IMIDAZO [1, 2-A] PYRAZINE DERIVATIVES AND THEIR USE AS ACID PUMP ANTAGONISTS

Abstract: This invention relates to compounds of the formula (I): or a pharmaceutically acceptable salt thereof, wherein: \( R^1, R^2, R^3, R^4, R^5, R^6, R^7 \). A and B are each as described herein or a pharmaceutically acceptable salt, and compositions containing such compounds and the method of treatment and the use, comprising such compounds for the treatment of a condition mediated by acid pump antagonistic activity such as, but not limited to, gastrointestinal disease, gastroesophageal disease, gastroesophageal reflux disease (GERD), laryngopharyngeal reflux disease, peptic ulcer, gastric ulcer, duodenal ulcer, NSAID-induced ulcers, gastritis, infection of Helicobacter pylori, dyspepsia, functional dyspepsia, Zollinger-Ellison syndrome, non-erosive reflux disease (NERD), visceral pain, cancer, heartburn, nausea, esophagitis, dysphagia, hypersalivation, airway disorders or asthma.
This invention relates to imidazopyrazine derivatives. These compounds have selective acid pump inhibitory activity. The present invention also relates to a pharmaceutical composition, method of treatment and use, comprising the above derivatives for the treatment of disease conditions mediated by acid pump modulating activity; in particular acid pump inhibitory activity.

It has been well established that proton pump inhibitors (PPIs) are prodrugs that undergo an acid-catalyzed chemical rearrangement that permits them to inhibit H⁺/K⁺-ATPase by covalently binding to its Cystein residues (Sachs, G. et al., Digestive Diseases and Sciences, 1995, 40, 3S-23S; Sachs et al., Annu Rev Pharmacol Toxicol, 1995, 35, 277-305.). However, unlike PPIs, acid pump antagonists inhibit acid secretion via reversible potassium-competitive inhibition of H⁺/K⁺-ATPase. SCH28080 is one of such reversible inhibitors and has been studied extensively. Other newer agents (revaprazan, soraprazan, AZD0865 and CS-526) have entered clinical trials confirming their efficacy in human (Pope, A.; Parsons, M., Trends in Pharmacological Sciences, 1993,14, 323-5; Vakil, N., Alimentary Pharmacology and Therapeutics, 2004, 19, 1041-1049.). In general, acid pump antagonists are found to be useful for the treatment of a variety of diseases, including gastrointestinal disease, gastroesophageal disease, gastroesophageal reflux disease (GERD), laryngopharyngeal reflux disease, peptic ulcer, gastric ulcer, duodenal ulcer, NSAID-induced ulcers, gastritis, infection of Helicobacter pylori, dyspepsia, functional dyspepsia, Zollinger-Ellison syndrome, non-erosive reflux disease (NERD), visceral pain, cancer, heartburn, nausea, esophagitis, dysphagia, hypersalivation, airway disorders or asthma (hereinafter, referred as "APA Diseases", Klijander, Toni O, American Journal of Medicine, 2003, 115 (Suppl. 3A), 6SS-71 S.).

WO04/074289 discloses compounds reported to be acid pump antagonists. They refer to certain compounds having imidazol[1,2-a]pyrazine structure.

There is a need to provide new acid pump antagonists that are good drug candidates and address unmet needs by PPIs for treating diseases. In particular, preferred compounds should bind potently to the acid pump whilst showing little affinity for other receptors and show functional activity as inhibitors of acid-secretion in stomach. They should be well absorbed from the gastrointestinal tract, be metabolically stable and possess favorable pharmacokinetic properties. They should be non-toxic.

Furthermore, the ideal drug candidate will exist in a physical form that is stable, non-hygroscopic and easily formulated.

**Summary of the Invention**

In this invention, it has now been found out that the new class of compounds having a chromane moiety and imidazol[1,2-a]pyrazine structure showed acid pump inhibitory activity and favorable properties as drug candidates, and thus are useful for the treatment of disease conditions mediated by acid pump inhibitory activity such as APA Diseases.

The present invention provides a compound of the following formula (I):
or a pharmaceutically acceptable salt thereof, wherein:

A-B represents -O-CH$_2$-, -CH$_2$-O-, -S-CH$_2$-, or -CH$_2$-S-;

R$^1$ represents a hydrogen atom or a d-C$_6$ alkyl group being unsubstituted or substituted with 1 to 2 substituents independently selected from the group consisting of a hydroxy group or a moiety convertible into a hydroxy group in vivo;

R$^2$ represents a C$_1$-C$_6$ alkyl group being unsubstituted or substituted with 1 to 2 substituents independently selected from the group consisting of a hydroxy group or a moiety convertible into a hydroxy group in vivo;

R$^3$ and R$^4$ independently represent a C$_1$-C$_6$ alkyl group or a C$_3$-C$_7$ cycloalkyl group, said C$_7$-C$_8$ alkyl group and said C$_3$-C$_7$ cycloalkyl group being unsubstituted or substituted with 1 to 3 substituents independently selected from the group consisting of a halogen atom, a hydroxy group, a C$_1$-C$_6$ alkoxy group and a C$_3$-C$_7$ cycloalkyl group; or R$^3$ and R$^4$ taken together with the nitrogen atom to which they are attached form a 4 to 7 membered heterocyclic ring unasc though or substituted with 1 to 3 substituents selected from the group consisting of a hydroxy group, a C$_1$-C$_6$ alkyl group, a C$_1$-C$_6$ alkoxy group and a hydroxy-C$_1$-C$_6$ alkyl group; and

R$_5$, R$_6$, R$^7$ and R$_8$ independently represent a hydrogen atom, a halogen atom or a C$_1$-C$_6$ alkyl group.

Also, the present invention provides a pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof, each as described herein, together with a pharmaceutically acceptable carrier for said compound.

Also, the present invention provides a pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof, each as described herein, further comprising other pharmacologically active agent(s).

Also, the present invention provides a method for the treatment of a condition mediated by acid pump modulating activity in a mammalian subject including a human, which comprises administering to a mammal in need of such treatment a therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof, each as described herein.

Examples of conditions mediated by acid pump modulating activity include, but are not limited to, APA Diseases.

Further, the present invention provides the use of a compound of formula (I) or a pharmaceutically acceptable salt thereof, each as described herein, for the manufacture of a medicament for the treatment of a condition mediated by acid pump inhibitory activity.

Further, the present invention provides a compound of formula (I) or a pharmaceutically
acceptable salt thereof, for use in medicine.

Preferably, the present invention also provides the use of a compound of formula (I) or a pharmaceutically acceptable salt thereof, each as described herein, for the manufacture of a medicament for the treatment of diseases selected from APA Diseases.

The compounds of the present invention may show good acid pump inhibitory activity, less toxicity, good absorption, good distribution, good solubility, less protein binding affinity other than acid pump, less drug-drug interaction and good metabolic stability.

**Detailed Description of the Invention**

In the compounds of the present invention:

Where $R^1$, $R^2$, $R^3$, $R^4$, $R^5$, $R^6$, $R^7$ and $R^8$ are the $C_{1-6}$ alkyl group, this $C_{1-6}$ alkyl group may be a straight or branched chain group having one to six carbon atoms, and examples include, but are not limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, 1-ethylpropyl and hexyl. Of these, $C_{1-2}$ alkyl is more preferred; methyl is more preferred.

Where $R^3$, $R^4$ and the substituent of $R^3$ and $R^4$ are the $C_3-C_7$ cycloalkyl group, this represents cycloalkyl group having three to seven carbon atoms, and examples include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl. Of these, $C_3-C_5$ cycloalkyl group is preferred; cyclopropyl is more preferred.

Where the substituent of $R^3$, $R^4$ and the 4 to 7 membered heterocyclic group are the $C_{1-6}$ alkoxy group, this represents the oxygen atom substituted with the said $C_{1-6}$ alkyl group, and examples include, but are not limited to, methoxy, ethoxy, propoxy, isopropanoxy, butoxy, isobutoxy, sec-butoxy, tert-butoxy, pentyloxy and hexyloxy. Of these, a $C_1-C_4$ alkoxy is preferred; a $C_1-C_2$ alkoxy is preferred; methoxy is more preferred.

