, 20 μm to 95 μm, 20 μm to 90 μm, 20 μm to 85 μm, 20 μm to 80 μm, 20 μm to 75 μm, 20 μm to 70 μm, 20 μm to 65 μm, 20 μm to 60 μm, 20 μm to 55 μm, 20 μm to 50 μm, 20 μm to 45 μm, 20 μm to 40 μm, 20 μm to 35 μm, 20 μm to 30 μm, 20 μm to 25 μm, 25 μm to 100 μm, 25 μm to 95 μm, 25 μm to 90 μm, 25 μm to 85 μm, 25 μm to 80 μm, 25 μm to 75 μm, 25 μm to 70 μm, 25 μm to 65 μm, 25 μm to 60 μm, 25 μm to 55 μm, 25 μm to 50 μm, 25 μm to 45 μm, 25 μm to 40 μm, 25 μm to 35 μm, 25 μm to 30 μm, 30 μm to 100 μm, 30 μm to 95 μm, 30 μm to 90 μm, 30 μm to 85 μm, 30 μm to 80 μm, 30 μm to 75 μm, 30 μm to 70 μm, 30 μm to 65 μm, 30 μm to 60 μm, 30 μm to 55 μm, 30 μm to 50 μm, 30 μm to 45 μm, 30 μm to 40 μm, 30 μm to 35 μm, 35 μm to 100 μm, 35 μm to 95 μm, 35 μm to 90 μm, 35 μm to 85 μm, 35 μm to 80 μm, 35 μm to 75 μm, 35 μm to 70 μm, 35 μm to 65 μm, 35 μm to 60 μm, 35 μm to 55 μm, 35 μm to 50 μm, 35 μm to 45 μm, 35 μm to 40 μm, 40 μm to 100 μm, 40 μm to 95 μm, 40 μm to 90 μm, 40 μm to 85 μm, 40 μm to 80 μm, 40 μm to 75 μm, 40 μm to 70 μm, 40 μm to 65 μm, 40 μm to 60 μm, 40 μm to 55 μm, 40 μm to 50 μm, 40 μm to 45 μm, 45 μm to 100 μm, 45 μm to 95 μm, 45 μm to 90 μm, 45 μm to 85 μm, 45 μm to 80 μm, 45 μm to 75 μm, 45 μm to 70 μm, 45 μm to 65 μm, 45 μm to 60 μm, 45 μm to 55 μm, 45 μm to 50 μm, 50 μm to 100 μm, 50 μm to 95 μm, 50 μm to 90 μm, 50 μm to 85 μm, 50 μm to 80 μm, 50 μm to 75 μm, 50 μm to 70 μm, 50 μm to 65 μm, 50 μm to 60 μm, 50 μm to 55 μm, 55 μm to 100 μm, 55 μm to 95 μm, 55 μm to 90 μm, 55 μm to 85 μm, 55 μm to 80 μm, 55 μm to 75 μm, 55 μm to 70 μm, 55 μm to 65 μm, 55 μm to 60 μm, 60 μm to 100 μm, 60 μm to 95 μm, 60 μm to 90 μm, 60 μm to 85 μm, 60 μm to 80 μm, 60 μm to 75 μm, 60 μm to 70 μm, 60 μm to 65 μm, 65 μm to 100 μm, 65 μm to 95 μm, 65 μm to 90 μm, 65 μm to 85 μm, 65 μm to 80 μm, 65 μm to 75 μm, 65 μm to 70 μm, 70 μm to 100 μm, 70 μm to 95 μm, 70 μm to 90 μm, 70 μm to 85 μm, 70 μm to 80 μm, 70 μm to 75 μm, 75 μm to 100 μm, 75 μm to 95 μm, 75 μm to 90 μm, 75 μm to 85 μm, 75 μm to 80 μm, 80 μm to 100 μm, 80 μm to 95 μm, 80 μm to 90 μm, 80 μm

to 85 μm, 85 μm to 100 μm, 85 μm to 95 μm, 85 μm to 90 μm, 90 μm to 100 μm, 90 μm to 95 μm, or 95 μm to 100 μm. In some embodiments, the D_{50} is 5 μm, 10 μm, 15 μm, 16 μm, 20 μm, 25 μm, 30 μm, 35 μm, 40 μm, 45 μm, 50 μm, 55 μm, 60 μm, 65 μm, 70 μm, 75 μm, 80 μm, 85 μm, 90 μm, 95 μm, or 100 μm. In some embodiments, the D_{50} is 10 μm, 11 μm, 12 μm, 13 μm, 14 μm, 15 μm, 16 μm, 17 μm, 18 μm, 19 μm, or 20 μm, or a value within a range defined by any of the preceding values. In some embodiments, the D_{50} value is 16 μm. The D_{50} value can be measured by conventional particle size measuring techniques well known to those skilled in the art. Such techniques include, for example, sedimentation field flow fractionation, photon correlation spectroscopy, light scattering, laser diffraction and disc centrifugation.

The SDDs of the present disclosure have a low residual solvent content. "Residual solvent content," as used herein, refers to the amount of solvent present in the SDD following spray drying, immediately upon exit from the spray dryer. The presence of solvent in the SDD lowers the glass transition temperature (T_g) of the dispersion. In some embodiments, mobility of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, in the SDD and its propensity to phase separate and crystallize decreases as the amount of residual solvent in the SDD decreases. In some embodiments, the SDDs have a residual solvent content of not more than about 10 wt%, such as not more than about 5 wt%, or not more than about 1 wt%. For example, the SDDs have a residual solvent content of about 2 wt%, about 1.9 wt%, about 1.8 wt%, about 1.7 wt%, about 1.6 wt%, about 1.5 wt%, about 1.4 wt%, about 1.3 wt%, about 1.2 wt%, about 1.1 wt%, about 1 wt%, about 0.9 wt%, about 0.8 wt%, about 0.7 wt%, about 0.6 wt%, about 0.5 wt%, or less. In some embodiments, the SDDs have a residual solvent content of less than about 2 wt%. In some embodiments, the SDDs have a residual solvent content of less than about 1 wt%. In some embodiments, the SDDs have a residual solvent content of about 0.5 wt% or less. In some embodiments, the SDDs have a residual solvent content of not more than 10 wt%, such as not more than 5 wt%, or not more than 1 wt%. For example, the SDDs may have a residual solvent content of 2 wt%, 1.9 wt%, 1.8 wt%, 1.7 wt%, 1.6 wt%, 1.5 wt%, 1.4 wt%, 1.3 wt%, 1.2 wt%, 1.1 wt%, 1 wt%, 0.9 wt%, 0.8 wt%, 0.7 wt%, 0.6 wt%, 0.5 wt%, or less. In some embodiments, the SDDs have a residual solvent content of less than 2 wt%. In some embodiments, the SDDs have a residual solvent content of less than 1 wt%. In some embodiments, the SDDs have a residual solvent content of 0.5 wt% or less.

In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, in the spray-dried dispersion is substantially amorphous. As used herein,

"substantially amorphous" means that the amount of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, in amorphous form is at least 60 wt% and that the amount of crystalline form present does not exceed 20 wt%. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, in the dispersion is "almost completely amorphous," meaning that at least 90 wt% of the drug is amorphous, or that the amount of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, in the crystalline form does not exceed 10 wt%. Amounts of crystalline drug can be measured by powder X-ray diffraction (PXRD), scanning electron microscope (SEM) analysis, differential scanning calorimetry (DSC), polarized light microscopy (PLM), or any other standard quantitative or qualitative measurement used to detect crystalline material. Without wishing to be bound by any theory, it is believed that the amorphous, or non-crystalline form, in combination with the polymer, leads to greater ease of dissolution and absorption in the desired location, for example, the intestines, resulting in enhanced bioavailability as compared to a crystalline form of the compound of Formula (I) without polymer.

Process for preparing spray-dried dispersions

Provided in the present disclosure are methods for preparing a spray-dried dispersion containing a polymer and the compound of Formula (I), or a pharmaceutically acceptable salt thereof, such as the SDDs described herein. In some embodiments, the method includes dissolving the compound of Formula (I), or a pharmaceutically acceptable salt thereof, and the polymer in an organic solvent to form a solution; and spray-drying the solution to produce the spray-dried dispersion, wherein the spray-drying forms homogeneous particles of the compound of Formula (I) and the polymer. In some embodiments, the product obtained by spray-drying is dried to remove the solvent or solvent mixture. In some embodiments, the organic solvent is acetone.

The SDDs disclosed herein can be obtained by spray-drying a mixture comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, one or more polymer(s), and an appropriate solvent or solvent mixture. Spray-drying involves atomization of a liquid mixture containing, for example, a solid and a solvent or solvent mixture, and removal of the solvent or solvent mixture. Atomization can be done, for example, through a two-fluid or pressure or electrosonic nozzle, or on a rotating disk.

Spray-drying converts a liquid feed to a dried particulate form. In some embodiments, spray-drying involves the atomization of a liquid feed solution into a spray of droplets and

contacting the droplets with hot air or gas in a drying chamber. The composition to be spray-dried can be any solution, coarse suspension, slurry, colloidal dispersion, or paste that can be atomized using the selected spray-drying apparatus. In some embodiments, the composition is a solution that is clear and free of undissolved solids. In some embodiments, the sprays are produced by either rotary (wheel) or nozzle atomizers. Evaporation of moisture from the droplets and formation of dry particles proceed under controlled temperature and airflow conditions. Commercially available types of apparatus can be used to conduct the spray-drying. For example, commercial spray dryers are manufactured by Buchi Ltd. and Niro (e.g., the PSD line of spray driers manufactured by Niro). Techniques and methods for spray-drying can also be found in *Perry's Chemical Engineering Handbook*, 6th Ed., R. H. Perry, D. W. Green & J. O. Maloney, eds., McGraw-Hill Book Co. (1984); and Marshall, "Atomization and Spray-Drying" 50, Chem. Eng. Prog. Monogr. Series 2 (1954).

In some embodiments, the spray-drying is conducted with an inlet temperature of from about 40°C to about 100°C, for example, from about 60°C to about 80°C, or from about 70°C to about 75°C. In some embodiments, the spray-drying is conducted with an inlet temperature of about 40°C, about 45°C, about 50°C, about 55°C, about 60°C, about 70°C, about 72°C, about 75°C, about 80°C, about 85°C, about 90°C, about 95°C, or about 100°C, or within a range defined by any of the preceding values. In some embodiments, the inlet temperature is about 60°C to about 80°C. In some embodiments, the spray-drying is conducted with an inlet temperature of about 61°C, about 63°C, about 65°C, about 67°C, about 69°C, about 71°C, about 73°C, about 75°C, about 77°C, about 79°C, about 81°C, about 83°C, or about 85°C, or within a range defined by any of the preceding values. In some embodiments, the inlet temperature is about 72°C.

In some embodiments, the spray-drying is conducted with an inlet temperature of from 40°C to 100°C, for example, from 60°C to 80°C, or from 70°C to 75°C. In some embodiments, the spray-drying is conducted with an inlet temperature of 40°C, 45°C, 50°C, 55°C, 60°C, 70°C, 72°C, 75°C, 80°C, 85°C, 90°C, 95°C, or 100°C, or within a range defined by any of the preceding values. In some embodiments, the inlet temperature is 60°C to 80°C. In some embodiments, the spray-drying is conducted with an inlet temperature of 61°C, 63°C, 65°C, 67°C, 69°C, 71°C, 73°C, 75°C, 77°C, 79°C, 81°C, 83°C, or 85°C, or within a range defined by any of the preceding values. In some embodiments, the inlet temperature is 72°C.

In some embodiments, the spray-drying is conducted with an outlet temperature of from about 20°C to about 75°C, for example, from about 25°C to about 50°C, or from about 30°C

to about 40°C. In some embodiments, the spray-drying is conducted with an outlet temperature of about 20°C, about 25°C, about 30°C, about 35°C, about 40°C, about 45°C, about 50°C, about 55°C, about 60°C, about 65°C, about 70°C, or about 75°C, or within a range defined by any of the preceding values. In some embodiments, the outlet temperature is about 25°C to about 45°C. In some embodiments, the spray-drying is conducted with an outlet temperature of about 24°C, about 26°C, about 28°C, about 30°C, about 32°C, about 34°C, about 36°C, about 38°C, about 40°C, about 42°C, about 44°C, or about 46°C, or within a range defined by any of the preceding values. In some embodiments, the outlet temperature is about 35°C.

In some embodiments, the spray-drying is conducted with an outlet temperature of from 20°C to 75°C, for example, from 25°C to 50°C, or from 30°C to 40°C. In some embodiments, the spray-drying is conducted with an outlet temperature of 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C, or 75°C, or within a range defined by any of the preceding values. In some embodiments, the outlet temperature is 25°C to 45°C. In some embodiments, the spray-drying is conducted with an outlet temperature of 24°C, 26°C, 28°C, 30°C, 32°C, 34°C, 36°C, 38°C, 40°C, 42°C, 44°C, or 46°C, or within a range defined by any of the preceding values. In some embodiments, the outlet temperature is 35°C.

In some embodiments, the method involves removing the organic solvent after formation of the spray-dried dispersion. In some embodiments, the organic solvent is removed by drying the SDD. In some embodiments, a secondary drying process, such as fluidized bed drying, vacuum drying, tray drying, microwave drying, rotary drum drying or biconical vacuum drying is used to remove or reduce residual solvents, such as the organic solvent, to pharmaceutically acceptable levels. In some embodiments, the SDD is dried with a convection tray dryer.