Where $R^3$ and $R^4$, taken together with the nitrogen atom to which they are attached form a 4 to 7 membered heterocyclic group, this 4 to 7 membered heterocyclic group represents a saturated heterocyclic group having three to six ring atoms selected from carbon atom, nitrogen atom, sulfur atom and oxygen atom other than said nitrogen atom, and examples include, but are not limited to, azetidinyl, pyrrolidinyl, imidazolidinyl, pyrazolidinyl, piperidinyl, piperazinyl, hexahydroazepinyl, hexahydrodiazepinyl, morpholino, thiomorpholino and homomorpholino. Of these, azetidinyl, pyrrolidinyl, morpholino and homomorpholino are preferred; morpholino is more preferred.

Where the substituent of the 4 to 7 membered heterocyclic group is a hydroxy-CrC alkyl group, this represents said $C_{1-6}$ alkyl group substituted with a hydroxy group, and examples include, but are not limited to, hydroxymethyl, 2-hydroxyethyl, 1-hydroxyethyl 3-hydroxypropyl, 2-hydroxypropyl, 2-hydroxy-1-methylethyl, 4-hydroxybutyl, 3-hydroxybutyl, 2-hydroxybutyl, 3-hydroxy-2-methylethyl, 3-hydroxy-1-methylethylpropyl, 5-hydroxypentyl and 6-hydroxyhexyl. Of these, hydroxy-d-Cs alkyl is preferred; hydroxymethyl is more preferred.

Where $R^5$, $R^6$, $R^7$, $R^8$ and the substituent of $R^2$ and $R^3$ are the halogen atom, it may be a fluorine, chlorine, bromine or iodine atom. Of these, a fluorine atom and a chlorine atom are preferred.

Where the "moiety convertible into a hydroxy group in vivo" means a moiety transformable in vivo by e.g. hydrolysis and/or by an enzyme, e.g. an esterase, into a hydroxy group. Examples of the
moiety include, but are not limited to, ester and ether groups which may be hydrolyzed easily in vivo. Such moieties have known to those skilled in the art as 'pro-moieties' as described, for example, in "Design of Prodrugs" by H. Bundgaard (Elsevier, 1985). Preferred moieties convertible in vivo into a hydroxy group are e.g. a C₁-C₆ alkoxy group, a C₇-C₈ alkyl-carbonyl-oxy group and a C₁-C₆ alkyl-carbonyl-oxy-methyl-oxy group.

Where -A-B- is -O-CH₂-, -A- corresponds -O- and -B- corresponds -CH₂O-.
Where -A-B- is -CH₂O-, -A- corresponds -CH₂ and -B- corresponds -O-.
Where -A-B- is -S-CH₂-, -A- corresponds -S- and -B- corresponds -CH₂S-.

The term "treating" and "treatment", as used herein, refers to curative, palliative and prophylactic treatment, including reversing, alleviating, inhibiting the progress of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition.

Preferred classes of compounds of the present invention are those compounds of formula (I) or a pharmaceutically acceptable salt thereof, each as described herein, in which:

(a) -A-B- is -O-CH₂ or -CH₂O-;
(b) -A-B- is -CH₂O-;
(c) R¹ is a C₁-C₆ alkyl group being unsubstituted or substituted with 1 substituent selected from the group consisting of a hydroxy group or a moiety convertible into a hydroxy group in vivo;
(d) R¹ is a hydroxy-CrC βalkyl group, C₁-C₆ 8lkOxy-C₁-C₆ alkyl group, C₁-C₆ alkyl-carbonyl-oxy-C₁-C₆ alkyl group or a C₁-C₆ alkyl group;
(e) R¹ is a hydroxy-CrCe alkyl group or a C₁-C₆ alkyl group;
(f) R¹ is a hydroxymethyl group or a C₁-C₆ alkyl group;
(g) R¹ is a hydroxymethyl group or a methyl group;
(h) R¹ is a hydroxymethyl group;
(i) R² is a C₁-C₆ alkyl group being unsubstituted or substituted with 1 substituent selected from the group consisting of a hydroxy group or a moiety convertible into a hydroxy group in vivo;
(j) R² is a hydroxy-CrC₁-C₆ alkyl group, C₁-C₆ 8lkOxy-C₁-C₆ alkyl group, C₁-C₆ alkyl-carbonyl-oxy-C₁-C₆ alkyl group or a C₁-C₆ alkyl group;
(k) R² is a hydroxy-CrC₁-C₆ alkyl group or a C₁-C₆ alkyl group;
(l) R² is a hydroxymethyl group or a C₁-C₆ alkyl group;
(m) R² is a C₁-C₆ alkyl group;
(n) R² is a methyl group;
(o) R³ is a C₁-C₆ alkyl group or a C₃-C₇ cycloalkyl group, said C₁-C₆ alkyl group and said C₃-C₇ cycloalkyl group being unsubstituted or substituted with 1 to 3 substituents independently selected from the group consisting of a halogen atom, a hydroxy group, a C₆ alkoxy group and a C₃-C₇ cycloalkyl group;
(p) R³ is a C₁-C₆ alkyl group;
(q) R³ is a C₁-C₆ alkyl group;
(r) R³ is a methyl group;
(s) R₄ is a C₁⁻C₆ alkyl group or a C₂⁻C₇ cycloalkyl group, said C₁⁻C₆ alkyl group and said C₂⁻C₇ cycloalkyl group being unsubstituted or substituted with 1 to 3 substituents independently selected from the group consisting of a halogen atom, a hydroxy group, a C₁⁻C₆ alkoxy group and a C₃⁻C₇ cycloalkyl group;

5 (t) R₄ is a C₁⁻C₆ alkyl group;
(u) R⁴ is a C₁⁻C₂ alkyl group;
(v) R⁴ is a methyl group;

(w) R³ and R⁴ taken together with the nitrogen atom to which they are attached form an azetidinyl group, a pyrrolidinyl group, a morpholino group or a homomorpholino group, said azetidinyl group, said pyrrolidinyl group, said morpholino group and said homomorpholino group being unsubstituted or substituted with 1 to 3 substituents selected from the group consisting of a hydroxy group, a C₁⁻C₆ alkyl group, a C₁⁻C₆ alkoxy group and a hydroxy-C₁⁻C₆ alkyl group;

10 (x) R³ and R⁴ taken together with the nitrogen atom to which they are attached form a pyrrolidinyl group, a morpholino group or a homomorpholino group, said pyrrolidinyl group, said morpholino group and said homomorpholino group being unsubstituted or substituted with a substituent selected from the group consisting of a hydroxy group, a C₁⁻C₆ alkyl group, a C₁⁻C₆ alkoxy group and a hydroxy-C₁⁻C₆ alkyl group;

(y) R⁵, R⁶, R⁷ and R⁸ are independently a hydrogen atom, a halogen atom or a C₁⁻C₆ alkyl group;
(z) R⁵ and R⁷ are independently a hydrogen atom, a halogen atom or a C₁⁻C₆ alkyl group;

20 (aa) R⁵ and R⁷ are independently a hydrogen atom, a halogen atom or a C₁⁻C₂ alkyl group;
(bb) R⁵ and R⁷ are independently a hydrogen atom, a fluorine atom, a chlorine atom or a methyl group;
(cc) R⁶ and R⁸ are independently a hydrogen atom or a halogen atom.
(dd) R⁶ and R⁸ are independently a hydrogen atom, a fluorine atom or a chlorine atom.

25 (ee) R⁵ is a hydrogen atom, a fluorine atom or a methyl group;

(gg) R⁷ is a hydrogen atom or a fluorine atom; and

(hh) R⁸ is a hydrogen atom;

Of these classes of compounds, any combination among (a) to (hh) is also preferred.

Preferred compounds of the present invention are those compounds of formula (l) or a pharmaceutically acceptable salt thereof, each as described herein, in which:

(A) -A-B- is -OCH₂- or -CH₂-O-; R¹ and R² are independently a hydroxy-d-C₆ alkyl group, C₁⁻C₆ alkyl, C₁⁻C₆ alkyl-carbonyl-oxy-C₁⁻C₆ alkyl group or a C₁⁻C₆ alkyl group; R³ and R⁴ are independently a C₁⁻C₆ alkyl group or a C₁⁻C₆ cyclicalkyl group, said C₁⁻C₆ alkyl group and said C₃⁻C₇ cycloalkyl group being unsubstituted or substituted with 1 to 3 substituents independently selected from the group consisting of a halogen atom, a hydroxy group, a C₁⁻C₆ alkoxy group and a C₃⁻C₇ cycloalkyl group; or R³ and R⁴ taken together with the nitrogen atom to which they are attached form an azetidinyl group, a pyrrolidinyl group, a morpholino group or a homomorpholino group, said azetidinyl group, said pyrrolidinyl group, said morpholino group and said homomorpholino group being unsubstituted or substituted with 1 to 3 substituents selected from the group consisting of a hydroxy group, a C₁⁻C₆ alkyl group, a C₁⁻C₆ alkoxy group and a hydroxy-C₁⁻C₆ alkyl group; and R⁵, R⁶,
R7 and R8 are independently a hydrogen atom, a halogen atom or a C1–C6 alkyl group;

(B) -A-B- is -CH2-O-; R1 and R2 are independently a hydroxymethyl group or a C1–C6 alkyl group; R3 and R4 are independently is a C1–C6 alkyl group; or R3 and R4 taken together with the nitrogen atom to which they are attached form an azetidinyl group, a pyrrolidinyl group, a morpholino group or a homomorpholino group, said azetidinyl group, said pyrrolidinyl group, said morpholino group and said homomorpholino group being unsubstituted or substituted with 1 to 3 substituents selected from the group consisting of a hydroxy group, a C1–C6 alkyl group, a C1–C6 alkoxy group and a hydroxy-C1–C6 alkyl group; R5 and R7 are independently a hydrogen atom, a halogen atom or a C1–C6 alkyl group; and R6 and R8 are independently a hydrogen atom or a halogen atom.

(C) -A-B- is -0-CH2- or -CH2-O-; R1 is a C1–C6 alkyl group being unsubstituted or substituted with 1 substituent selected from the group consisting of a hydroxy group or a moiety convertible into a hydroxy group in vivo; R2 is a C1–C6 alkyl group being unsubstituted or substituted with 1 substituent selected from the group consisting of a hydroxy group or a moiety convertible into a hydroxy group in vivo; R3 and R4 are independently a C1–C6 alkyl group or a C3–C7 cycloalkyl group, said C1–C6 alkyl group and said C3–C7 cycloalkyl group being unsubstituted or substituted with 1 to 3 substituents independently selected from the group consisting of a halogen atom, a hydroxy group, a C1–C6 alkoxy group and a C3–C7 cycloalkyl group; or R3 and R4 taken together with the nitrogen atom to which they are attached form an azetidinyl group, a pyrrolidinyl group, a morpholino group or a homomorpholino group, said azetidinyl group, said pyrrolidinyl group, said morpholino group and said homomorpholino group being unsubstituted or substituted with 1 to 3 substituents selected from the group consisting of a hydroxy group, a CrC6 alkyl group, a C1–C6 alkoxy group and a hydroxy-CrC6 alkyl group; and R5, R6, R7 and R8 are independently a hydrogen atom, a halogen atom or a C1–C6 alkyl group.

(D) -A-B- is -0-CH2- or -CH2-O-; R1 is a hydroxymethyl group or a C1–C6 alkyl group; R2 is a C1–C6 alkyl group being unsubstituted or substituted with 1 substituent selected from the group consisting of a hydroxy group or a moiety convertible into a hydroxy group in vivo; R3 and R4 are independently a C1–C6 alkyl group or a C3–C7 cycloalkyl group, said C1–C6 alkyl group and said C3–C7 cycloalkyl group being unsubstituted or substituted with 1 to 3 substituents independently selected from the group consisting of a halogen atom, a hydroxy group, a C1–C6 alkoxy group and a C3–C7 cycloalkyl group; or R3 and R4 taken together with the nitrogen atom to which they are attached form an azetidinyl group, a pyrrolidinyl group, a morpholino group or a homomorpholino group, said azetidinyl group, said pyrrolidinyl group, said morpholino group and said homomorpholino group being unsubstituted or substituted with 1 to 3 substituents selected from the group consisting of a hydroxy group, a C1–C6 alkyl group, a C1–C6 alkoxy group and a hydroxy-C1–C6 alkyl group; and R5, R6, R7 and R8 are independently a hydrogen atom, a halogen atom or a C1–C6 alkyl group.

(E) -A-B- is -0-CH2- or -CH2-O-; R1 is a C1–C6 alkyl group being unsubstituted or substituted with 1 substituent selected from the group consisting of a hydroxy group or a moiety convertible into a hydroxy group in vivo; R2 is a C1–C6 alkyl group being unsubstituted or substituted with 1 substituent selected from the group consisting of a hydroxy group or a moiety convertible into a hydroxy group in vivo; R3 and R4 are independently a C1–C6 alkyl group or a C3–C7 cycloalkyl group, said C1–C6 alkyl group and said C3–C7 cycloalkyl group being unsubstituted or substituted with 1 to 3 substituents
independently selected from the group consisting of a halogen atom, a hydroxy group, a C₁-C₆ alkoxy group and a C₂-C₇ cycloalkyl group; or R² and R⁴ taken together with the nitrogen atom to which they are attached form an azetidinyl group, a pyrrolidinyl group, a morpholino group or a homomorpholino group, said azetidinyl group, said pyrrolidinyl group, said morpholino group and said homomorpholino group being unsubstituted or substituted with 1 to 3 substituents selected from the group consisting of a hydroxy group, a C₁-C₆ alkyl group, a C₁-C₆ alkoxy group and a hydroxy-CᵢCᵢ alky group; and R⁷ and R⁸ are independently a hydrogen atom, a fluorine atom, a chlorine atom or a methyl group; R⁵ and R⁶ are independently a hydrogen atom, a fluorine atom or a chlorine atom.

The compounds of formula (I) containing one or more asymmetric carbon atoms can exist as two or more stereoisomers.

Included within the scope of the present invention are all stereoisomers and geometric isomers of the compounds of formula (I), including compounds exhibiting more than one type of isomerism, and mixtures of one or more thereof. Also included are acid addition salts wherein the counterion is optically active, for example, D-lactate or L-lysine, or racemate, DL-tartrate or DL-arginine.

One embodiment of the invention provides a compound selected from the group consisting of:

(-)-8-[(5,7-Difluoro-3,4-dihydro-2/-/-chromen-4-yl)amino]-3-(hydroxymethyl)-N,N,2-trimethylimidazo[1,2-a]pyrazine-6-carboxamide;

(+)-8-[(5,7-Difluoro-3,4-dihydro-2H-chromen-4-yl)amino]-3-(hydroxymethyl)-N,N,2-trimethylimidazo[1,2-a]pyrazine-6-carboxamide;

(S)-(-)-3-(Hydroxymethyl)-N,N,2-trimethyl-8-[(5-methyl-3,4-dihydro-2H-chromen-4-yl)amino]imidazo[1,2-a]pyrazine-6-carboxamide;

(R)-(+)3-(Hydroxymethyl)-N,N,2-trimethyl-8-[(5-methyl-3,4-dihydro-2/-/-chromen-4-yl)amino]imidazo[1,2-a]pyrazine-6-carboxamide;

(-)-8-[(5,7-Difluoro-3,4-dihydro-2/-/-chromen-4-yl)amino]-N,N,2,3-tetramethylimidazo[1,2-a]pyrazine-6-carboxamide;

(+)-8-[(5,7-Difluoro-3,4-dihydro-2/-/-chromen-4-yl)amino]-N,N,2,3-tetramethylimidazo[1,2-a]pyrazine-6-carboxamide;

(-J,N,N,S-tetramethyl-S^-S-methyl-S^-dihydro^-H-chromen^-yOaminolimidazoti^-alpyrazine-e-carboxamide; and

(+)-N,N,2,3-tetramethyl-8-[(5-methyl-3,4-dihydro-2H-chromen-4-yl)amino]imidazo[1,2-a]pyrazine-6-carboxamide;

or a pharmaceutically acceptable salt thereof.

Pharmaceutically acceptable salts of a compound of formula (I) include the acid addition salts (including disalts) thereof.

Suitable acid addition salts are formed from acids which form non-toxic salts. Examples include the acetate, adipate, aspartate, benzoate, besylate, bicarbonate/carbonate, bisulphate/sulphate, borate, camsylate, citrate, cyclamate, edisylate, esylate, formate, fumarate, gluconate, glucuronate, hexafluorophosphate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, isethionate, lactate, malate, maleate, malonate, mesylate, methylsulphate,
naphthylate, 2-napsylate, nicotinate, nitrate, orotate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, pyroglutamate, saccharate, stearate, succinate, tannate, tartrate, tosylate, trifluoroacetate and xinofoate salts.