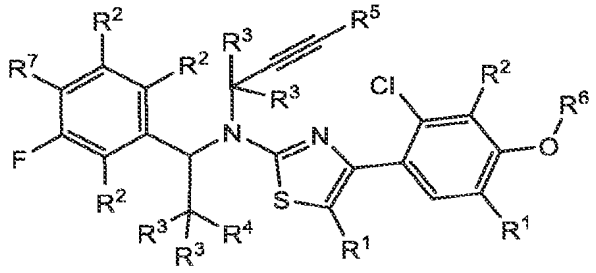
In some embodiments, the homogeneous particles produced by the methods of the present disclosure have a bulk density of less than about 0.2 g/mL, or less than about 0.15 g/mL. In some embodiments, the homogeneous particles produced by the methods of the present disclosure have a bulk density of about 0.19 g/mL, about 0.18 g/mL, about 0.17 g/mL, about 0.16 g/mL, about 0.15 g/mL, about 0.14 g/mL, about 0.13 g/mL, about 0.12 g/mL, about 0.11 g/mL, about 0.1 g/mL, about 0.09 g/mL, about 0.08 g/mL, about 0.07 g/mL, about 0.06 g/mL or about 0.05 g/mL, or within a range defined by any of the preceding values. In some embodiments, the homogeneous particles produced by the methods of the present disclosure have a bulk density of less than 0.2 g/mL, or less than 0.15 g/mL. In some embodiments, the homogeneous particles produced by the methods of the present disclosure have a bulk density

of 0.19 g/mL, 0.18 g/mL, 0.17 g/mL, 0.16 g/mL, 0.15 g/mL, 0.14 g/mL, 0.13 g/mL, 0.12 g/mL, 0.11 g/mL, 0.1 g/mL, 0.09 g/mL, 0.08 g/mL, 0.07 g/mL, 0.06 g/mL or 0.05 g/mL, or within a range defined by any of the preceding values. The term "bulk density," as used herein, refers to a property of powders and is defined as the mass of many particles of the material divided by the total volume they occupy. The total volume includes particle volume, inter-particle void volume and internal pore volume.

In some embodiments, the homogeneous particles produced by the methods of the present disclosure have a tapped density of less than about 0.3 g/mL, or less than about 0.25 g/mL. In some embodiments, the homogeneous particles produced by the methods of the present disclosure have a tapped density of about 0.29 g/mL, about 0.28 g/mL, about 0.27 g/mL, about 0.26 g/mL, about 0.25 g/mL, about 0.24 g/mL, about 0.23 g/mL, about 0.22 g/mL, about 0.21 g/mL, about 0.2 g/mL, or about 0.19 g/mL, or within a range defined by any of the preceding values. In some embodiments, the homogeneous particles produced by the methods of the present disclosure have a tapped density of less than 0.3 g/mL, or less than 0.25 g/mL. In some embodiments, the homogeneous particles produced by the methods of the present disclosure have a tapped density of 0.29 g/mL, 0.28 g/mL, 0.27 g/mL, 0.26 g/mL, 0.25 g/mL, 0.24 g/mL, 0.23 g/mL, 0.22 g/mL, 0.21 g/mL, 0.2 g/mL, or 0.19 g/mL, or within a range defined by any of the preceding values. The term "tap density" or "tapped density," as used herein, refers to a measure of the density of a powder. The tapped density of a pharmaceutical powder is determined using a tapped density tester, which is set to tap the powder at a fixed impact force and frequency. Tapped density by the USP method is determined by a linear progression of the number of taps.

Deuterated Compounds

Also disclosed herein are compounds having the structure of the following formula (II):



(II)

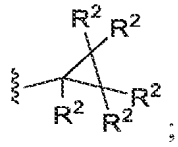
or a pharmaceutically acceptable salt thereof, wherein:

each R^1 is independently $C(R^A)_3$;

each R^A is independently hydrogen or deuterium;

each R^2 is independently hydrogen or deuterium;

each R^3 is independently hydrogen or deuterium;

R^4 is  ;

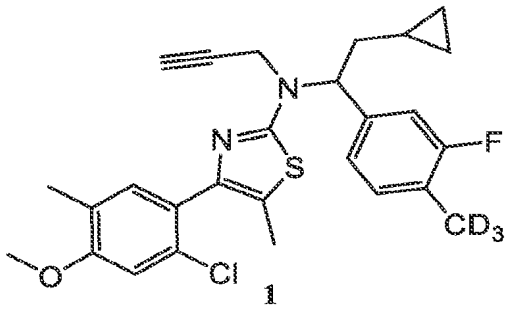
R^5 is hydrogen or deuterium;

R^6 is $C(R^A)_3$; and

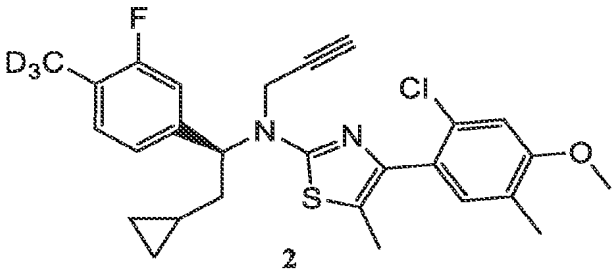
R^7 is $C(R^B)_3$, wherein at least one of R^A, R^B, R^2, R^3 and R^5 is deuterium.

With regard to the compounds provided herein, when a particular atomic position is designated as having deuterium or "D" or "d", it is understood that the abundance of deuterium at that position is substantially greater than the natural abundance of deuterium, which is about 0.015%. A position designated as having deuterium typically has a minimum isotopic enrichment factor of, in certain embodiments, at least 3500 (52.5% deuterium incorporation), at least 4000 (60% deuterium incorporation), at least 4500 (67.5% deuterium incorporation), at least 5000 (75% deuterium incorporation), at least 5500 (82.5% deuterium incorporation), at least 6000 (90% deuterium incorporation), at least 6333.3 (95% deuterium incorporation), at least 6466.7 (97% deuterium incorporation), at least 6600 (99% deuterium incorporation), or at least 6633.3 (99.5% deuterium incorporation) at each designated deuterium position.

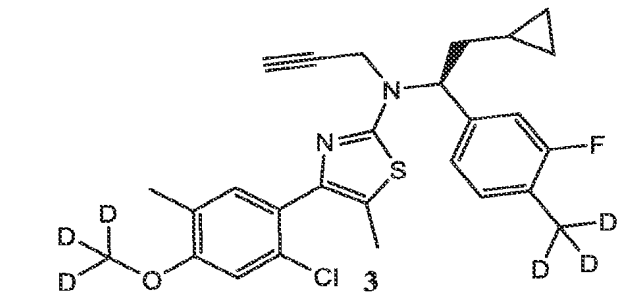
In some embodiments, the compound of Formula (II) may be one of the following, or a pharmaceutically acceptable salt thereof:



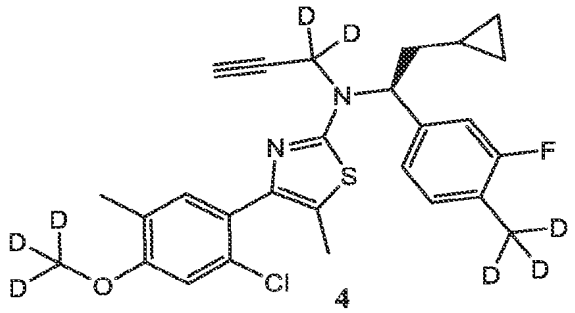
1



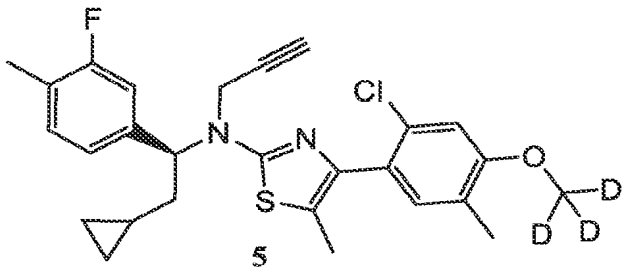
2



3



4



5

Pharmaceutical compositions

The methods and uses disclosed herein can comprise administering the compound of Formula (I) as a pharmaceutical composition.

In some embodiments of the methods described herein, the 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof is administered in a pharmaceutical composition further comprising one or more pharmaceutically acceptable excipients.

Also provided herein is a pharmaceutical composition comprising 4-(2-chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-prop-2-ynyl-1,3-thiazol-2-amine, or a pharmaceutically acceptable salt thereof, for use in any of the methods described herein.

In some embodiments, the methods and uses described herein comprise administering a pharmaceutical composition that does not comprise a spray-dried dispersion of the compound

of Formula (I), as specified, e.g., in Example 1. Accordingly, in some embodiments, the pharmaceutical composition does not comprise any of the following polymers: hydroxypropylmethylcellulose acetate succinate-L (HPMCAS-L); polyvinyl pyrrolidone vinyl acetate 64 (PVP/VA 64); HPMCAS-M; and methyl methacrylate copolymer (1:1) (Eudragit® L100).

In some embodiments, the methods and uses described herein comprise administering a pharmaceutical composition that is not the reference formulation described in Example 9. Accordingly, in some embodiments, the pharmaceutical composition does not comprise at least three of the excipients selected from caprylic/capric triglyceride (Labrafac® Lipophile, Gattefossé, France); propylene glycol dicrapolate/dicaprate (Labrafac® PG, Gattefossé, France); oleoyl polyoxyl-6 glycerides (Labrafil® M 1944 CS, Gattefossé, France); polysorbate 20; polyoxyl castor oil (Kolliphor® RH 40, BASF, Germany); polyoxyl 15 hydroxystearate (Kolliphor® HS 15, BASF, Germany); lauroyl polyoxyl-32 glycerides (Gelucire® 44/14, Gattefossé, France); d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS); and diethylene glycol monoethyl ether (Transcutol®, Gattefossé, France).

In some embodiments, the methods and uses described herein comprise administering a pharmaceutical composition that is the formulation described in Example 9. In some embodiments, the methods and uses described herein comprise administering a pharmaceutical composition that is the formulation described in Example 11. In some embodiments, the methods and uses described herein comprise administering a pharmaceutical composition that is the formulation described in Example 12. In some embodiments, the methods and uses described herein comprise administering a pharmaceutical composition that is the formulation described in Example 13.

In some embodiments, the pharmaceutical compositions include a spray-dried dispersion containing a polymer and the compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the pharmaceutical composition includes the SDD comprising a compound of Formula (I) and one or more pharmaceutically acceptable excipients. In some embodiments, the SDD is present in the pharmaceutical composition in an amount of about 20% to about 90% w/w of the composition, such as about 20% to about 85%, about 20% to about 80%, about 20% to about 75%, about 20% to about 70%, about 20% to about 65%, about 20% to about 60%, about 20% to about 55%, about 20% to about 50%, about 20% to about 45%, about 20% to about 40%, about 20% to about 35%, about 20% to about 30%, about 20%

to about 25%, about 25% to about 90%, about 25% to about 85%, about 25% to about 80%, about 25% to about 75%, about 25% to about 70%, about 25% to about 65%, about 25% to about 60%, about 25% to about 55%, about 25% to about 50%, about 25% to about 45%, about 25% to about 40%, about 25% to about 35%, about 25% to about 30%, about 30% to about 90%, about 30% to about 85%, about 30% to about 80%, about 30% to about 75%, about 30% to about 70%, about 30% to about 65%, about 30% to about 60%, about 30% to about 55%, about 30% to about 50%, about 30% to about 45%, about 30% to about 40%, about 30% to about 35%, about 35% to about 90%, about 35% to about 85%, about 35% to about 80%, about 35% to about 75%, about 35% to about 70%, about 35% to about 65%, about 35% to about 60%, about 35% to about 55%, about 35% to about 50%, about 35% to about 45%, about 35% to about 40%, about 40% to about 90%, about 40% to about 85%, about 40% to about 80%, about 40% to about 75%, about 40% to about 70%, about 40% to about 65%, about 40% to about 60%, about 40% to about 55%, about 40% to about 50%, about 40% to about 45%, about 45% to about 90%, about 45% to about 85%, about 45% to about 80%, about 45% to about 75%, about 45% to about 70%, about 45% to about 65%, about 45% to about 60%, about 45% to about 55%, about 45% to about 50%, about 50% to about 90%, about 50% to about 85%, about 50% to about 80%, about 50% to about 75%, about 50% to about 70%, about 50% to about 65%, about 50% to about 60%, about 50% to about 55%, about 55% to about 90%, about 55% to about 85%, about 55% to about 80%, about 55% to about 75%, about 55% to about 70%, about 55% to about 65%, about 55% to about 60%, about 60% to about 90%, about 60% to about 85%, about 60% to about 80%, about 60% to about 75%, about 60% to about 70%, about 60% to about 65%, about 65% to about 90%, about 65% to about 85%, about 65% to about 80%, about 65% to about 75%, about 65% to about 70%, about 70% to about 90%, about 70% to about 85%, about 70% to about 80%, about 70% to about 75%, about 75% to about 90%, about 75% to about 85%, about 75% to about 80%, about 80% to about 90%, about 80% to about 85%, or about 85% to about 90% w/w of the composition. In some embodiments, the SDD is present in an amount of about 40% to about 90% w/w of the composition. In some embodiments, the SDD is present in an amount of about 40% to about 80% w/w of the composition. In some embodiments, the SDD is present in the pharmaceutical composition in an amount of about 60% to about 80% w/w of the composition. In some embodiments, the SDD is present in an amount of about 80% w/w of the composition. In some embodiments, the SDD is present in the pharmaceutical composition in an amount of about 1% to about 20% w/w of the composition, such as about 13% w/w of the composition.