For a review on suitable salts, see "Handbook of Pharmaceutical Salts: Properties, Selection, and Use" by Stahl and Wermuth (Wiley-VCH, Weinheim, Germany, 2002). A pharmaceutically acceptable salt of a compound of formula (I) may be readily prepared by mixing together solutions of the compound of formula (I) and the desired acid or base, as appropriate. The salt may precipitate from solution and be collected by filtration or may be recovered by evaporation of the solvent. The degree of ionization in the salt may vary from completely ionized to almost non-ionized.

Pharmaceutically acceptable salts of the compounds of the invention include both unsolvated and solvated forms. The term "solvate" is used herein to describe a molecular complex comprising a compound of the invention and one or more pharmaceutically acceptable solvent molecules, for example, ethanol. The term 'hydrate' is employed when said solvent is water.

Pharmaceutically acceptable solvates in accordance with the invention include hydrates and solvates wherein the solvent of crystallization may be isotopically substituted, e.g. D$_2$O, cVAcetone, c$_6$DMSO.

Included within the scope of the invention are complexes such as clathrates, drug-host inclusion complexes wherein, in contrast to the aforementioned solvates, the drug and host are present in stoichiometric or non-stoichiometric amounts. Also included are complexes of the drug containing two or more organic and/or inorganic components which may be in stoichiometric or non-stoichiometric amounts. The resulting complexes may be ionized, partially ionized, or non-ionized. For a review of such complexes, see J Pharm Sci, 64 (8), 1269-1288 by Halebian (August 1975).

The compounds of formula (I) may exist in one or more crystalline forms. These polymorphs, including mixtures thereof are also included within the scope of the present invention.

The compounds of formula (I) containing one or more asymmetric carbon atoms can exist as two or more stereoisomers.

Included within the scope of the present invention are all stereoisomers of the compounds of formula (I), including compounds exhibiting more than one type of isomerism, and mixtures of one or more thereof.

The present invention includes all pharmaceutically acceptable isotopically-labeled compounds of formula (I) wherein one or more atoms are replaced by atoms having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number usually found in nature.

Examples of isotopes suitable for inclusion in the compounds of the invention include isotopes of hydrogen, such as $^2$H and $^3$H, carbon, such as $^{11}$C, $^{12}$C and $^{14}$C, chlorine, such as $^{35}$Cl, fluorine, such as $^{19}$F, iodine, such as $^{123}$I and $^{125}$I, nitrogen, such as $^{14}$N and $^{15}$N, oxygen, such as $^{16}$O, $^{17}$O and $^{18}$O, phosphorus, such as $^{32}$P, and sulphur, such as $^{35}$S.

Certain isotopically-labeled compounds of formula (I), for example, those incorporating a radioactive isotope, are useful in drug and/or substrate tissue distribution studies. The radioactive isotopes tritium, i.e. $^3$H, and carbon-14, i.e. $^{14}$C, are particularly useful for this purpose in view of their ease of incorporation and ready means of detection.
Substitution with heavier isotopes such as deuterium, \textit{i.e.} $^2\text{H}$, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased \textit{in vivo} half-life or reduced dosage requirements, and hence may be preferred in some circumstances.

Substitution with positron emitting isotopes, such as $^{11}\text{C}$, $^{18}\text{F}$, $^{15}\text{O}$ and $^{13}\text{N}$, can be useful in \textbf{Positron Emission Topography (PET)} studies for examining substrate receptor occupancy.

Isotopically-labeled compounds of formula (I) can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the accompanying examples and preparations using an appropriate isotopically-labeled reagents in place of the non-labeled reagent previously employed.

All of the compounds of the formula (I) can be prepared by the procedures described in the general methods presented below or by the specific methods described in the examples section and the preparations section, or by routine modifications thereof. The present invention also encompasses any one or more of these processes for preparing the compounds of formula (I), in addition to any novel intermediates used therein.

\textbf{General Synthesis}

The compounds of the present invention may be prepared by a variety of processes well known for the preparation of compounds of this type, for example as shown in the following \textbf{Method A}.

Unless otherwise indicated, $R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$, $R_7$, $R_8$, $A$ and $B$ in the following methods are as defined above. All starting materials in the following general syntheses may be commercially available or obtained by conventional methods known to those skilled in the art.

\textbf{Method A}

This illustrates the preparation of compound of formula (I).

\textbf{Reaction Scheme A}
In Reaction Scheme A, R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, A and B are each as defined above; Hal is a halogen atom, preferably a bromine atom; Alk is a C₁-C₆ alkyl group, preferably a methyl group; R¹ₐ is R¹ as defined above or R¹ wherein hydroxy group may be protected by a hydroxy-protecting group; R²ₐ is R² as defined above or R² wherein hydroxy group may be protected by a hydroxy-protecting group; R³ₐ is R³ as defined above or R³ wherein hydroxy group may be protected by a hydroxy-protecting group; R⁴ₐ is R⁴ as defined above or R⁴ wherein hydroxy group may be protected by a hydroxy-protecting group. The same shall apply hereinafter.

The term "hydroxy-protecting groups", as used herein, signifies a protecting group capable of being cleaved by various means to yield a hydroxy group, such as hydrogenolysis, hydrolysis, electrolysis or photolysis, and such hydroxy-protecting groups are described in Protective Groups in Organic Synthesis edited by T. W. Greene et al. (John Wiley & Sons, 1999). Such as for example, C₁-C₄ alkoxy carbonyl, C₁-C₄ alkyl carbonyl, W-C₁-C₄ alkylsilyl or tri-C₁-C₄ alkylaryl silyl groups, and C₁-C₄ alkoxy-C₁-C₄ alkyl groups. Suitable hydroxy-protecting groups include benzyl, 4-methoxy benzyl and tert-butyl dimethyl silyl.

(Step A1)

In this step, the compound of formula (IV) is prepared by nucleophilic substitution of the compound of formula (II), which is commercially available or may be prepared by the methods as described in J. Med. Chem., 1983, 26, 357. with the compound of formula (III), which is commercially available or may be prepared by the methods described in the following Method B and C. The reaction is carried out under the same conditions as described in WO2005/004607 and WO2004/074289.

The reaction is normally and preferably effected in the presence or absence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent.

Examples of suitable solvents include: ethers, such as tetrahydrofuran (THF), ethylene glycol dimethyl ether and dioxane; amides, such as N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMA) and
N-methyl-2-pyrrolidinone (NMP); nitriles, such as acetonitrile; alcohols, such as 2-methyl-2-propanol, 1-butanol, 1-propanol, 2-propanol, ethanol and methanol; and sulfoxide, such as dimethyl sulfoxide (DMSO). Of these solvents, amides and nitriles are preferred. N-methyl-2-pyrrolidinone and acetonitrile are more preferred.

In this reaction, a base can accelerate the reaction. The reaction may be carried out with or without a base. There is likewise no particular restriction on the nature of the bases used, and any base commonly used in reactions of this type may equally be used here. Examples of such bases include: alkali metal alkoxides, such as potassium ferb-butoxide; alkali metal carbonates, such as lithium carbonate, sodium carbonate (Na₂CO₃), cesium carbonate and potassium carbonate (K₂CO₃); alkali metal hydrogencarbonates, such as sodium hydrogen carbonate (NaHCO₃) and potassium hydrogen carbonate; and organic amines, such as triethylamine, tripopyramine, tributylamine, dicyclohexylamine, N,N-diisopropylethylamine, N-methylpiperidine, N-methylmorpholine, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 1,5-diazabicyclo[4.3.0]non-5-ene (DBN). Of these, triethylamine is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 0 °C to about 250 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 5 minutes to about 72 hours will usually suffice.

In this reaction, microwave can be employed to accelerate the reaction. In the case of employing microwave in sealed tube, the reaction at a temperature may be from about 50 °C to about 250 °C, and the reaction time from about 5 minutes to about 12 hours will usually suffice.