In some embodiments, the pharmaceutical composition includes the SDD comprising a compound of Formula (I) and one or more pharmaceutically acceptable excipients. In some embodiments, the SDD is present in the pharmaceutical composition in an amount of 20% to 90% w/w of the composition, such as 20% to 85%, 20% to 80%, 20% to 75%, 20% to 70%, 20% to 65%, 20% to 60%, 20% to 55%, 20% to 50%, 20% to 45%, 20% to 40%, 20% to 35%, 20% to 30%, 20% to 25%, 25% to 90%, 25% to 85%, 25% to 80%, 25% to 75%, 25% to 70%, 25% to 65%, 25% to 60%, 25% to 55%, 25% to 50%, 25% to 45%, 25% to 40%, 25% to 35%, 25% to 30%, 30% to 90%, 30% to 85%, 30% to 80%, 30% to 75%, 30% to 70%, 30% to 65%, 30% to 60%, 30% to 55%, 30% to 50%, 30% to 45%, 30% to 40%, 30% to 35%, 35% to 90%, 35% to 85%, 35% to 80%, 35% to 75%, 35% to 70%, 35% to 65%, 35% to about 60%, 35% to 55%, 35% to 50%, 35% to 45%, 35% to 40%, 40% to 90%, 40% to 85%, 40% to 80%, 40% to 75%, 40% to 70%, 40% to 65%, 40% to 60%, 40% to 55%, 40% to 50%, 40% to 45%, 45% to 90%, 45% to 85%, 45% to 80%, 45% to 75%, 45% to 70%, 45% to 65%, 45% to 60%, 45% to 55%, 45% to 50%, 50% to 90%, 50% to 85%, 50% to 80%, 50% to 75%, 50% to 70%, 50% to 65%, 50% to 60%, 50% to 55%, 55% to 90%, 55% to 85%, 55% to 80%, 55% to 75%, 55% to 70%, 55% to 65%, 55% to 60%, 60% to 90%, 60% to 85%, 60% to 80%, 60% to 75%, 60% to 70%, 60% to 65%, 65% to 90%, 65% to 85%, 65% to 80%, 65% to 75%, 65% to 70%, 70% to 90%, 70% to 85%, 70% to 80%, 70% to 75%, 75% to 90%, 75% to 85%, 75% to 80%, 80% to 90%, 80% to 85%, or about 85% to 90% w/w of the composition. In some embodiments, the SDD is present in an amount of 40% to 90% w/w of the composition. In some embodiments, the SDD is present in an amount of 40% to 80% w/w of the composition. In some embodiments, the SDD is present in the pharmaceutical composition in an amount of 60% to 80% w/w of the composition. In some embodiments, the SDD is present in an amount of 80% w/w of the composition. In some embodiments, the SDD is present in the pharmaceutical composition in an amount of about 1% to about 20% w/w of the composition, such as about 13% w/w of the composition.

In some embodiments of the pharmaceutical compositions disclosed herein (e.g., a composition including an SDD), the pharmaceutically acceptable excipient is selected from the group consisting of a filler, a lubricant, and combinations thereof. In some embodiments, the pharmaceutical excipients are selected from the group consisting of a glidant, a filler, a disintegrant, a lubricant, and a combination thereof.

In some embodiments, the pharmaceutical composition includes a filler. In some embodiments, the filler is selected from among binders, diluents, disintegrants, glidants, surfactants, and combinations thereof.

In some embodiments, the filler include saccharides (e.g., sugars, starch, and cellulose), gelatin, calcium carbonate, and synthetic polymers (e.g., polyvinylpyrrolidone, polyethylene glycol, and poloxamers (e.g., Poloxamer 188, a copolymer of polyoxyethylene and polyoxypropylene)). Exemplary fillers include, but are not limited to, glucose, sucrose, lactose, a starch, including modified starches such as sodium starch glycolate (e.g., Explotab®), xylitol, dextrin, saccharose, sorbitol, mannitol (e.g., Parteck® M 200 (mannitol with an average particle size of about 50 μm to about 500 μm) or Parteck® M 100 (mannitol with an average particle size of less than 212 μm)), a cellulose, a polyvinylpyrrolidone, a polyethylene glycol, a polyvinyl alcohol, a polymethacrylate, dibasic calcium phosphate, magnesium stearate, calcium stearate, sodium stearate, stearic acid, hydrogenated vegetable oils, a mineral oil, sodium lauryl sulfate, magnesium lauryl sulfate, glyceryl palmitostearate, sodium benzoate, sodium stearyl fumarate, colloidal silicon dioxide, sodium benzoate, sodium oleate, sodium acetate, aliginic acid, alginates (e.g., sodium alginate), calcium silicate, and ion exchange resins. Exemplary cellulose fillers include microcrystalline cellulose (e.g., Avicel® PH-101 (microcrystalline cellulose with an average particle size of approximately 50 μm) or Avicel® PH 200 (microcrystalline cellulose with an average particle size of approximately 180 μm)), methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, and hydroxypropylmethylcellulose. Exemplary fillers include cross-linked polyvinylpyrrolidone such as with an average particle size of 90 μm to 130 μm) or with an average particle size of 10 μm to 30 μm). Other fillers known to those of skill in the art are also contemplated as being useful when formulated in the pharmaceutical compositions described herein.

In some embodiments, the filler is a binder. Binders include agents that hold the active pharmaceutical ingredient (e.g., spray-dried dispersion containing a polymer and the compound of Formula (I), or a pharmaceutically acceptable salt thereof) and inactive ingredients together in a cohesive mix. Exemplary binders include, but are not limited to, glucose, sucrose, lactose, a starch, including modified starches such as sodium starch glycolate (Explotab®), xylitol, dextrin, saccharose, sorbitol, mannitol (e.g., Parteck® M 200 (mannitol with an average particle size of about 50 μm to about 500 μm), Parteck® M 100 (mannitol with an average particle size of less than 212 μm)), gelatin, gum tragacanth, acacia mucilage, a cellulose, a polyvinylpyrrolidone, a polyethylene glycol, a polyvinyl alcohol, a

polymethacrylate, and sodium starch glycolate. Exemplary cellulose fillers include microcrystalline cellulose (e.g., Avicel® PH-101 (microcrystalline cellulose with an average particle size of approximately 50 μm) or Avicel® PH 200 (microcrystalline cellulose with an average particle size of approximately 180 μm)), cellulose ethers, methyl cellulose, ethyl cellulose, croscarmellose sodium, sodium carboxy methyl cellulose starches, hydroxypropyl cellulose, and hydroxypropyl methyl cellulose. Exemplary polyvinylpyrrolidone fillers include cross-linked polyvinylpyrrolidone such as Kollidon® CL (crospovidone with an average particle size of 90 μm to 130 μm) or Kollidon® CL-SF (crospovidone with an average particle size of 10 μm to 30 μm). Other binders known to those of skill in the art are also contemplated as being useful when formulated in the compositions described herein.

In some embodiments, the filler is a diluent. Suitable diluents include, but are not limited to, lactose, mannitol, isomalt, sucrose, dextrose, and sorbitol.

In some embodiments, the filler is a disintegrant. Disintegrants include any agent that promotes breakup of the formulation in an aqueous environment, for example, to promote more rapid release of the active pharmaceutical ingredient (e.g., the compound of Formula (I), or a pharmaceutically acceptable salt thereof). Exemplary disintegrants include, but are not limited to, starch and modified starches, such as corn starch, potato starch, sodium starch glycolate or croscarmellose sodium, alginic acid, alginates, such as sodium alginate, polyvinylpyrrolidone, bentonite, methylcellulose, agar, carboxymethylcellulose, crospovidone, acid-carbonate effervescent systems, such as citric acid with bicarbonate salts, and ion exchange resins. Other disintegrants known to those of skill in the art are also contemplated as being useful when formulated in the compositions described herein.

In some embodiments, the pharmaceutical composition comprises a disintegrant. In some embodiments, the pharmaceutical composition comprises about 1 w/w% to about 30 w/w% of the disintegrant. In some embodiments, the pharmaceutical composition comprises about 5 w/w% to about 15 w/w% of the disintegrant. In some embodiments, the pharmaceutical composition comprises about 10 w/w% of the disintegrant. In some embodiments, the disintegrant is selected from croscarmellose sodium, sodium starch glycolate, crospovidone, and sodium bicarbonate. In some embodiments, the disintegrant is croscarmellose sodium.

In some embodiments, the filler is a glidant. Glidants can be used to improve the flowability of a powder or granules or both. Glidants include, but are not limited to, silicone dioxide, such as colloidal silicon dioxide or hydrated silicon dioxide, magnesium silicate,

magnesium aluminometasilicate, talc, starch, calcium silicate, light anhydrous silicic acid, and silicon dioxide aerogels.

In some embodiments, the pharmaceutical composition comprises a glidant. In some embodiments, the pharmaceutical composition comprises about 0.1 w/w% to about 5 w/w% of the glidant. In some embodiments, the pharmaceutical composition comprises about 0.1 w/w% to about 1 w/w% of the glidant. In some embodiments, the pharmaceutical composition comprises about 0.67 w/w% of the glidant. In some embodiments, the glidant is selected from calcium silicate, silicon dioxide, and talc. In some embodiments, the glidant is calcium silicate.

In some embodiments, the filler is a surfactant, wetting agent, solubilizer, or combination thereof. Examples include, but are not limited to, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers (e.g., macrogol ethers such as cetomacrogol 1000), polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters (e.g., Tween®), polyoxyethylene stearates, sodium dodecylsulfate, tyloxapol (a nonionic liquid polymer of the alkyl aryl polyether alcohol type, also known as superinone or triton). Other examples include, but are not limited to, poloxamers such as Pluronic® F68, F127, and F108, which are block copolymers of ethylene oxide and propylene oxide, and polyxamines such as Tetronic® 908 (also known as Poloxamine® 908), which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine (available from BASF), dextran, lecithin, dialkylesters of sodium sulfosuccinic acid, such as Aerosol® OT, which is a dioctyl ester of sodium sulfosuccinic acid (available from American Cyanimid), Duponol® P, which is a sodium lauryl sulfate (available from DuPont), Triton® X-200, which is an alkyl aryl polyether sulfonate (available from Rohm and Haas), Tween® 20 and Tween® 80, which are polyoxyethylene sorbitan fatty acid esters (available from ICI Specialty Chemicals), Carbowax™ 3550 and 934, which are polyethylene glycols (available from Union Carbide), Crodesta™ F-110, which is a mixture of sucrose stearate and sucrose distearate, and Crodesta™ SL-40 (both available from Croda Inc.), and SA90HCO, which has the chemical formula $C_{18}H_{37}—CH_2(CON(CH_3)CH_2(CHOH)_4CH_2OH)_2$.

In some embodiments, the pharmaceutical composition comprises a filler. In some embodiments, the pharmaceutical composition comprises about 30 w/w% to about 99 w/w% of the filler. In some embodiments, the pharmaceutical composition comprises about 50 w/w% to about 90 w/w% of the filler. In some embodiments, the pharmaceutical composition comprises about 75.5 w/w% of the filler. In some embodiments, the filler is selected from

mannitol, microcrystalline cellulose, lactose, starch, isomalt, silicified microcrystalline cellulose, Dicalcium Phosphate, maltodextrin, and a combination thereof. In some embodiments, the filler is a combination of mannitol and microcrystalline cellulose. In some embodiments, the pharmaceutical composition comprises about 30 w/w% to about 80 w/w% of mannitol. In some embodiments, the pharmaceutical composition comprises about 50 w/w% to about 60 w/w% of mannitol. In some embodiments, the pharmaceutical composition comprises about 56 w/w% of mannitol. In some embodiments, the pharmaceutical composition comprises about 1 w/w% to about 50 w/w% of microcrystalline cellulose. In some embodiments, the pharmaceutical composition comprises about 10 w/w% to about 30 w/w% of microcrystalline cellulose. In some embodiments, the pharmaceutical composition comprises about 20 w/w% of microcrystalline cellulose. In some embodiments, the pharmaceutical composition comprises about 56 w/w% of mannitol and about 20 w/w% of microcrystalline cellulose.

In some embodiments, the pharmaceutical composition includes a lubricant. Lubricants are agents added to pharmaceutical formulations to reduce friction during processing and prevent ingredients from clumping together. Exemplary lubricants include, but are not limited to, talc, starch, magnesium stearate, calcium stearate, sodium stearate, zinc stearate, stearic acid, vegetable stearin, adipic acid, waxy fatty acids, such as glyceryl behenate, a hydrogenated vegetable oil, a mineral oil, a polyethylene glycol, lycopodium, sodium lauryl sulfate, magnesium lauryl sulfate, glyceryl palmitostearate, sodium benzoate, sodium chloride, sterotex, glycerol monostearate, sodium stearyl fumarate, colloidal silicon dioxide, sodium benzoate, sodium oleate, and sodium acetate. Other lubricants known to those of skill in the art are also contemplated as being useful when formulated in the compositions described herein.

In some embodiments, the pharmaceutical composition comprises a lubricant. In some embodiments, the pharmaceutical composition comprises about 0.1 w/w% to about 10 w/w% of the lubricant. In some embodiments, the pharmaceutical composition comprises about 0.1 w/w% to about 1 w/w% of the lubricant. In some embodiments, the pharmaceutical composition comprises about 0.5 w/w% of the lubricant. In some embodiments, the pharmaceutical lubricant is selected from sodium stearyl fumarate, magnesium stearate, stearic acid sodium lauryl sulfate, sodium oleate, glyceryl behenate, and talc. In some embodiments, the lubricant is sodium stearyl fumarate.

In some embodiments, the pharmaceutical composition comprises:

(a) the spray-dried dispersion comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, and a polymer;

(b) a glidant;

(c) a filler; and

(d) a disintegrant.

In some embodiments, the pharmaceutical composition comprises:

(a) about 1 w/w% to about 20 w/w% of the spray-dried dispersion comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, and a polymer;

(b) about 0.1 w/w% to about 1 w/w% of a glidant;

(c) about 50 w/w% to about 90 w/w% of a filler; and

(d) about 5 w/w% to about 0.2 w/w% of a disintegrant.

In some embodiments, the pharmaceutical composition comprises:

(a) about 13 w/w% of the spray-dried dispersion comprising the compound of Formula (I), or a pharmaceutically acceptable salt thereof, and a polymer;

(b) about 0.67 w/w% of a glidant;

(c) about 75.5 w/w% of a filler; and

(d) about 10 w/w% of a disintegrant.

In some embodiments, the pharmaceutical composition comprises:

(a) the spray-dried dispersion of Example 3;

(b) calcium silicate;

(c) a combination of mannitol and microcrystalline cellulose; and

(d) croscarmellose sodium.

In some embodiments, the pharmaceutical composition comprises:

(a) about 1 w/w% to about 20 w/w% of the spray-dried dispersion of Example 3;

(b) about 0.1 w/w% to about 1 w/w% of calcium silicate;

(c) about 50 w/w% to about 60 w/w% of mannitol and about 10 w/w% to about 30 w/w% of microcrystalline cellulose; and

(d) about 5 w/w% to about 0.2 w/w% of croscarmellose sodium.

In some embodiments, the pharmaceutical composition comprises:

(a) about 13 w/w% of the spray-dried dispersion of Example 3;

(b) about 0.67 w/w% of calcium silicate;

(c) about 56 w/w% of mannitol and about 20 w/w% of microcrystalline cellulose; and

(d) about 10 w/w% of croscarmellose sodium.

Additional excipients can be included in the pharmaceutical formulations of the present disclosure. Further examples of excipients include, but are not limited to, pigments, colorants, flavoring agents, preservatives, and sweeteners. Flavors and colors can be added to improve the taste or appearance of a formulation. Examples of preservatives used in pharmaceutical compositions are aromatic alcohols, such as benzyl or phenol alcohol, antioxidants such as vitamin A, vitamin E, vitamin C, and selenium, amino acids such as cysteine and methionine, citric acid and sodium citrate, or synthetic preservatives such as methyl paraben and propyl paraben. Sweeteners can be added to make the ingredients more palatable, especially in chewable tablets or liquids like syrups.

Also provided are methods for preparing a pharmaceutical composition, such as a pharmaceutical composition of the present disclosure. In some embodiments, the method includes combining the spray-dried dispersion described herein with one or more pharmaceutically acceptable excipients, e.g., a pharmaceutically acceptable excipient described herein.

In some embodiments, the method for preparing the pharmaceutical composition comprises:

(a) blending the spray-dried dispersion described herein, with a glidant;

(b) further blending the blend of step (a) with a filler and a disintegrant;

(c) screening the blend of step (b) to break up aggregates and assist with blend uniformity;

(d) further blending the intragranular blend of step (c);

(e) compaction of the intragranular blend of step (d) via roller compaction to form granules; and

(f) milling of ribbon from roller compaction into granules of step (e).

In some embodiments, the spray-dried dispersion is the spray-dried dispersion described in Example 3.

The pharmaceutical compositions of the present disclosure are formulated for oral administration. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media can be employed. For solid oral preparations such as, for example, powders, capsules, caplets, gelcaps, and tablets, suitable carriers and additives include starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like. Suitable binders include, without limitation, starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum and the like.

Oral pharmaceutical dosage forms can be solid, gel, or liquid. In some embodiments, the dosage form is a solid dosage form. In some embodiments, the solid dosage form is a pill, tablet, capsule, caplet, gelcaps, granules, powder, sachet, melting strip, or melting film. In some embodiments, the solid dosage form is coated. In some embodiments, the coating is an enteric coating, a sugar coating, or a film coating. In some embodiments, the solid dosage form is a coated particle, coated tablet, enterocoated tablet, or enterocoated capsule. In some embodiments, the solid dosage form is a pill or tablet. Types of oral tablets include compressed, chewable lozenges and tablets which may be enteric coated, sugar coated or film coated. In some embodiments, the pharmaceutical composition is formulated as a capsule. In some embodiments, the pharmaceutical composition is formulated as a powder, solution, or suspension (e.g., in propylene carbonate, vegetable oils, PEG's, poloxamer 124 or triglycerides), or is encapsulated in a capsule (gelatin or cellulose base capsule). Capsules can be hard or soft gelatin capsules, while granules and powders can be provided in non-effervescent or effervescent form with a combination of other ingredients known to those skilled in the art.

The pharmaceutical compositions of the present disclosure can contain, per dosage unit, e.g., tablet, capsule, powder, and the like, an amount of the active ingredient necessary to deliver an effective dose as described above.

In some embodiments, the pharmaceutical compositions of the present disclosure are formulated in unit dosage form. In some embodiments, the compound of Formula (I), or pharmaceutically acceptable salt thereof, is present in an amount of about 5 mg to about 200 mg in the unit dosage form. For example, about 5 mg to about 175 mg, about 5 mg to about 150 mg, about 5 mg to about 125 mg, about 5 mg to about 100 mg, about 5 mg to about 75 mg,

about 5 mg to about 50 mg, about 5 mg to about 25 mg, about 25 mg to about 200 mg, about 25 mg to about 175 mg, about 25 mg to about 150 mg, about 25 mg to about 125 mg, about 25 mg to about 100 mg, about 25 mg to about 75 mg, about 25 mg to about 50 mg, about 50 mg to about 200 mg, about 50 mg to about 175 mg, about 50 mg to about 150 mg, about 50 mg to about 125 mg, about 50 mg to about 100 mg, about 50 mg to about 75 mg, about 75 mg to about 200 mg, about 75 mg to about 175 mg, about 75 mg to about 150 mg, about 75 mg to about 125 mg, about 75 mg to about 100 mg, about 100 mg to about 200 mg, about 100 mg to about 175 mg, about 100 mg to about 150 mg, about 100 mg to about 125 mg, about 125 mg to about 200 mg, about 125 mg to about 175 mg, about 125 mg to about 150 mg, about 150 mg to about 200 mg, about 150 mg to about 175 mg, or about 175 mg to about 200 mg in the unit dosage form. In some embodiments, the compound of Formula (I), or pharmaceutically acceptable salt thereof, is present in an amount of about 25 mg to about 125 mg in the unit dosage form. In some embodiments, the compound of Formula (I), or pharmaceutically acceptable salt thereof, is present in an amount of about 75 mg to about 150 mg in the unit dosage form. In some embodiments, the compound of Formula (I), or pharmaceutically acceptable salt thereof, is present in an amount of about 5 mg, about 10 mg, about 25 mg, about 35 mg, about 50 mg, about 65 mg, about 75 mg, about 90 mg, about 100 mg, about 125 mg, about 150 mg, about 175 mg, or about 200 mg in the unit dosage form, or within a range defined by any of the preceding values. In some embodiments, the compound of Formula (I), or pharmaceutically acceptable salt thereof, is present in an amount of about 50 mg in the unit dosage form. In some embodiments, the compound of Formula (I), or pharmaceutically acceptable salt thereof, is present in an amount of about 100 mg in the unit dosage form. . In some embodiments, the compound of Formula (I), or pharmaceutically acceptable salt thereof, is present in an amount of about 25 mg in the unit dosage form. In some embodiments, the compound of Formula (I), or pharmaceutically acceptable salt thereof, is present in an amount of 5 mg to 250 mg in the unit dosage form. For example, 5 mg to 175 mg, 5 mg to 150 mg, 5 mg to 125 mg, 5 mg to 100 mg, 5 mg to 75 mg, 5 mg to 50 mg, 5 mg to 25 mg, 25 mg to 200 mg, 25 mg to 175 mg, 25 mg to 150 mg, 25 mg to 125 mg, 25 mg to 100 mg, 25 mg to 75 mg, 25 mg to 50 mg, 50 mg to 200 mg, 50 mg to 175 mg, 50 mg to 150 mg, 50 mg to 125 mg, 50 mg to 100 mg, 50 mg to 75 mg, 75 mg to 200 mg, 75 mg to 175 mg, 75 mg to 150 mg, 75 mg to 125 mg, 75 mg to 100 mg, 100 mg to 200 mg, 100 mg to 175 mg, 100 mg to 150 mg, 100 mg to 125 mg, 125 mg to 200 mg, 125 mg to 175 mg, 125 mg to 150 mg, 150 mg to 200 mg, 150 mg to 175 mg, or 175 mg to 200 mg in the unit dosage form. In some embodiments, the

compound of Formula (I), or pharmaceutically acceptable salt thereof, is present in an amount of 25 mg to 125 mg in the unit dosage form. In some embodiments, the compound of Formula (I), or pharmaceutically acceptable salt thereof, is present in an amount of 75 mg to 150 mg in the unit dosage form. In some embodiments, the compound of Formula (I), or pharmaceutically acceptable salt thereof, is present in an amount of 5 mg, 10 mg, 25 mg, 35 mg, 50 mg, 65 mg, 75 mg, 90 mg, 100 mg, 125 mg, 150 mg, 175 mg, or 200 mg in the unit dosage form, or within a range defined by any of the preceding values. In some embodiments, the compound of Formula (I), or pharmaceutically acceptable salt thereof, is present in an amount of 50 mg in the unit dosage form. In some embodiments, the compound of Formula (I), or pharmaceutically acceptable salt thereof, is present in an amount of 100 mg in the unit dosage form. In some embodiments, the pharmaceutical compositions of the present disclosure are formulated as a tablet. In some embodiments, the tablet is coated. In some embodiments, the pharmaceutical compositions of the present disclosure are formulated as capsules. In some embodiments, the pharmaceutical compositions are in sachet form. In some embodiments, the pharmaceutical compositions are in granule form.

In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in a dose of about 25 mg, based on the weight of the free base. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in a dose of about 50 mg, based on the weight of the free base. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in a dose of about 75 mg, based on the weight of the free base. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered in a dose of about 100 mg, based on the weight of the free base.

In some embodiments, the pharmaceutical composition is administered in a dose of about 25 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the pharmaceutical composition is administered in a dose of about 50 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the pharmaceutical composition is administered in a dose of about 75 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the pharmaceutical composition is administered in a dose of about 100 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base.

In some embodiments, the pharmaceutical composition comprises about 25 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the pharmaceutical composition comprises about 50 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the pharmaceutical composition comprises about 75 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the pharmaceutical composition comprises about 100 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base.

The daily dosage of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, in a pharmaceutical composition as described in the present disclosure can be varied over a wide range from about 1.0 mg to about 10,000 mg per adult human per day, or higher, or any range therein. For oral administration, the compositions can be provided in the form of tablets containing, for example, about 0.01 mg, about 0.05 mg, about 0.1 mg, about 0.5 mg, about 1.0 mg, about 2.5 mg, about 5.0 mg, about 10.0 mg, about 15.0 mg, about 25.0 mg, about 50.0 mg, about 75.0 mg, about 100 mg, about 150 mg, about 200 mg, about 250 or about 500 milligrams of the compound of Formula (I), or pharmaceutically acceptable salt thereof, for the symptomatic adjustment of the dosage to the subject to be treated. In some embodiments, an effective amount of the compound of Formula (I), or pharmaceutically acceptable salt thereof, can be supplied at a dosage level of from about 0.1 mg/kg to about 1000 mg/kg of body weight per day, or any range therein, for example, the range can be from about 0.5 mg/kg to about 500 mg/kg, about 1.0 mg/kg to about 250 mg/kg, about 0.1 mg/kg to about 100 mg/kg, about 0.1 mg/kg to about 50.0 mg/kg of body weight per day, about 0.1 mg/kg to about 15.0 mg/kg of body weight per day, about 0.5 mg/kg to about 7.5 mg/kg of body weight per day, or any amount to range therein. In some embodiments, an effective amount of the compound of Formula (I), or pharmaceutically acceptable salt thereof, can be supplied at a dosage level of from 0.1 mg/kg to 1000 mg/kg of body weight per day, or any range therein, for example, the range can be from 0.5 mg/kg to 500 mg/kg, 1.0 mg/kg to 250 mg/kg, 0.1 mg/kg to 100 mg/kg, 0.1 mg/kg to 50.0 mg/kg of body weight per day, 0.1 mg/kg to 15.0 mg/kg of body weight per day, 0.5 mg/kg to 7.5 mg/kg of body weight per day, or any amount to range therein. A pharmaceutical composition as provided herein can be administered on a regimen of 1 to 4 times per day or in a single daily dose.

In some embodiments, the daily dose of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is about 25 mg, based on the weight of the free base. In some embodiments, the daily dose of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is about 50 mg, based on the weight of the free base. In some embodiments, the daily dose of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is about 75 mg, based on the weight of the free base. In some embodiments, the daily dose of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is about 100 mg, based on the weight of the free base. In some embodiments, the daily dose of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is about 150 mg, based on the weight of the free base. In some embodiments, the daily dose of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is about 200 mg, based on the weight of the free base.

In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered twice daily in a dose of about 25 mg, based on the weight of the free base. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered twice daily in a dose of about 50 mg, based on the weight of the free base. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered twice daily in a dose of about 75 mg, based on the weight of the free base. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered twice daily in a dose of about 100 mg, based on the weight of the free base.

In some embodiments, the daily dose of the pharmaceutical composition is about 25 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the daily dose of the pharmaceutical composition is about 50 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the daily dose of the pharmaceutical composition is about 75 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the daily dose of the pharmaceutical composition is about 100 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the daily dose of the pharmaceutical composition is about 150 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the daily dose of the

pharmaceutical composition is about 200 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base.

In some embodiments, the pharmaceutical composition is administered twice daily in a dose of about 25 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the pharmaceutical composition is administered twice daily in a dose of about 50 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, is administered twice daily in a dose of about 75 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the pharmaceutical composition is administered twice daily in a dose of about 100 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base.

In some embodiments, the method comprises administering a daily dose of the pharmaceutical composition comprising about 25 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the method comprises administering a daily dose of the pharmaceutical composition comprising about 50 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the method comprises administering a daily dose of the pharmaceutical composition comprising about 75 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the method comprises administering a daily dose of the pharmaceutical composition comprising about 100 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the method comprises administering a daily dose of the pharmaceutical composition comprising about 150 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the method comprises administering a daily dose of the pharmaceutical composition comprising about 200 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base.

In some embodiments, the method comprises administering the pharmaceutical composition twice daily in a dose of about 25 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some

embodiments, the method comprises administering the pharmaceutical composition twice daily in a dose of about 50 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the method comprises administering the compound of Formula (I), or a pharmaceutically acceptable salt thereof, twice daily in a dose of about 75 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base. In some embodiments, the method comprises administering the pharmaceutical composition twice daily in a dose of about 100 mg of the compound of Formula (I), or a pharmaceutically acceptable salt thereof, based on the weight of the free base.