(Step A2)

In this step, the compound of formula (VI) is prepared by cyclization of the compound of formula (IV) and the compound of formula (V), which may be commercially available or may be prepared by the methods described in Synthetic communications., 1994, 24, 2557, Synthetic communications., 1995, 25, 3923, Synthetic communications., 1995, 25, 1045, Tetrahedron Letters., 1993, 34, 1733. or Tetrahedron Letters., 1999, 40, 2231.

The reaction is normally and preferably effected in the presence or absence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: halogenated hydrocarbons, such as dichloromethane, chloroform, carbon tetrachloride and 1,2-dichloroethane; ethers, such as diethyl ether, disopropyl ether, tetrahydrofuran and dioxane; aromatic hydrocarbons, such as benzene, toluene and nitrobenzene; amides, such as formamide, N.N-dimethylformamide, N.N-dimethylacetamide, N-methyl-2-pyrrolidinone and hexamethylphosphoric triamide; amines, such as N-methylmorpholine, triethylamine, tripopyramine, tributylamine, disopropylethylamine, dicyclohexylamine, N-methylpiperidine, pyridine, 4-pyrrolindinopyridine, N,N-dimethylanline and N,N-diethylanline; nitriles, such as acetonitrile and
benzonitrile; sulfoxides, such as dimethyl sulfoxide and sulfolane; and ketones, such as acetone, cyclohexanone and diethylketone. Of these solvents, dioxane is preferred.

The reaction may be carried out in the presence or absence of reagent, such as an acid or a base. There is likewise no particular restriction on the nature of the acids or bases used, and any acid or base commonly used in reactions of this type may equally be used here. Examples of such acids include: acids, such as hydrochloric acid, sulfuric acid, hydrobromic acid and p-toluenesulfonic acid. Of these, p-toluenesulfonic acid or the absence of acid is preferred. Examples of such bases include: alkali metal hydrogenocarbonates, such as sodium hydrogenocarbonate and potassium hydrogenocarbonate; alkali metal carbonates, such as sodium carbonate and potassium carbonate; and amines, such as triethylamine, pyridine and diisopropylethylamine. Of these, triethylamine or the absence of base is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 20 °C to about 180 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 30 minutes to about 120 hours, will usually suffice. (Step A3)

In this step, the compound of formula (VII) is prepared by the ester formation reaction of the compound of formula (VI) with alcohol under the carbon monoxide atmosphere.

The reaction is normally and preferably effected in the presence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: ethers, such as diethyl ether, diisopropyl ether, tetrahydrofuran and dioxane; aromatic hydrocarbons, such as benzene, toluene and nitrobenzene; amides, such as formamide, \( \Lambda,\Lambda \)-dimethylformamide, \( \Lambda,\Lambda \)-dimethylacetamide, \( \Lambda \)-methyl-2-pyrrolidinone, and hexamethylenephosphoric triamide; nitriles, such as acetonitrile and benzonitrile; and ketones, such as acetone and diethylketone. Of these solvents, \( \Lambda,\Lambda \)-dimethylformamide is preferred.

The reaction is carried out in the presence of a palladium catalyst. There is no particular restriction on the nature of the palladium catalyst to be employed, and any palladium catalyst commonly used in reactions of this type may equally be used here. Examples of such palladium catalysts include: palladium metal, palladium-carbon, palladium (II) acetate, tris(dibenzylideneacetone)dipalladiumchloroform, \( [1,2\text{-bis(diphenylphosphino)}\text{ethane}]\text{palladium dichloride,} \)

\( \text{bis(tri-o-tolylphosphine)palladium dichloride,} \) \( \text{bis(triphenylphosphine)palladium dichloride,} \) \( \text{tetrakis(diphenylphosphine)} \text{palladium, dichloro[}1,1\text{-bis(diphenylphosphino)}\text{ferrocene}]\text{palladium,} \) or a catalyst produced in solution by adding a ligand into the reaction solution of these. The ligand added into the reaction solution may be a phosphoric ligand such as \( 1,1\text{-bis(diphenylphosphino)}\text{ferrocene,} \)

\( \text{bis(2-diphenylphosphinophenyl)} \text{ether,} \) \( 2,2\text{-bis(diphenylphosphino)}\text{-1,1'-binaphthol,} \)

\( 1,3\text{-bis(diphenylphosphino)}\text{propane,} \) \( 1,4\text{-bis(diphenylphosphino)}\text{butane,} \) tri-o-tolylphosphine,
triphenylphosphine, 2-diphenylphosphino-2'-methoxy-1',1'-binaphthyl or 2,2'-bis
diphenylphosphino)-1',1'-binaphthyl. The above palladium catalysts are preferably
preferred. The reaction may be carried out with or without a base. There is likewise no particular
restriction on the nature of the bases used, and any base commonly used in reactions of this type may
equally be used here. Examples of such bases include: amines, such as \( N \)-methylmorpholine,
triethylamine, diisopropylethylamine, \( N \)-methylpipерidine and pyridine. Of these, triethylamine is
preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction
temperature is not critical to the invention. The preferred reaction temperature will depend upon such
factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to
carry out the reaction at a temperature of from about 20 °C to about 180 °C. The time required for the
reaction may also vary widely, depending on many factors, notably the reaction temperature and the
nature of the starting materials and solvent employed. However, provided that the reaction is effected
under the preferred conditions outlined above, a period of from about 30 minutes to about 72 hours, will
usually suffice.

(Step A4)

In this step, the compound of formula (I) is prepared by (A4a1) hydrolysis of the compound of
formula (VII), prepared as described in Step A3, followed by (A4a2) condensing reaction with the
compound of formula (VIII) or (A4b) substituting the reaction of the compound of formula (VII) with the
compound of formula (VIII).

(A4a1) hydrolysis

The reaction is normally and preferably effected in the presence of solvent. There is no
particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on
the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples
of suitable solvents include: ether, such as tetrahydrofuran and dioxane; amides, such as
\( \Lambda, \Lambda \)-dimethylformamide; alcohols, such as ethanol and methanol; and water; or mixed solvents thereof.
Of these solvents, methanol, tetrahydrofuran, and water are preferred.

The reaction is carried out in the presence of a base. There is likewise no particular restriction
on the nature of the bases used, and any base commonly used in reactions of this type may equally be
used here. Examples of such bases include: alkali metal hydroxides, such as lithium hydroxide (LiOH),
sodium hydroxide (NaOH) and potassium hydroxide (KOH). Of these, sodium hydroxide is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction
temperature is not critical to the invention. The preferred reaction temperature will depend upon such
factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to
carry out the reaction at a temperature of from about 0 °C to about 100 °C. The time required for the
reaction may also vary widely, depending on many factors, notably the reaction temperature and the
nature of the starting materials and solvent employed. However, provided that the reaction is effected
under the preferred conditions outlined above, a period of from about 5 minutes to about 12 hours will
usually suffice.
The reaction is normally and preferably effected in the presence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: halogenated hydrocarbons, such as dichloromethane, chloroform, and 1,2-dichloroethane; ethers, such as tetrahydrofuran and dioxane; amides, such as \( \Lambda,\Lambda' \)-dimethylformamide and \( \Lambda,\Lambda' \)-dimethylacetamide; and nitriles, such as acetonitrile. Of these solvents, halogenated hydrocarbons and amides are preferred. Dichloromethane and \( \Lambda,\Lambda' \)-dimethylformamide are more preferred.

The reaction is carried out in the presence of a condensing agent. There is likewise no particular restriction on the nature of the condensing agents used, and any condensing agents commonly used in reactions of this type may equally be used here. Examples of such condensing agents include: azodicarboxylic acid di-lower alkyl ester-triphenylphosphines, such as diethyl azodicarboxylate-triphenylphosphine; 2-halo-1-lower alkyl pyridinium halides, such as 2-chloro-1-methyl pyridinium iodide and 2-bromo-1-ethylpyridinium tetrafluoroborate (BEP); \( \Lambda \)-diaryloxythiophosphorocyanidates, such as diphenylphosphorylazide (DPPA); chloroformates, such as ethyl chloroformate and isobutyl chloroformate; phosphorocyanidates, such as diethyl phosphorocyanidate (DEPC); imidazole derivatives, such as \( \Lambda,\Lambda' \)-carbonyldimdiamazole (CDI); carbodiimide derivatives, such as \( \Lambda,\Lambda' \)-dicyclohexylcarbodiimide (DCC) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI); iminium salts, such as 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and tetramethylfluoroformamidinium hexafluoro phosphate (TFFH); and phosphonium salts, such as benzotriazol-1-yloxythi(dimethylamino)phosphonium hexafluorophosphate (BOP) and bromo-ths-pyrrolidinophosphonium hexafluorophosphate (PyBop). Of these, EDCI and HBTU are preferred.