Factors associated with the particular subject being treated, including subject age, weight, diet, and time of administration, can result in the need to adjust dosages. In some embodiments, the subject is a human adult. In some embodiments, the subject is a pediatric subject.

One skilled in the art will recognize that both *in vivo* and *in vitro* trials using suitable, known, and generally accepted cell and/or animal models are predictive of the ability of a test compound to treat or prevent a given disorder. One skilled in the art will further recognize that human clinical trials including first-in-human, dose ranging and efficacy trials, in healthy subjects and/or those suffering from a given disorder, can be completed according to methods well known in the clinical and medical arts. For example, determining proper dosages for pediatric subjects can be determined using known methods, including weight, age, and models such as Simcyp® Pediatric Simulation modeling (CERTARA, Princeton, N.J.) which can be used to establish a pharmacokinetic approach for dosing that takes into account subject age, ontogeny of the clearance pathways that a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and body surface area (BSA).

In some embodiments, the pharmaceutical compositions of the present disclosure are stable for at least 3 months. In some embodiments, the pharmaceutical compositions are stable for at least 6 months. In some embodiments, the pharmaceutical compositions are stable for at least 9 months. In some embodiments, the pharmaceutical compositions are stable for at least 12 months. For example, the compositions do not exhibit a change (e.g., greater than 5%) in appearance, pH, percent impurities, activity (as measured by in vitro assays), or osmolarity over time, e.g., at least 3 months, 6 months, 9 months, or at least 12 months as compared to the original composition after manufacturing. In some embodiments, the pharmaceutical compositions do not exhibit a significant change, as defined by the International Conference

on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), in one or more of appearance, pH, percent impurities, activity (as measured by *in vitro* assays), or osmolarity over time, e.g., at least 12 months as compared to the original pharmaceutical composition after manufacturing.

Kits

Also provided are kits. Typically, a kit includes one or more pharmaceutical compositions as described herein, e.g., a pharmaceutical composition containing, e.g., a spray-dried dispersion as described in Examples 1-4, or the formulation described in Example 9. In certain embodiments, a kit can include one or more delivery systems, e.g., for delivering or administering the pharmaceutical composition as provided herein, and directions for use of the kit (e.g., instructions for treating a subject). In some embodiments, the kit can include a pharmaceutical composition as described herein and a label that indicates that the contents are to be administered to a subject with congenital adrenal hyperplasia. The actual dose of the compound of Formula (I), or pharmaceutically acceptable salt thereof, provided herein depends on the specific formulation, the weight of the patient, and on the condition to be treated.

EXAMPLES

Example 1: Spray-dried dispersion formulations containing the compound of Formula (I) and various polymers

Spray-dried dispersion formulations

A series of spray-dried dispersion (SDD) formulations containing the compound of Formula (I) and a polymer were prepared. The SDD formulations included: (1) 10% compound of Formula (I)/90% hydroxypropylmethylcellulose acetate succinate-L (HPMCAS-L); (2) 25% compound of Formula (I)/75% HPMCAS-L; (3) 40% compound of Formula (I)/60% HPMCAS-L; (4) 25% compound of Formula (I)/75% polyvinyl pyrrolidone vinyl acetate 64 (PVP/VA 64); (5) 25% compound of Formula (I)/60% Cabosil (fumed silica)/15% HPMCAS-L; (6) 25% compound of Formula (I)/75% HPMCAS-M; and (7) 25% compound of Formula (I)/75% methyl methacrylate copolymer (1:1) (Eudragit® L100).

The PVP/VA polymer was a copolymer of 1-vinyl-2-pyrrolidone and vinyl acetate with a ratio of 60:40 by weight 1-vinyl-2-pyrrolidone:vinyl acetate with an average molecular weight of 45,000-70,000 (copovidone, sold as Kollidon® VA 64, BASF, Florham Park, NJ). The HPMCAS was a mixture of acetic acid and monosuccinic acid esters of hydroxypropylmethyl cellulose that was either grade L (HPMCAS-L), with an acetyl content of 5-9%, a succinoyl content of 14-18%, a methoxyl content of 20-24%, and a hydroxypropoxy content of 5-9% (sold by Shin-Etsu, Japan); or grade M (HPMCAS-M), with an acetyl content of 7-11%, a succinoyl content of 10-14%, a methoxyl content of 21-25%, and a hydroxypropoxy content of 5-9% (sold by Shin-Etsu, Japan).

Dissolution performance

Dissolution performance of several of the SDD formulations described above was tested (see FIG. 1). 1000 μgA/mL of each SDD was tested in 0.5 wt% simulated intestinal fluid (SIF) in PBS, pH 6.5. Samples were tested at 5, 10, 20, 45, 90, and 1200 minutes. A lipid formulation containing 10% of the compound of Formula (I) was used as a control. The results are shown in Table 4, below.

Table 4. Dissolution data of various SDDs

Sample	C_{max90} (µg/mL)	AUC_{90} (min*µg/mL)	C_{max90} (µg/mL)	$Ultra_{90}$ (µg/mL)	C_{1200} (µg/mL)	$Ultra_{1200}$ (µg/mL)
2	762	66,080	743	210	671	166
4	322	27,330	306	109*	268	199
6	718	62,240	708	202	632	217
7	742	60,600	742	113*	674	194
Control	802	69,580	800	253	799	270

*Large variability between replicates, high value discarded

Non-sink dissolution

A membrane flux assay was performed (see, e.g., Stewart et al., Mol. Pharm. (2017) 14:2032-2046) and non-sink dissolution data was collected for several of the SDD formulations described above and compared to the compound of Formula (I) and several reference formulations, including a semi-solid lipidic formulation (Reference Formulation 1) and two self-emulsifying drug delivery system (SEDDS) formulations (Reference Formulations 2 and 3). The components of the Reference Formulations are shown in Table 5, below, and include, in addition to the compound of Formula (I), caprylic/capric triglyceride (Labrafac® Lipophile, Gattefossé, France); propylene glycol dicrapolate/dicaprate (Labrafac® PG, Gattefossé, France); oleoyl polyoxyl-6 glycerides (Labrafil® M 1944 CS, Gattefossé, France); polysorbate 20; polyoxyl castor oil (Kolliphor® RH 40, BASF, Germany); polyoxyl 15 hydroxystearate (Kolliphor® HS 15, BASF, Germany); lauroyl polyoxyl-32 glycerides (Gelucire® 44/14, Gattefossé, France); d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS); and diethylene glycol monoethyl ether (Transcutol®, Gattefossé, France).

Table 5. Reference formulations (capsules)

Formulation (mg/caps)	Ref. Formulation 1	Ref. Formulation 2	Ref. Formulation 3
Formula (I)	50.0	50.0	50.0
Labrafac® Lipophile	196.0	100.0	100.0
Labrafac® PG	102.0	-	-
Labrafil® M 1944 CS	-	135.0	46.0
Polysorbate 20	-	-	89.9
Kolliphor® RH 40	-	-	100.0
Kolliphor® HS 15	-	165.0	-
Gelucire® 44/14	95.0	-	-
TPGS	57.0	-	65.0
Transcutol®	-	50.0	50.0
Total	500.0	500.0	500.0

The assay measured the flux across simulated gastric and intestinal walls via UV spectroscopy (μDiss ProfilerTM, Pion Inc., Billerica, MA). Briefly, the assay was performed as follows. A vertical membrane flux cell consisting of a donor compartment and a receiver compartment, and separated by an Accurel PP 1E (55% porous, 100 μm thickness) polypropylene membrane (3M, Maplewood, MN) (FIG. 2), was impregnated with 50 μL of Pion GIT-0 lipid solution consisting of 20% w/w phospholipid dissolved into dodecane (Pion Inc., Billerica, MA) and attached to the receiver vessel. Both the donor and receiver compartments were agitated by magnetic stirring. The receiver compartment contained a plastic spacer and grating to elevate the stir bar above the membrane. Samples were introduced to the donor vessel by pre-weighing directly into the donor vessel and subsequently adding dissolution medium. Once the dissolution medium was added to the donor vessel, the receiver vessel was inserted into the donor vessel and suspended vertically 5 mm above the donor compartment by a plastic sleeve. For this assay, the simulated gastric (feed) media was 0.1 N HCl, pH 2 and included 200 μgA/mL of each SDD, and the simulated intestinal (receiver) media was 0.5 wt% SIF in PBS, pH 6.5 and included 100 μgA/mL of each SDD. The temperature for the assay was maintained at 44.5°C. UV probes (10 mm path length) connected to a Rainbow UV spectrometer (Pion Inc.) system were used to determine the apparent drug

concentration in the receiver vessels. Samples of the donor compartment were removed with a disposable pipet for centrifugation followed by HPLC and DLS analysis of the supernatant. The results are shown in FIG. 3 and Table 6, below.

Table 6. Non-sink dissolution data

Sample	C_{maxGB} (µg/mL)	$C_{max90\ IB}$ (µg/mL)	$AUC_{4\text{-}90IB}$ (min*µg/mL)	C_{90} (µg/mL)	$Ultra_{90}$ (µg/mL)	C_{1200} (µg/mL)
Formula (I)	0	1	10	0	0	3
1	6	80	6,800	80	79	90
2	17	74	6,240	73	73	86
4	6	4	200	4	5	36
5	23	55	3,180	55	54	83
6	35	71	6,070	71	77	83
Ref. Formulation 1	205	109	9,050	109	-	-
Ref. Formulation 2	249	120	10,160	120	-	-
Ref. Formulation 3	218	107	9,100	107	-	-

The membrane flux of 1 mg/mL gastric barrier/intestinal barrier (GB/IB) 0.5 wt% SIF doses of the compound of Formula (I) and spray-dried dispersions (2) 25% compound of Formula (I)/75% HPMCAS-L and (4) 25% compound of Formula (I)/75% PVP/VA 64 were also determined. The results are shown in FIG. 4 as receiver concentration vs. time and flux vs. time (smoothed derivative of receiver concentration x volume/surface area).

Example 2: Characterization of a spray-dried dispersion containing 25% of the compound of Formula (I) and 75% of a polyvinyl pyrrolidone vinyl acetate (PVP/VA) polymer

SDD stability screening

Several of the SDDs described in Example 1 were tested for chemical and physical stability. Wet SDD stability studies were performed, with samples stored at both 5°C and 25°C. Measurements were taken after 1 week and 2 weeks of storage. The results are shown in Table 7 below. The column with a retention time of 32.36 min correlates with the compound of Formula (I).

Table 7. Wet SDD stability data

Retention time (min)			11.04	16.79	17.26	30.94	32.26			
Relative retention time			0.34	0.52	0.53	0.96	1.00			
	Storage temp	**Timepoint**						**Total impurities**	**Potency (mgA/g)**	**Std Dev**
Ref. Std.						0.37	99.63	0.37		
Formula (I)						0.26	99.74	0.26		
Sample 1		initial		0.13	0.16	0.25	99.46	0.54	100	1.3
	5°C	1 week		0.03	0.03	0.26	99.69	0.31	99	0.0
		2 weeks		0.12	0.16	0.28	99.45	0.55	99	0.5
	25°C	1 week		0.03	0.04	0.26	99.67	0.33	99	0.3
		2 weeks		0.21	0.27	0.28	99.24	0.76	98	0.8
Sample 2		initial	<LOQ	0.07	0.08	0.26	99.59	0.41	247	0.3
	5°C	1 week	<LOQ	0.02	0.03	0.26	99.70	0.30	248	1.3
		2 weeks	<LOQ	0.22	0.27	0.27	99.24	0.76	246	0.3
	25°C	1 week	<LOQ	0.02	0.03	0.26	99.69	0.31	248	0.9
		2 weeks	<LOQ	0.23	0.28	0.26	99.23	0.77	246	1.4

LOQ = limit of quantification

Solution stability studies were also performed, with samples stored at both 5°C and 25°C. Measurements were taken after 1 week and 2 weeks of storage. The results are shown in Table 8 below. The column with a retention time of 32.36 min correlates with the compound of Formula (I).

Table 8. SDD solution stability data

Retention time (min)			31.51	32.26	
Relative retention time			0.97	1.00	
	Storage temp	**Timepoint**			**Total impurities**
Ref. Std.			0.74	99.26	0.74
Formula (I)			0.26	99.74	0.26
Sample 1		initial	0.33	99.67	0.33
	5°C	2 weeks	0.29	99.71	0.29
	25°C	2 weeks	0.38	99.62	0.38
Sample 2		initial	0.25	99.75	0.25
	5°C	2 weeks	0.26	99.74	0.26
	25°C	2 weeks	0.33	99.67	0.33

Stability studies were also performed for the SDD containing 25% of the compound of Formula (I) and 75% PVP/VA 64, with samples stored at both 5°C (closed with desiccant), 25°C (60% RH, closed with desiccant), and 30°C (65% RH, closed with desiccant). Measurements were taken after storage for 1 month, 2 months, 3 months, 6 months, and 12 months. No change in purity was observed after 12 months of storage. The results are shown in Table 9 below. The column with a retention time of 30.2 min correlates with the compound of Formula (I).

Table 9. SDD stability data

Retention time (min)			28.7	30.2		
Relative retention time			0.95	1.00		
	Storage conditions	**Timepoint**			**Total impurities**	**Potency (mgA/g)**
Crystalline Formula (I)			0.26	99.74	0.26	1001
Sample 4 (25% Formula (I):75% PVP/VA 64)		initial	0.26	99.74	0.26	245
	5°C (closed w/ desiccant)	1 month	0.25	99.75	0.25	247
		2 months	0.25	99.75	0.25	244
		3 months	0.26	99.74	0.26	246
		6 months	0.25	99.75	0.25	245
		12 months	0.25	99.75	0.25	248
	25°C/60% RH (closed with desiccant)	1 month	0.25	99.75	0.25	245
		2 months	0.25	99.75	0.25	247
		3 months	0.25	99.75	0.25	246
		6 months	0.25	99.75	0.25	242
		12 months	0.25	99.75	0.25	245
	30°C/65% RH (closed with desiccant)	1 month	0.25	99.75	0.25	249
		2 months	0.25	99.75	0.25	242
		3 months	0.25	99.75	0.25	246
		6 months	0.25	99.73	0.25	243
		12 months	0.25	99.75	0.25	242

While Samples 1 and 2 showed degradation after about 2 weeks of storage, the SDD containing 25% of the compound of Formula (I) and 75% PVP/VA 64 (Sample 4) was found to be both chemically and physically stable and was further screened and characterized as described below.