Reagents, such as 4-(\( \Lambda,\Lambda' \)-dimethylamino)pyridine (DMAP), and \( \Lambda' \)-hydroxybenztriazole (HOBt), may be employed for this step. Of these, HOBt is preferred.

The reaction may be carried out with or without a base. There is likewise no particular restriction on the nature of the bases used, and any base commonly used in reactions of this type may equally be used here. Examples of such bases include: amines, such as \( \Lambda' \)-methylmorpholine, triethylamine, diisopropylethylamine, \( \Lambda \)-methylpiperidine and pyridine. Of these, triethylamine and \( \Lambda' \)-methylmorpholine are preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 0 °C to about 80 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 5 minutes to about 24 hours will usually suffice.

(A4b) substituting reaction

The reaction can be carried out by heating the reactants in the neat amino compound or in an
inert solvent under standard condition. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: ethers, such as ethylene glycol dimethyl ether, tetrahydrofuran and dioxane; amides, such as \( \Lambda.\Lambda'\)-dimethylformamide and \( \Lambda.\Lambda'\)-dimethylacetamide; nitriles, such as acetonitrile; and alcohols such as 2-methyl-2-propanol, 1-butanol, 1-propanol, 2-propanol, ethanol and methanol. Of these solvents, ethers and alcohols are preferred. Tetrahydrofuran is more preferred.

The reaction may be carried out with or without a catalyst. There is likewise no particular restriction on the nature of the catalysts used, and any catalysts commonly used in reactions of this type may equally be used here. Examples of such catalysts include: sodium cyanide or potassium cyanide. Of these, sodium cyanide is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 40 °C to about 200 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 30 minutes to about 24 hours will usually suffice.

(Deprotection of hydroxy-protecting group)

In cases where \( R^{1a}, R^{2a} \) has a protected hydroxy group, the deprotection reaction will follow to yield a hydroxy group. This reaction is described in detail by T. W. Greene et al., Protective Groups in Organic Synthesis, 369-453, (1999), the disclosures of which are incorporated herein by reference. The following exemplifies a typical reaction involving the protecting group tert-butyldimethylsilyl.

The deprotection of the hydroxy groups is carried out with an acid, such as acetic acid, hydrogen fluoride, hydrogen fluoride-pyridine complex, or fluoride ion, such as tetrabutylammonium fluoride (TBAF).

The deprotection reaction is normally and preferably effected in the presence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include, but are not limited to: alcohol, such as methanol, ethanol or mixed solvents thereof.

The deprotection reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 0 °C to about 100 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 10 minutes to about 24 hours, will usually suffice.

(Introduction of hydroxymethyl)
In the case of the compound wherein \( R^1a \) is H and \( R^1 \) is hydroxymethyl, the following reaction will be carried out to yield a hydroxymethyl group of formula (I).

The hydroxymethyl reaction is carried out with formaldehyde, paraformaldehyde or 1,3,5-thioxane.

The reaction is carried out in the presence or absence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: aliphatic hydrocarbons, such as hexane, heptane and petroleum ether; halogenated hydrocarbons, such as dichloromethane, chloroform, carbon tetrachloride and 1,2-dichloroethane; ethers, such as diethyl ether, disopropyl ether, tetrahydrofuran and dioxane; aromatic hydrocarbons, such as benzene, toluene and nitrobenzene; amides, such as formamide, \( \Lambda,\Lambda' \)-dimethylformamide and hexamethylenophosphoric thiamide; amines, such as \( \Lambda \)-methylmorpholine, triethylamine, N-propylamine, N,N-dimethylamine, pyridine, 4-pyrrolidinopyridine, \( \Lambda,\Lambda' \)-dimethylaniline and \( \Lambda,\Lambda' \)-diethylaniline; alcohols, such as methanol, ethanol, propanol, 2-propanol and 1-butanol; nitriles, such as acetonitrile and benzonitrile; sulfoxides, such as dimethyl sulfoxide and sulfolane; and water. Of these solvents, acetonitrile and water are preferred.

The reaction is carried out in the presence of reagent, such as an acid or a base. There is likewise no particular restriction on the nature of the acids or bases used, and any acid or base commonly used in reactions of this type may equally be used here. Examples of such acids include: carboxylic acids, such as acetic acid and propionic acid; inorganic acids, such as hydrochloric acid and sulfuric acid; organic acids, such as p-toluenesulfonic acid and thifluoro acetic acid; and Lewis acids, such as BF\(_3\), AlCl\(_3\), FeCl\(_3\), AgCl, ZnI\(_2\), Fe(NO\(_3\))\(_3\), CF\(_3\)SO\(_2\)Si(CH\(_3\))\(_3\), Yb(CF\(_3\)SO\(_3\))\(_3\) and SnCl\(_4\). Of these, acetic acid is preferred. Examples of such bases include: alkali metal acetates, such as lithium acetate, sodium acetate, potassium acetate and cesium acetate; alkali metal hydroxides, such as lithium hydroxide, sodium hydroxide and potassium hydroxide; alkali metal alkoxydes, such as sodium methoxide, sodium ethoxide and potassium 1-butoxide; alkali metal carbonates, such as lithium carbonate, sodium carbonate and potassium carbonate; alkali metal hydrogen carbonates, such as lithium hydrogen carbonate, sodium hydrogen carbonate and potassium hydrogen carbonate; and amines, such as \( \Lambda' \)-methylmorpholine, triethylamine, tripropylamine, tributylamine, disopropyl amine, diethylamine, N-methylpyrrolidine, pyridine, 4-pyrrolidinopyridine, picoline, 4-(\( \Lambda,\Lambda' \)-dimethylamino)pyridine, 2,6-di(f-butyl)-4-methylpyridine, quinoline, \( \Lambda,\Lambda' \)-dimethylaniline, \( \Lambda,\Lambda' \)-diethylaniline, DBN, 1,4-diazabicyclo[2.2.2]octane (DABCO), imidazole and DBU. Of these, sodium acetate is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 0 °C to about 250 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 5 minutes to about 72 hours will
usually suffice.

(Step A5)

In this step, the compound of formula (I) is prepared by the amidation of the compound of formula (VI) with the compound of formula (VIII) under the carbon monoxide atmosphere.

The reaction is normally and preferably effected in the presence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: ethers, such as diethyl ether, diisopropyl ether, tetrahydrofuran and dioxane; aromatic hydrocarbons, such as benzene, toluene and nitrobenzene; amides, such as formamide, \(N, N\)-dimethylformamide, \(N, N\)-dimethylacetamide, \(N\)-methyl-2-pyrrolidinone, and hexamethylphosphoric triamide; nitriles, such as acetonitrile and benzonitrile; and ketones, such as acetone and diethylketone. Of these solvents, tetrahydrofuran is preferred.

The reaction is carried out in the presence of a palladium catalyst. There is no particular restriction on the nature of the palladium catalyst to be employed, and any palladium catalyst commonly used in reactions of this type may equally be used here. Examples of such palladium catalysts include: palladium metal, palladium-carbon, palladium (II) acetate, tris(dibenzylideneacetone)dipalladiumchloroform, \(\{1,2\text{-bis(diphenylphosphino)ethane}\}\text{palladium dichloride,}
\text{bis(tri-o-tolylphosphine)palladium dichloride, bis(triphenylphosphine)palladium dichloride, tetrakis(triphenylphosphine) palladium, dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium, or a catalyst produced in solution by adding a ligand into the reaction solution of these.}
\text{The ligand added into the reaction solution may be a phosphoric ligand such as 1,1'-bis(diphenylphosphino)ferrocene, bis[2-diphenylphosphinophenyl] ether, 2,2'-bis(diphenylphosphino)-1,1'-binaphthol, 1,3-bis(diphenylphosphino)propane, 1,4-bis(diphenylphosphino)butane, tri-o-tolylphosphine, triphenylphosphine, 2-diphenylphosphino-2'-methoxy-1,1'-binaphthyl or 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl.}
\text{The above palladium catalysts are preferably tetrakis(triphenylphosphine) palladium and palladium (II) acetate - triphenylphosphine.}

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 20 °C to about 120 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 60 minutes to about 72 hours, will usually suffice.

(Deprotection of hydroxy-protecting group)

In cases where \(R^{1a}, R^{2a}\) has a protected hydroxy group, the deprotection reaction will be carried out to yield a hydroxy group. The deprotection of the hydroxy-protecting group may be carried out under the same condition described in Step A4 of method A.

(Introduction of hydroxymethyl)

In the case of the compound of wherein \(R^{1a}\) is H and \(R^{1}\) is hydroxymethyl, the following reaction
will be carried out to yield a hydroxymethyl group of formula (I). The reaction may be carried out under the same reaction condition in Step A4 of Method A.

Method B

5 This illustrates the preparation of compounds of formula (Ilia) wherein A is CH₂.

Reaction Scheme B

In Reaction Scheme B, Hal is a halogen atom, Rªalk is a hydrogen atom or a Ci-C₆ alkyl group and the same shall apply hereinafter.

(Step B1)

In this step, the compound of formula (XIII) is prepared by Michael reaction (B1 a) of the compound of formula (IX) with the compound of formula (X), by alkylation reaction (B1 b) of the compound of formula (IX) with the compound of formula (XI), or by coupling reaction (B1 c) of the compound of formula (IX) with the compound of formula (XII) followed by the hydrogenation (B1 d). The compound of formula (IX), (X), (XI) and (XII) are commercially available.

(B1 a) Michael reaction

The reaction is normally and preferably effected in the presence or the absence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: ethers, such as diethyl ether, diisopropyl ether, tetrahydrofuran and dioxane; amides, such as formamide, N,N-dimethylformamide, N,N-dimethylacetamide, N-methyl-2-pyrrolidinone, and hexamethylenephosphohc thamide; alcohols, such as methanol, ethanol, propanol, 2-propanol and butanol; nitriles, such as acetonitrile and benzonitrile; sulfoxides, such as dimethyl sulfoxide and sulfolane; or mixed solvents thereof. Of these, the reaction in the absence of solvent is preferred.

The reaction is carried out in the presence of a base. There is likewise no particular restriction on the nature of the bases used, and any base commonly used in reactions of this type may equally be used here. Examples of such bases include: alkali metal hydroxides, such as lithium hydroxide, sodium hydroxide and potassium hydroxide; alkali metal hydrides, such as lithium hydride, sodium hydride and potassium hydride; alkali metal alkoxides, such as sodium methoxide, sodium ethoxide and potassium tert-butoxide; alkali metal carbonates, such as lithium carbonate, sodium carbonate and potassium
carbonate; amines, such as N-methylmorpholine, triethylamine, tripropylamine, tributylamine, diisopropylethylamine, dicyclohexylamine, N,N-methylpiperidine, pyridine, 4-pyridinopyridine, picoline, 4-(N,N-dimethylamino)pyridine, 2,6-di(fert-butyl)-4-methylpyridine, quinoline, N,N-dimethylaniline, N,N-diethylaniline, DBN, DABCO, DBU and benzyltrimethylammonium hydroxide; alkali metal amides, such as lithium amide, sodium amide, potassium amide, lithium diisopropyl amide, potassium diisopropyl amide, sodium diisopropyl amide, lithium bis(trimethylsilyl)amide and potassium bis(trimethylsilyl)amide. Of these, benzyltrimethylammonium hydroxide or sodium methoxide is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 20 °C to about 120 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 60 minutes to about 48 hours, will usually suffice.

After the above procedure, hydrolysis is carried out by adding an acid in a solvent to produce the compound of formula (XIII), and may be carried out in a usual hydrolysis condition. The acid may include, for example, inorganic acids such as hydrochloric acid, hydrobromic acid and sulfuric acid. It is preferably hydrochloric acid. The solvent may include, for example, water; alcohols such as methanol, ethanol, propanol and fert-butanol; ethers such as diethyl ether, dimethoxyethane, tetrahydrofuran, diethoxymethane and dioxane; or mixed solvents thereof. It is preferably water. The reaction temperature varies depending on the starting compound, the reagent and the solvent, however, it is usually from 20 °C to the reflux temperature. The reaction time varies depending on the starting compound, the reagent, the solvent and the reaction temperature, however, it is usually from 60 minutes to 24 hours.

(B1 b) alkylation reaction

The reaction is normally and preferably effected in the presence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: ethers, such as diethyl ether, diisopropyl ether, tetrahydrofuran and dioxane; aromatic hydrocarbons, such as benzene, toluene and nitrobenzene; amides, such as formamide, N,N'-dimethylformamide, N,N-dimethylacetamide, N'-methyl-2-pyrlidinone, and hexamethylphosphoric triamide; alcohols, such as methanol, ethanol, propanol, 2-propanol and butanol; nitriles, such as acetonitrile and benzonitrile; sulfoxides, such as dimethyl sulfoxide and sulfolane; ketones, such as acetone and diethylketone; water; or mixed solvents thereof. Of these, water is preferred.

The reaction is carried out in the presence of a base. There is likewise no particular restriction on the nature of the bases used, and any base commonly used in reactions of this type may equally be used here. Examples of such bases include: alkali metal hydroxides, such as lithium hydroxide, sodium hydroxide and potassium hydroxide; alkali metal hydrides, such as lithium hydride, sodium hydride and potassium hydride; alkali metal alkoxides, such as sodium methoxide, sodium ethoxide and potassium fert-butoxide; alkali metal carbonates, such as lithium carbonate, sodium carbonate and potassium...
carbonate; alkali metal amides, such as lithium amide, sodium amide, potassium amide, lithium diisopropyl amide, potassium diisopropyl amide, sodium diisopropyl amide, lithium bis(trimethylsilyl)amide and potassium bis(trimethylsilyl)amide. Of these, sodium hydroxide is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 20 °C to about 100 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 60 minutes to about 24 hours, will usually suffice.

(B1c) coupling reaction

The reaction is normally and preferably effected in the presence of a base. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: halogenated hydrocarbons, such as dichloromethane, chloroform, carbon tetrachloride and 1,2-dichloroethane; ethers, such as diethyl ether, diisopropyl ether, tetrahydrofuran and dioxane; aromatic hydrocarbons, such as benzene, toluene and nitrobenzene; amides, such as formamide, \( \Lambda, \Lambda \)-dimethylformamide, \( \Lambda, \Lambda \)-dimethylacetamide and hexamethylphosphoronic triamide; amines, such as \( \Lambda \)-methylmorpholine, triethylamine, tripropylamine, tributylamine, disopropylethylamine, \( \Lambda \)-methylpiperidine, pyridine, 4-pyrrolidinopyridine, \( \Lambda, \Lambda \)-dimethylaniline and \( \Lambda, \Lambda \)-diethylaniline; alcohols, such as methanol, ethanol, propanol, 2-propanol and butanol; nitriles, such as acetonitrile and benzonitrile; sulfones, such as dimethyl sulfoxide and sulfolane; and ketones, such as acetone and diethylketone. Of these solvents, acetonitrile and tetrahydrofuran are preferred.

The reaction is carried out in the presence of a base. There is likewise no particular restriction on the nature of the bases used, and any base commonly used in reactions of this type may equally be used here. Examples of such bases include: alkali metal hydroxides, such as lithium hydroxide, sodium hydroxide and potassium hydroxide; alkali metal hydrides, such as lithium hydride, sodium hydride and potassium hydride; alkali metal alkoxides, such as sodium methoxide, sodium ethoxide and potassium tert-butoxide; alkali metal carbonates, such as lithium carbonate, sodium carbonate and potassium carbonate; alkali metal hydrogencarbonates, such as lithium hydrogencarbonate, sodium hydrogencarbonate and potassium hydrogencarbonate; amines, such as \( \Lambda \)-methylmorpholine, triethylamine, tripropylamine, tributylamine, disopropylethylamine, \( \Lambda \)-methylpiperidine, pyridine, 4-(\( \Lambda, \Lambda \)-dimethylamino)pyridine and DBU; and tetraalkylammonium fluorides, such as tetra-n-butylammonium fluoride (TBAF). Of these, TBAF is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 0 °C to about 100 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the
nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 5 minutes to about 72 hours will usually suffice.