25% Formula (I)/75% PVP/VA 64 SDD process parameter screening manufacture Round 1

The 25% Formula (I)/75% PVP/VA 64 SDD was prepared on a Pharmaceutical Spray Dryer with 100 kg/hr drying gas capacity (PSD-1). The manufacturing summary is shown in Table 10, below.

Table 10. Manufacturing summary of process parameters

Formulation	25% Formula (I):75% PVP/VA 64
Solids Loading (wt%)	10
Batch Size (kg)	1.5
Solvent	Acetone
Atomizer (Pressure Swirl)	SK 80-16
Solution Flow-rate (g/min)	160
Atomization Pressure (psig)	480
Inlet Temperature (°C)	94
Outlet Temperature (°C)	40
Calculated Outlet Acetone Saturation (% RS)	6.2
Dry Yield (%)	73

Based on the 73% yield observed in the first round of process screening, three sprays were performed to investigate the effect of reducing solution throughput and outlet temperature on product yield. All sprays were conducted at a reduced flow-rate of 110 g/min. The outlet temperature was varied at 40°C (Lot A), 35°C (Lot B), and 30°C (Lot C). The outlet temperature was decreased while maintaining a low outlet acetone saturation to increase the difference between the chamber outlet temperature and the wet SDD T_g, thus improving product yields. The spray dryer chamber and outlet ductwork were cleaned between all manufactures. A manufacturing summary is shown in Table 11.

Table 11. Manufacturing summary for process parameters (1.5 kg batch size)

Description	Low Flow-Rate	Low Flow-Rate/Low Outlet Temperature	Low Flow-Rate/Lower Outlet Temperature
Lot	A	B	C
Solids Loading (wt%)	10	10	10
Batch Size (kg)	1.5	1.5	1.5
Solvent	Acetone	Acetone	Acetone
Atomizer (Pressure Swirl)	Steinen A75	Steinen A75	Steinen A75
Solution Flow-Rate (g/min)	110	110	110
Atomization Pressure (psig)	275	285	285
Inlet Temperature (°C)	79	72	63
Outlet Temperature (°C)	40	35	30
Calculated Outlet Acetone Saturation (% RS)	4.3	5.2	6.4
Calculated wet SDD T_g (°C)	72	71	69
Dry Yield (%)	55	80	43

The conditions used for Lot B were found to give the highest yield. One additional spray was then performed at the same processing conditions as Lot B while increasing the batch size from 1.5 kg to 3.5 kg to evaluate process consistency and to determine if product yield would continue to improve over time. The averaged process conditions for this lot are shown in Table 12.

Table 12. Manufacturing summary of process parameters (1.5 kg and 3.5 kg batch sizes)

Description	Low Flow-Rate/Low Outlet Temperature	Low Flow-Rate/Low Outlet Temperature/Larger Batch Size
Lot	B	D
Solids Loading (wt%)	10	10
Batch Size (kg)	1.5	3.5
Solvent	Acetone	Acetone
Atomizer (Pressure Swirl)	Steinen A75	Steinen A75
Solution Flow-Rate (g/min)	110	110
Atomization Pressure (psig)	285	285
Inlet Temperature (°C)	72	72
Outlet Temperature (°C)	35	35
Calculated Outlet Acetone Saturation (% RS)	5.2	5.2
Calculated wet SDD T_g (°C)	71	71
Dry Yield (%)	80	84

The 1.5 kg batch size (Lot D) was sprayed with an 84% yield compared to the 80% yield of the 3.5 kg batch (Lot B).

25% Formula (I)/75% PVP/VA 64 SDD process parameter screening characterization

The 25% Formula (I)/75% PVP/VA 64 SDDs manufactured to evaluate processing parameters were characterized for powder properties, performance, and physical and chemical properties. Testing included particle size distribution by Malvern, determination of bulk and tapped density, microcentrifuge dissolution, modulated differential scanning calorimetry (mDSC), powder x-ray diffraction (PXRD), scanning electron microscope (SEM), and assay and related substances. The results did not show any significant differences between the lots.

The particle size distribution (PSD) and tabulated powder properties data of the 25% Formula (I)/75% PVP/VA 64 SDDs are shown in Table 13. All 25% Formula (I)/75% PVP/VA 64 SDDs were observed to have a very similar PSD with a D_{50} of approximately 16 µm. All 25% Formula (I)/75% PVP/VA 64 SDDs were observed to have low bulk and tapped densities.

Table 13. Powder properties of process parameter screening PVP/VA-64 SDDs

Sample	Lot	D_{10} (µm)	D_{50} (µm)	D_{90} (µm)	$D_{(3,2)}$ (µm)	$D_{(4,3)}$ (µm)	Span	Bulk density (g/mL)	Tapped density (g/mL)
40°C Outlet	A	5	15	34	8	17	1.93	0.12	0.25
35°C Outlet	B	5	16	36	9	19	1.97	0.11	0.23
30°C Outlet	C	5	15	32	7	17	1.86	0.12	0.27
35°C Outlet, 3.5 kg batch	D	5	16	38	9	19	1.98	0.12	0.24

The 3.5 kg batch size lot was analyzed and compared to process parameter Lot A. Dissolution performance was similar for each of these lots. Dissolution was rapid to C_{max} and high free drug was sustained through 90 minutes. These data are shown in Table 14.

Table 14. Dissolution performance of Lot A (1.5 kg batch size) vs. Lot D (3.5 kg batch size)

Sample	C_{max90} (µg/mL)	AUC_{90} (min*µg/mL)	C_{90} (µg/mL)	$Ultra_{90}$ (µg/mL)
Lot A	447	37,740	437	319
Lot D	437	37,120	433	301

The 25% Formula (I)/75% PVP/VA 64 SDDs were also evaluated by DSC, PXRD, and SEM. The DSC thermograms showed a single T_g at 84°C, indicating homogeneous dispersions. PXRD diffractograms showed no evidence of crystals in the SDDs. SEM images showed inflated sphere morphology with some broken particles and some very small particles.

Additional testing on Lot B was carried out, which included assessing the chemical/physical stability of both spray solution and SDD prior to secondary drying (wet

SDD) to establish maximum in-process hold times. Residual acetone concentration as a function of secondary drying time in a convection tray dryer was also evaluated to nominate tray drying conditions to ensure the SDD is dried below International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines for acetone.

Residual acetone content as a function of drying time was assessed by drying wet SDD in a tray dryer and collecting samples over a 24-hour period. Wet SDD was dried at 40°C/15% relative humidity (RH) and was observed to dry below ICH acetone guidelines (0.5 wt%, 5000 ppm) by four hours.

Spray solution hold time was determined by making up a representative solution that contained 2.5 wt% compound of Formula (I), 7.5 wt% PVP/VA 64, and 90 wt% acetone. These solutions were analyzed initially for related substances. and then aged at 5°C and 25°C. Aliquots were taken and analyzed for related substances periodically for 14 days. Results showed no change in impurity profile at either condition through 14 days.

Wet SDD was analyzed for impurities after storage at 5°C and 25°C for 1 and 2 weeks and compared to the impurity profiles of the ingoing compound of Formula (I) and the SDD that was secondarily dried immediately after spray drying. The impurity profiles were similar to that of the initial dried sample and the ingoing compound of Formula (I) through 2 weeks of storage.

The wet SDD stability samples were characterized for physical stability by DSC, PXRD, and SEM. DSC thermograms showed a single T_g at 81°C, indicative of a homogeneous dispersion with no phase separation. The PXRD diffractograms did not show any evidence of crystals after storage at either condition. SEM images showed a typical morphology of mostly inflated spheres with some broken particles.

Example 3: Preparation of a 1000 g batch of a spray-dried dispersion containing 25% of the compound of Formula (I) and 75% PVP/VA 64

A 1000 g batch of the spray-dried dispersion containing 25% of the compound of Formula (I) and 75% PVP/VA 64 was prepared as described in Example 2 for the 1.5 kg and 3.5 kg batches. Briefly, acetone (90% (w/w) of the total mixture) was added to the mixing tank followed by the addition of 250.0 g of the compound of Formula (I) (2.5% (w/w) of the total mixture). The mixture was mixed for 30 minutes in the dark at a temperature range of 15°C to 27°C. At the end of the mixing period, the solution was clear and free of undissolved solids.

The PVP/VA 64 (750.0 g, 7.5% (w/w) of the total mixture) was then added and the mixture was stirred for an additional 30 minutes in the dark at a temperature range of 15°C to 27°C. At the end of the mixing period, the solution was clear and free of undissolved solids.

The solution was pumped and atomized in a drying chamber. The spray-dried dispersions were prepared in a Pharmaceutical Spray Dryer with 100 kg/hr drying gas capacity (PSD-1). The inlet temperature was set at 75°C (varied between 60°C-90°C). The outlet temperature was set at 35°C (varied between 32°C-38°C). The feed pressure was set at 280 psig (varied between 230-330 psig). The feed rate was set at 110 g/min (varied between 90-130 g/min). The spray dried powder was then dried in a convection tray dryer with a bed depth of ≤ 2.5 cm at 40°C (±5°C) and 15% relative humidity (±10%) for 24 hours under amber light. The residual acetone after drying was < 0.5 wt% (5000 ppm). FIG. 5 is a flow diagram of the manufacturing process.

Example 4: Preparation of spray-dried dispersion formulations of the compound of Formula (I) for clinical use

The spray-dried dispersion (SDD) containing 25% compound of Formula (I) and 75% PVP/VA 64, prepared as described above, was formulated as a suspension or a capsule for clinical use.

Suspension preparation

A suspension that contained 50 mg of the SDD was prepared as follows. A 30 mL amber dosing bottle was tared on a balance. 200.0 mg SDD (50 mgA) ± 5% was then weighed into the dosing bottle. Using a 10-mL syringe, 5.0 mL of water (purified, USP) was added to the dosing bottle and the bottle was capped and shaken moderately for 30 seconds. The SDD suspension was stored in an amber vial at 2-8°C prior to use, and dosed within 24 hours of preparation.

Capsule preparation

An empty hard gelatin capsule, size 0 (Capsugel, Morristown, NJ), was placed on a balance and the weight was recorded. 200.0 mg SDD (50 mgA) ± 5% was then weighed onto weigh paper or an equivalent. All contents were transferred to the capsule using a ProFunnel device for Size 0 capsules. The filled capsule was placed on the balance and the weight was recorded. The weight of the empty capsule was subtracted from the filled weight, ensuring that the weight of the SDD within the capsule was 200.0 mg SDD ± 5%, or from 190.0 mg to 210.0 mg. The capsule was securely closed with the head, assuring it clicked into place. The capsules

were stored in an amber vial at 2-8°C prior to use, and were dosed within 24 hours of preparation.

Example 5: Dog relative bioavailability and food effect study

Four spray-dried dispersions (SDDs), formulated in 0.25% methylcellulose as a suspension, were prepared: (1) 25% compound of Formula (I)/75% HPMCAS-L; (2) 10% compound of Formula (I)/90% HPMCAS-L; (3) 25% compound of Formula (I)/75% methyl methacrylate copolymer (1:1) (Eudragit® L100); and (4) 25% compound of Formula (I)/75% PVP/VA 64. A clinical capsule formulation was prepared as a reference formulation (Reference Formulation 1 from Table 5, above).

Dogs (two cohorts, six dogs in each) were dosed in six sessions, including fasted state sessions and fed sessions (high fat diet), with 50 mg dose of one of the SDDs or reference per dog in a 3-way crossover design. Each session had a 3-day washout in between. All formulations were well tolerated. The study design is shown in Table 15, below.

Table 15. Study design

Cohort 1	**Session 1 (Fasted)**	**Session 2 (Fed)**	**Session 3 (Fasted)**	**Session 4 (Fed)**	**Session 5 (Fasted)**	**Session 6 (Fed)**
Dog 1001	Reference	Reference	SDD 1	SDD 1	SDD 2	SDD 2
Dog 1002	Reference	Reference	SDD 1	SDD 1	SDD 2	SDD 2
Dog 2001	SDD 1	SDD 1	SDD 2	SDD 2	Reference	Reference
Dog 2002	SDD 1	SDD 1	SDD 2	SDD 2	Reference	Reference
Dog 3001	SDD 2	SDD 2	Reference	Reference	SDD 1	SDD 1
Dog 3002	SDD 2	SDD 2	Reference	Reference	SDD 1	SDD 1
Cohort 2	**Session 1 (Fasted)**	**Session 2 (Fed)**	**Session 3 (Fasted)**	**Session 4 (Fed)**	**Session 5 (Fasted)**	**Session 6 (Fed)**
Dog 4001	Reference	Reference	SDD 3	SDD 3	SDD 4	SDD 4
Dog 4002	Reference	Reference	SDD 3	SDD 3	SDD 4	SDD 4
Dog 5001	SDD 3	SDD 3	SDD 4	SDD 4	Reference	Reference
Dog 5002	SDD 3	SDD 3	SDD 4	SDD 4	Reference	Reference
Dog 6001	SDD 4	SDD 4	Reference	Reference	SDD 3	SDD 3
Dog 6002	SDD 4	SDD 4	Reference	Reference	SDD 3	SDD 3

The area under the plasma concentration versus time curve from 0 hours extrapolated to infinity ($AUC_{0-\infty}$), maximum plasma concentration (C_{max}), the apparent terminal half-life ($t_{½}$), and the time to achieve maximum plasma concentration (t_{max}) were calculated. Results are shown in Table 16, below, and in FIGS. 6A and 6B.

Table 16. Pharmacokinetic results

Cohort	Form	Fasted [Mean (%CV)]		Fed [Mean (%CV)]		Ratio of Fed/Fasted [Mean (%CV)]	
		C_{max} (ng/mL)	AUC_{inf} (hr*ng/mL)	C_{max} (ng/mL)	AUC_{inf} (hr*ng/mL)	C_{max}	AUC
1 (N=4)*	Ref	652 (55.5)	5170 (69.4)	3650 (19.5)	20100 (22)	6.9 (48)	5.5 (69.2)
	SDD 1	524 (62.7)	2870 (62.9)	5760 (25.6)	22800 (14.6)	15.4 (66.9)	8 (89.6)
	SDD 2	487 (45.7)	3950 (26.4)	4220 (27.4)	18100 (26.8)	16.5 (40.7)	6.3 (34.6)
2 (N=6)	Ref	854 (40.2)	5520 (59.1)	4220 (27.4)	18100 (26.8)	5.8 (46.5)	4.1 (49.2)
	SDD 3	453 (28.3)	2830 (21.6)	5320 (13.5)	23800 (26.1)	12.6 (30.3)	8.6 (22.2)
	SDD 4	353 (20.1)	1840 (21.9)	3060 (20.7)	17200 (20.5)	8.9 (29.3)	8.4 (46.1)

*Animals 2001 and 2002 were excluded from summary statistics due to emesis in all 3 fed sessions, which resulted in notably lower exposures

The results showed that $t_{1/2}$ and t_{max} were similar among the formulations, and comparable between the fed and fasted states. Under the fed state with a high fat meal, exposures increased and inter-animal variability decreased. The food effect was more notable with the spray-dried dispersion formulations, especially for peak exposure (C_{max}).

Compared to the reference form, SDD 4 (25% compound of Formula (I)/75% PVP/VA 64) appeared to have lower inter-animal variability, lower exposures under the fasted state, and slightly lower C_{max} but relatively comparable AUC in the fed state.

Example 6: Phase 1 study to evaluate the pharmacokinetics, effect of food on pharmacokinetics, and safety of the compound of Formula (I) in healthy adult subjects

The present study was designed to evaluate the pharmacokinetics (PK) of the compound of Formula (I) as well as to evaluate the effect of a fed condition on the PK of the compound of Formula (I). The 50 mg dose was chosen for this study because it was within the tested dose range in completed Phase 1 and Phase 2 trials and was well tolerated in those studies. The objectives of the study were: to evaluate the PK of the compound of Formula (I) 50 mg in healthy adult subjects; to evaluate the effect of food on the PK of the compound of Formula (I) 50 mg; and to evaluate the safety and tolerability of the compound of Formula (I) 50 mg.

Study Design

This was a Phase 1, open-label, randomized, 2-period crossover study of the PK and the effect of food on the PK of the compound of Formula (I) in 16 healthy male and female adult subjects, 18 to 55 years of age.

After providing informed consent, subjects were screened for eligibility to participate in the study up to 28 days prior to Day 1 of treatment period 1. Eligible subjects were admitted to the clinical unit on Day -1 and randomized to 1 of the 2 treatment sequences (16 subjects [8 males and 8 females]; see Table 17, below). On Day 1 of each treatment period, subjects received a single dose of the compound of Formula (I) 50 mg under fasted or fed conditions. There were 21 days between doses.

Table 17. Treatment sequences

Treatment sequence	Treatment period 1	Treatment period 2
1	Formula (I) – fasted	Formula (I) – fed
2	Formula (I) – fed	Formula (I) – fasted

Subjects were required to fast for at least 4 hours before check-in on Day -1. In the fasted condition, subjects were required to fast overnight for at least 10 hours prior to dosing and continued to fast for an additional 4 hours after dosing. In the fed condition, subjects were required to fast overnight for at least 10 hours and then ingest a liquid dietary supplement with study drug (liquid dietary supplement was consumed within 30 minutes) and not consume any other food for 4 hours after dosing. During both treatment periods, water was not permitted for

1 hour before dosing until 2 hours after dosing except for the water/liquid dietary supplement provided for study drug dosing. Vanilla-flavored Ensure Plus® was used as the liquid dietary supplement.

On Day 1 of each treatment period, subjects were dosed with the compound of Formula (I) 50 mg. Blood samples were collected for PK analysis over a period of 36 hours during the in-house stay. Subjects remained in the unit on the day of dosing and were discharged on Day 2 of each treatment period, following completion of all required procedures. On the mornings of Days 8 and 15 of each treatment period, subjects returned to the clinical unit on an outpatient basis for PK blood sample collection and safety assessments. On Day 21 of treatment period 1, subjects arrived at the site and had Day 21 assessments completed in the evening, and they stayed overnight at the site and began Day 1 of treatment period 2 the following day. A final follow-up study visit was conducted on Day 22 of treatment period 2 (21 ± 2 days after treatment period 2 dosing) or upon early termination.

During each treatment period, blood samples for PK analysis were collected within 45 minutes before dosing, and at approximately 30 minutes, and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, 24, 36, 168, 336, and 504 hours after dosing.

Safety assessments (including clinical safety laboratory tests, vital sign measurements, physical examinations, and electrocardiograms (ECGs)) were conducted at scheduled times throughout the study. Adverse events (AEs) and the use of concomitant medications were monitored throughout the study. FIG. 7 illustrates the study design.

Test product, dose and mode of administration

The compound of Formula (I) was supplied as an encapsulated, lipidic semi-solid containing 50 mg of the compound of Formula (I) as free base equivalent for oral administration. Subjects swallowed a single capsule with approximately 240 mL of water in the fasted condition. Subjects swallowed a single capsule with a liquid dietary supplement (Ensure Plus® [237 mL container]) with up to an additional 120 mL of water in the fed condition.

Duration of treatment

The duration of study participation for each adult subject was approximately 10 weeks, including up to 28 days of screening, 2 days of dosing separated by 21 days, and a final follow-up study visit 21 days after receiving the last dose of study drug during treatment period 2.

Criteria for Evaluation

Pharmacokinetics

The following plasma PK parameters were calculated for the compound of Formula (I):

- Area under the plasma concentration versus time curve from 0 hours to last measurable concentration ($AUC_{0\text{-}tlast}$)
- Area under the plasma concentration versus time curve from 0 to 24 hours ($AUC_{0\text{-}24}$)
- Area under the plasma concentration versus time curve from 0 hours extrapolated to infinity ($AUC_{0\text{-}\infty}$)
- Maximum plasma concentration (C_{max})
- Time to achieve maximum plasma concentration (t_{max})
- Delay time between time of dosing and time of appearance of measurable test article (T_{lag})
- Apparent terminal half-life ($t_{½}$)
- Apparent terminal rate constant (λz)
- Apparent mean residence time (MRT)
- Molar AUC ratio of the hydroxylated metabolite of the compound of Formula (I) to the parent drug the compound of Formula (I)

The following plasma PK parameters were calculated only for the compound of Formula (I):

- Apparent systemic clearance after oral administration (CL/F)
- Apparent volume of distribution during terminal phase after oral administration (Vz/F)

Safety

Safety was monitored throughout the study and included the following assessments:

- Adverse events (AEs)
- Clinical laboratory tests (hematology, coagulation, clinical chemistry, and urinalysis)
- Vital sign measurements (including orthostatic blood pressure and pulse rate)
- Physical examinations
- 12-lead electrocardiograms (ECGs)

Statistical Methods

Pharmacokinetic parameters were calculated using noncompartmental methods and summarized by condition (fed or fasted) using descriptive statistics. Two-sided 90% confidence intervals were calculated for the ratio under the fed condition vs. under the fasted condition for $AUC_{0-\infty}$, $AUC_{0-tlast}$, and C_{max} for the compound of Formula (I) and the hydroxylated metabolite of the compound of Formula (I).

Safety data were summarized with descriptive statistics.

Pharmacokinetics

Pharmacokinetic assessments

PK plasma samples for analyses of the compound of Formula (I) and the hydroxylated metabolite of the compound of Formula (I) were collected at the following times during each treatment period:

- Day 1: within 45 minutes before dosing, and at approximately 30 minutes, and 1, 2, 3, 4, 5,6, 7, 8, 9, 10, 12, and 16 hours after dosing.
- Day 2: approximately 24 and 36 hours after dosing.
- Day 8: approximately 168 hours after dosing.
- Day 15: approximately 336 hours after dosing.
- Approximately 504 hours after dosing (for treatment period 1, this sample was collected in the morning at least 30 minutes prior to the predose sample on Day 1 of treatment period 2).
- Final study visit for subjects who terminate early: 1 sample.

Blood samples on Day 1 were collected within 5 minutes of the scheduled sampling times (other than the predose sample). Blood samples on Days 2, 8, and 15 were collected within 2 hours of the scheduled sampling time. The 504 hour blood sample for treatment period 2 had a ± 2-day window. A PK sample was to be collected from subjects who terminated early. The exact time of sampling in hour and minutes was recorded.

Bioanalytical methods

Plasma samples were analyzed for the compound of Formula (I) and for its hydroxylated metabolite by inVentiv Health, Princeton NJ, in compliance with Good Laboratory Practice (GLP) and relevant Standard Operating Procedures (SOPs).

The concentrations of the compound of Formula (I) and the hydroxylated metabolite of the compound of Formula (I) were quantified in plasma samples according to validated

methods using tandem mass spectrometry in positive ion mode. This method was validated for the analysis of the compound of Formula (I) and the hydroxylated metabolite of the compound of Formula (I) in 25.0 µL dipotassium ethylenediaminetetraacetic acid (K_2-EDTA) human plasma samples over concentration ranges of 5.00 to 2500 ng/mL and 0.500 and 250 ng/mL, respectively. All analytical results were within acceptable limits. Incurred sample reanalysis (ISR) was successfully conducted in this study for both analytes.

Results

Pharmacokinetic results

Eight male and eight female subjects were enrolled. The mean age was 37.1 years (range, 21 to 55 years). The majority of subjects were White (93.8%) and of Hispanic ethnicity (81.3%). The mean weight at screening was 160.28 lbs (range, 102.0 to 222.2 lbs) and mean BMI was 25.50 kg/m^2 (range, 20.7 to 30.5 kg/m^2). The randomization was well balanced with respect to demographics and baseline characteristics.

All 16 subjects were included in the safety analysis set. No subjects were excluded from the safety analysis set and no subject had his or her PK data excluded from analysis.

The mean plasma concentration versus time profiles for the compound of Formula (I) under fasted and fed conditions are presented in FIGS. 8A and 8B, respectively. The compound of Formula (I) was slowly absorbed after oral administration in both fasted and fed conditions. Mean plasma concentrations were lower in the fasted than in the fed condition.

PK parameters for the compound of Formula (I) after treatment with the compound of Formula (I) under fasted and fed conditions are summarized in Table 18, below, where AUC_{0-24}=area under the plasma concentration vs. time curve from 0 to 24 hours, $AUC_{0-tlast}$=AUC from 0 hours to last measurable concentration, $AUC_{0-\infty}$=AUC from 0 hours extrapolated to infinity, CL/F=apparent systemic clearance after oral administration, CV=coefficient of variation, C_{max}=maximum plasma concentration, CV(%)=coefficient of variation, max=maximum, min=minimum, MRT=apparent mean residence time, PK=pharmacokinetic, SD=standard deviation, $t_{½}$=apparent terminal half-life, T_{lag}=delay time between time of dosing and time of appearance of measurable test article, t_{max}=time to maximum plasma concentration, VZ/F=apparent volume of distribution during the terminal phase after oral administration.

The PK data for t_{max}, T_{lag}, $t_{½}$, MRT, and Vz/F were rounded to 2 significant figures and all other parameters (AUC_{0-24}, $AUC_{0-tlast}$, $AUC_{0-\infty}$, C_{max}, and CL/F) were rounded to 3

significant figures. The last significant figure was rounded up if the digit to the right of it was ≥5, and was rounded down if the digit to the right of it was ≤4.

Table 18. Summary of Formula (I) PK parameters (safety analysis set)

Parameter Statistic	Fasted Formula (I) (50 mg) (N=16)	Fed Formula (I) (50 mg) (N=15)
$AUC_{0\text{-}24}$ (ng×hr/mL) Mean (SD) Geometric CV%	 5590 (2230) 32.7	 9950 (2540) 26.2
$AUC_{0\text{-}tlast}$ (ng×hr/mL) Mean (SD) Geometric CV%	 8020 (5110) 53.6	 16200 (5450) 39.7
$AUC_{0\text{-}\infty}$ (ng×hr/mL) Mean (SD) Geometric CV%	 9440 (2990) [n=7] 39.7	 17800 (4990) [n=12] 28.1
C_{max} (ng/mL) Mean (SD) Geometric CV%	 731 (301) 36.6	 1550 (392) 24.2
t_{max} (hours) Median (min, max)	 6.0 (3.0, 6.0)	 5.0 (3.0, 6.0)
T_{lag} (hours) Mean (SD)	 0.63 (0.22)	 0.94 (0.37)
$t_{½}$ (hours) Mean (SD) Geometric CV%	 33 (17) [n=7] 99	 42 (6.8) [n=12] 15
MRT (hours) Mean (SD) Geometric CV%	 28 (11) [n=7] 49	 30 (5.0) [n=12] 16
CL/F (L/hr) Mean (SD) Geometric CV%	 6.0 (2.7) [n=7] 40	 3.0 (0.82) [n=12] 28
VZ/F (L) Mean (SD) Geometric CV%	 240 (120) [n=7] 67	 180 (57) [n=12] 30

As seen in Table 18, above, administration of the compound of Formula (I) 50 mg under fed compared with fasted conditions resulted in a higher mean C_{max} of the compound of Formula (I) (approximately 2-fold higher; 1550 vs. 731 ng/mL), a longer $t_{½}$ (42 vs. 33 hours), a slightly shorter median t_{max} (5.0 vs. 6.0 hours), and a higher mean $AUC_{0\text{-}\infty}$ (approximately 2-fold higher; 17800 vs. 9440 ng×hr/mL). The compound of Formula (I) geometric mean ratios for C_{max} and $AUC_{0\text{-}tlast}$ for fed vs. fasted conditions were 218.6% and 215.2%, respectively, indicating that the compound of Formula (I) absorption was approximately 2-fold greater when

administered with food. The upper and lower 90% confidence interval (CI) bounds for both C_{max} (187.4% and 255.1%, respectively) and $AUC_{0\text{-tlast}}$ (182.9% and 253.1%, respectively) were outside of the "no-effect" range of 80.00% to 125.00%, indicating that there was a food effect on the compound of Formula (I) exposure.

Frequency distribution of T_{lag} and t_{max} values are presented in Table 19 and Table 20, respectively. Spaghetti plots for the compound of Formula (I) $AUC_{0\text{-tlast}}$, $AUC_{0\text{-}\infty}$ and C_{max} are shown in FIGS. 9A, 9B, and 9C, respectively.

Table 19. Frequency distribution of plasma Formula (I) T_{lag} values by treatment (safety analysis set)

T_{lag} (hr)	**Statistic**	**Fasted (Formula (I) 50 mg) (N=16)**	**Fed (Formula (I) 50 mg) (N=15)**
0.50	n (%)	11 (68.8%)	3 (20.0%)
0.53	n (%)	1 (6.3%)	0 (0.0%)
0.55	n (%)	0 (0.0%)	1 (6.7%)
1.00	n (%)	4 (25.0%)	8 (53.3%)
1.02	n (%)	0 (0.0%)	1 (6.7%)
1.03	n (%)	0 (0.0%)	1 (6.7%)
2.00	n (%)	0 (0.0%)	1 (6.7%)

Table 20. Frequency distribution of plasma Formula (I) T_{max} values by treatment (safety analysis set)

T_{max} (hr)	Statistic	Fasted (Formula (I) 50 mg) (N=16)	Fed (Formula (I) 50 mg) (N=15)
3.00	n (%)	1 (6.3%)	2 (13.3%)
4.02	n (%)	1 (6.3%)	0 (0.0%)
5.00	n (%)	4 (25.0%)	8 (53.3%)
5.03	n (%)	0 (0.0%)	1 (6.7%)
5.05	n (%)	0 (0.0%)	1 (6.7%)
6.00	n (%)	10 (62.5%)	2 (13.3%)
6.02	n (%)	0 (0.0%)	1 (6.7%)

The compound of Formula (I) was slowly absorbed after oral administration in the fasted and fed conditions. In the fasted condition, the mean compound of Formula (I) C_{max} was approximately 53% lower than in the fed condition (731 vs. 1550 ng/mL). Due to a prolonged elimination phase, $t_{½}$ values and therefore, $AUC_{0-\infty}$ values could not be determined for some the compound of Formula (I) concentration-time profiles. Mean $AUC_{0-\infty}$ was approximately 47% lower in the fasted condition than in the fed condition (9440 vs. 17800 ng×hr/mL) for those subjects for whom $AUC_{0-\infty}$ could be determined. Mean $AUC_{0-tlast}$ was approximately 50% lower in the fasted condition than in the fed condition (8020 vs. 16200 ng×hr/mL). Median t_{max} was slightly longer in the fasted condition than in the fed condition (6.0 vs. 5.0 hours) and mean $t_{½}$ was shorter in the fasted condition than in the fed condition (33 vs. 42 hours) for those subjects for whom a $t_{½}$ could be determined. Variability in the compound of Formula (I) PK (geometric CV%) for AUC, C_{max}, $t_{½}$, and MRT was lower in the fed condition compared with the fasted condition.

The geometric mean ratios and associated 90% CIs for $AUC_{0-tlast}$ and C_{max} for the compound of Formula (I) after treatment with the compound of Formula (I) for the fed vs. fasted condition are provided in Table 21, below, where $AUC_{0-tlast}$= AUC from 0 hours to last measurable concentration, C_{max}=maximum plasma concentration, and PK=pharmacokinetic.

Table 21. Formula (I) geometric mean ratios for PK exposure parameters under fed vs. fasted conditions (safety analysis set)

Parameter	Ratio[a] (%) (Fed vs. Fasted Condition)	90% Confidence Interval[b]
$AUC_{0\text{-tlast}}$ (ng×hr/mL)	215.2	182.9, 253.1
C_{max} (ng/mL)	218.6	187.4, 255.1

[a] Ratio of geometric least-squares means was based on a mixed model using log-transformed (base 10) data.

[b] The 90% confidence interval for geometric mean ratio was based on least-squares means using log-transformed (base 10) data.

The compound of Formula (I) geometric mean ratios for C_{max} and $AUC_{0\text{-tlast}}$ for the fed vs. fasted conditions were 218.6% and 215.2%, respectively, indicating that the compound of Formula (I) absorption was approximately 2-fold greater when administered with food. The upper and lower 90% CI bounds for both C_{max} (187.4%, 255.1%) and $AUC_{0\text{-tlast}}$ (182.9%, 253.1%) were outside of the "no-effect" range of 80.00% to 125.00%, indicating that there was a food effect on the compound of Formula (I) exposure. Due to the missing $AUC_{0-\infty}$ values, the food effect on overall exposure was not assessed using $AUC_{0-\infty}$ values.

Conclusion

Administration of the compound of Formula (I) 50 mg under fed compared with fasted conditions resulted in a higher mean C_{max} of the compound of Formula (I) (approximately 2-fold higher; 1550 vs. 731 ng/mL), a longer $t_{½}$ (42 vs. 33 hours), a slightly shorter median t_{max} (5.0 vs. 6.0 hours), and a higher mean $AUC_{0-\infty}$ (approximately 2-fold higher; 17800 vs. 9440 ng×hr/mL). The compound of Formula (I) geometric mean ratios for C_{max} and $AUC_{0\text{-tlast}}$ for fed vs. fasted conditions were 218.6% and 215.2%, respectively, indicating that the compound of Formula (I) absorption was approximately 2-fold greater when administered with food. The upper and lower 90% CI bounds for both C_{max} (187.4%, 255.1%) and $AUC_{0\text{-tlast}}$ (182.9%, 253.1%) were outside of the "no-effect" range of 80.00% to 125.00%, indicating that there was a food effect on the compound of Formula (I) exposure. Similar results were observed with the hydroxylated metabolite of the compound of Formula (I). Overall, these results indicate that the compound of Formula (I) 50 mg was well tolerated in healthy subjects when administered under fasted or fed conditions and that the total AUC and C_{max} were increased when the compound of Formula (I) was taken with food.

Example 7: Phase 1 study to evaluate the relative bioavailability, effect of food on pharmacokinetics, and safety of formulations of the compound of Formula (I) in healthy adult subjects

A Phase 1 study to compare the relative bioavailability of a 50 mg dose of different formulations of the compound of Formula (I) as well as to evaluate the effect of fasting and fed conditions on the pharmacokinetics (PK) of the compound of Formula (I) was designed. The 50 mg dose was chosen for this study because it is within the tested dose range in completed Phase 1 and Phase 2 trials and was well tolerated in those studies. The objectives of the study are: to evaluate the PK and compare the relative bioavailability of the compound of Formula (I) 50 mg formulations in healthy adult subjects; to evaluate the effect of food on the PK of the compound of Formula (I) 50 mg formulations; and to evaluate the safety and tolerability of the compound of Formula (I) 50 mg formulations.

Study Design

This is a Phase 1, open-label, randomized, three-period crossover study of the relative bioavailability and the effect of food on the PK of the compound of Formula (I) 50 mg in healthy adult subjects. During treatment period 1 and treatment period 2, subjects will receive a single dose of the compound of Formula (I) 50 mg administered as an encapsulated, lipidic semi-solid (reference) and 1 of 2 different spray-dried dispersion (SDD) test formulations (suspension or capsule) under fed conditions, and during treatment period, three subjects will receive the same SDD test formulation under fasted conditions.

A total of 36 healthy adult subjects will be randomized to 1 of 4 treatment sequences (9 subjects per sequence; approximately equal distribution of males and females per sequence; see Table 22, below). There will be 21 days between each dose.

Table 22. Treatment sequences

Treatment sequence	Treatment period 1 (Fed)	Treatment period 2 (Fed)	Treatment period 3 (Fasted)
1	Reference	SDD suspension	SDD suspension
2	SDD suspension	Reference	SDD suspension
3	Reference	SDD capsule	SDD capsule
4	SDD capsule	Reference	SDD capsule

After providing informed consent, subjects will be screened for eligibility to participate in the study. Screening will begin up to 28 days prior to Day 1 of treatment period 1. Eligible subjects will be admitted to the clinical unit on Day -1, and randomized to 1 of the 4 treatment sequences on Day 1 of treatment period 1. During treatment periods 1 and 2, subjects will fast overnight for at least 10 hours and then ingest a liquid dietary supplement (vanilla-flavored Ensure Plus®, 237 mL container) with the study drug and not consume any other food for 4 hours after dosing. During treatment period 3, subjects will fast overnight for at least 10 hours prior to dosing and continue to fast for an additional 4 hours after dosing. During all treatment periods, water will not be permitted for 1 hour before dosing until 2 hours after dosing except for the water/liquid dietary supplement provided for study drug dosing.

On Day 1 of each treatment period, subjects will be dosed with the compound of Formula (I) 50 mg and have blood samples collected for PK analysis. Subjects will complete a taste satisfaction questionnaire after study drug ingestion on Day 1 of treatment period 3 (only for subjects who receive the SDD suspension under the fasted condition). Subjects will remain in the unit on the day of dosing and will be discharged on Day 2 of each treatment period, following completion of all required procedures, including collection of the 36-hour PK sample. On the mornings of Days 8 and 15 of each treatment period, subjects will return to the clinical unit on an outpatient basis for PK blood sample collection and safety assessments. On Day 21 of treatment period 1 and treatment period 2, subjects will arrive at the site and have Day 21 assessments completed, and they will stay overnight at the site and begin Day 1 of treatment period 2 or treatment period 3 the following day. A final follow-up study visit will be conducted on Day 22 of treatment period 3 (21 ± 2 days after treatment period 3 dosing) or upon early termination.

Blood samples for PK analysis and safety assessments will be collected/conducted at scheduled times throughout the study. The study design schematic is shown in FIG. 10.

Duration of treatment

The expected duration of study participation for each healthy adult subject will be approximately 13 weeks, including up to 28 days of screening, 3 doses each separated by 21 days, and a final follow-up study visit 21 days after receiving the last dose of study drug during treatment period 3.

Test product, dose, and mode of administration

The compound of Formula (I) will be supplied as two different test formulations for oral administration: as powder in bottles for constitution into a suspension (20 mL) and as

powder-filled capsules. The compound of Formula (I) test formulations will contain 50 mg of the compound of Formula (I) as free base equivalent. Subjects must swallow the study drug with a liquid dietary supplement (Ensure Plus® [237 mL container]) with an additional 100 mL of water (SDD capsule formulation) or with an additional 80 mL of water (SDD suspension formulation) during treatment period 1 or 2. Subjects must swallow the study drug with 330 mL of water (SDD capsule formulation) or 310 mL of water (SDD suspension formulation) during treatment period 3.

Reference therapy, dose, and mode of administration

The compound of Formula (I) reference formulation (encapsulated, lipidic semi-solid formulation) will be supplied as capsules for oral administration. The compound of Formula (I) reference capsules will contain 50 mg of the compound of Formula (I) as free base equivalent. Subjects must swallow a single capsule with a liquid dietary supplement (Ensure Plus [237 mL container]) with an additional 100 mL of water during treatment period 1 or 2.

Criteria for Evaluation

Pharmacokinetics

Blood samples for assessment of plasma concentrations of the compound of Formula (I) and metabolites will be collected within 45 minutes before dosing, and at approximately 30 minutes, and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, 24, 36, 168, 336, and 504 hours after dosing.

The following plasma PK parameters will be calculated for the compound of Formula (I) and metabolites:

- Area under the plasma concentration versus time curve from 0 hours to the time of the last measurable concentration ($AUC_{0\text{-}tlast}$)
- Area under the plasma concentration curve extrapolated from 0 hours to infinity ($AUC_{0\text{-}\infty}$)
- Maximum plasma concentration (C_{max})
- Time to achieve maximum plasma concentration (t_{max})
- Delay time between time of dosing and time of appearance of measurable test article (T_{lag})
- Apparent terminal half-life ($t_{½}$)
- Apparent terminal rate constant (λz)
- Apparent mean residence time (MRT)

- Molar AUC ratio of primary metabolite(s) to the parent drug the compound of Formula (I)

The following plasma PK parameters will be calculated only for the compound of Formula (I):

- Apparent systemic clearance after oral administration (CL/F)
- Apparent volume of distribution during terminal phase after oral administration (Vz/F)

Other assessment

A taste satisfaction questionnaire will be administered.

Safety assessments

Safety will be monitored throughout the study and will include the following assessments:

- AEs
- Clinical laboratory tests (hematology, coagulation, clinical chemistry, and urinalysis)
- Vital sign measurements (including orthostatic blood pressure and pulse rate)
- Physical examinations
- 12-lead electrocardiogram (ECG)

Statistical methods

Pharmacokinetic parameters will be calculated using noncompartmental methods and summarized by formulation using descriptive statistics. Two-sided 90% confidence intervals will be calculated for the ratio of each test formulation (SDD suspension and SDD capsule) vs. the reference formulation for $AUC_{0-\infty}$, $AUC_{0-tlast}$, and C_{max} for the compound of Formula (I) and metabolites under fed conditions. Further, two-sided 90% confidence intervals will be calculated for the ratio of each test formulation under fasted conditions vs. under fed conditions for $AUC_{0-\infty}$, $AUC_{0-tlast}$, and C_{max} for the compound of Formula (I) and metabolites.

Safety and taste satisfaction questionnaire data will be summarized with descriptive statistics.

Results

Pharmacokinetic results

Pharmacokinetic results are shown in Tables 23-26, below.