(Bid) hydrogenation

The reaction is normally and preferably effected in the presence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: aromatic hydrocarbons, such as toluene; alcohols, such as methanol and ethanol; esters, such as ethyl acetate; and carboxylic acids, such as acetic acid. Of these solvents, alcohols and carboxylic acids are preferred.

The reaction is carried out under hydrogen atmosphere and in the presence of a catalyst. There is likewise no particular restriction on the nature of the catalysts used, and any catalysts commonly used in reaction of this type may equally be used here. Examples of such catalysts include: palladium on carbon, palladium hydroxide, platinum and Raney nickel. Of these catalysts, palladium on carbon is preferred.

In case that hydrodehalogenation (of substituent "Hal" in Reaction Scheme B) is a serious problem, the reaction may be carried out in the presence of an additive, which reduces activity of the catalyst employed. The additive is selected from substances known to show poisonous effect in some extent against the catalyst. Examples of such additives include: halide ion source, such as tetra-n-butylammonium bromide and sodium bromide; and sulfoxides, such as dimethyl sulfoxide. Of these, sodium bromide is preferred.

The reaction can take place under a wide range of pressures, and precise pressure is not critical to the invention. The preferred pressure will depend upon such factors as the nature of the starting materials, and the solvent. However, in general, it is convenient to carry out the reaction at a pressure of from 1 atm to about 10 atm. The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 0 °C to about 50 °C. The time required for the reaction may also vary widely, depending on many factors, notably the pressure of hydrogen, the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred condition outlined above, a period of from about 30 minutes to about 12 hours will usually suffice.

(Step B2)

In this step, the compound of formula (XIV) is prepared by Friedel Crafts reaction (B2a) after halogenation (B2b) or by cyclization (B2c) of the compound of formula (XIII) when R is a hydrogen atom, or by acidic cyclization (B2d) of the compound of formula (XIII) when R is a C1-C6 alkyl group.

(B2a) Friedel Crafts reaction

The reaction is normally and preferably effected in the presence or the absence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: halogenated hydrocarbons, such as dichloromethane,
chloroform, carbon tetrachloride, 1,1,2,2-tetrachloroethane and 1,2-dichloroethane; aromatic hydrocarbons, such as benzene, toluene and nitrobenzene; carbon disulfide; or mixed solvents thereof. Of these, dichloromethane or carbon disulfide is preferred.

The reaction is carried out in the presence of an acid. There is likewise no particular restriction on the nature of the acids used, and any acid commonly used in reactions of this type may equally be used here. Examples of such acids include: Lewis acids, such as BF₃, AlCl₃, AlBr₃, FeCl₃, AgCl, ZnI₂, ZnCl₂, Fe(NO₃)₃, CF₃SO₂Si(CH₃)₃, Yb(CF₃SO₂)₃ and SnCl₄. Of these, AlCl₃ is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 0 °C to about 150 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 30 minutes to about 24 hours, will usually suffice.

(B2b) halogenation

The reaction is normally and preferably effected in the presence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: halogenated hydrocarbons, such as dichloromethane, chloroform, carbon tetrachloride and 1,2-dichloroethane; ethers, such as diethyl ether, disopropyl ether, tetrahydrofuran and dioxane; amides, such as formamide, 4,4'-dimethylformamide, 4,4'-dimethylacetamide and hexamethylphosphor triamide; and nitriles, such as acetonitrile and benzonitrile; or mixed solvents thereof. Of these, 1,2-dichloroethane or dichloromethane is preferred.

The reaction is carried out in the presence of a halogenating agent. There is likewise no particular restriction on the nature of the halogenating agents used, and any halogenating agent commonly used in reactions of this type may equally be used here. Examples of such halogenating agents include: thionyl chloride, oxalyl chloride and phosphorus oxychloride. Of these, thionyl chloride is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 0 °C to about 80 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 10 minutes to about 12 hours will usually suffice.

(B2c) cyclization

The reaction is normally and preferably effected in the presence or absence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent.
Examples of suitable solvents include: halogenated hydrocarbons, such as dichloromethane, chloroform, carbon tetrachloride and 1,2-dichloroethane; ethers, such as diethyl ether, diisopropyl ether, tetrahydrofuran and dioxane; aromatic hydrocarbons, such as benzene, toluene and nitrobenzene; and amides, such as formamide, $\Lambda,N$-dimethylformamide, $\Lambda,N$-dimethylacetamide, $N$-methyl-2-pyrrolidinone, and hexamethylphosphoric triamide; or mixed solvents thereof. Of these, dichloromethane or the absence of solvent is preferred.

The reaction is carried out in the presence of an acid. There is likewise no particular restriction on the nature of the acids used, and any acid commonly used in reactions of this type may equally be used here. Examples of such acids include: acids, such as hydrochloric acid, sulfuric acid, or hydrobromic acid; acids, such as trifluoro acetic acid, or polyphosphoric acid. Of these, polyphosphoric acid is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 20 °C to about 150 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 30 minutes to about 24 hours, will usually suffice.

(B2d) acidic cyclization

The reaction is normally and preferably effected in the presence of an acid, which functions as solvent and reagent. There is no particular restriction on the nature of the acid to be employed, provided that it has no adverse effect on the reaction and that it can dissolve substrate, at least to some extent. Examples of suitable acids include: sulfuric acid and trifluoromethanesulfonic acid. Of these, trifluoromethanesulfonic acid is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 0 °C to about 150 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 30 minutes to about 24 hours, will usually suffice.

(Step B3)

In this step, the compound (XV) is prepared by reduction of the carbonyl group of the compound of formula (XIV). In case of employing the optically active reducing agent, the resulting compound of formula (XV) may be obtained as an optically active compound.

The reaction is normally and preferably effected in the presence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: halogenated hydrocarbons, such as dichloromethane, chloroform, carbon
tetrachloride and 1,2-dichloroethane; ethers, such as diethyl ether, diisopropyl ether, tetrahydrofuran and dioxane; aromatic hydrocarbons, such as benzene, toluene and nitrobenzene; sulfoxides, such as dimethyl sulfoxide and sulfolane; and alcohols, such as methanol, ethanol, propanol, 2-propanol and butanol; or mixed solvents thereof. Of these, methanol is preferred.

The reaction is carried out in the presence of a reducing agent. There is likewise no particular restriction on the nature of the reducing agents used, and any reducing agent commonly used in reactions of this type may equally be used here. Examples of such reducing agents include: metal borohydrides, such as sodium borohydride, lithium borohydride and sodium cyanoborohydride; hydride compounds, such as lithium aluminum hydride and diisobutyl aluminum hydride; and borane reagents, such as boran-tetrahydrofuran complex, boran-dimethyl sulfide complex (BMS) and 9-borabicyclo[3,3,1]nonane (9-BBN). Of these, sodium borohydride is preferred.

Concerning an optically active reducing agent, there is likewise no particular restriction on the nature of the reducing agents used, and any reducing agent commonly used in reactions of this type may equally be used here. Examples of such reducing agents include: the combination of (S) or (R)-tetrahydro-1-methyl-3,3-diphenyl-1 H,3H-pyrrolo[1,2-c][1,3,2]oxazaborole and BMS; the combination of the optically active ruthenium catalyst and hydrogen gas. Examples of the optically active ruthenium catalyst includes; dichloro[(S)-2,2'-bis(diphenylphosphino)-1 ,1'-binaphthyl][(S)-1 ,1'-bis(p-methoxyphenyl)-2-isopropyl-1 ,2-et hanediamine]ruthenium(II),
dichloro[(R)-2,2'-bis(diphenylphosphino)-1 ,1'-binaphthyl][(R)-1,1'-bis(p-methoxyphenyl)-2-isopropyl-1,2-et hanediamine]ruthenium(II). The ruthenium catalyst is used in the presence of a catalytic amount of potassium tert-butoxide. Of these, the combination of (S) or (R)-tetrahydro-1-methyl-3,3-diphenyl-1 H,3H-pyrrolo[1,2-c][1,3,2]oxazaborole and BMS is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about -78 °C to about 80 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 10 minutes to about 8 hours will usually suffice. (Step B4)

In this step, the compound of formula (XVI) is prepared by the azide displacement of the hydroxy group of the compound of formula (XV).

The reaction is normally and preferably effected in the presence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: aliphatic hydrocarbons, such as hexane, heptane and petroleum ether; ethers, such as diethyl ether, diisopropyl ether, tetrahydrofuran and dioxane; aromatic hydrocarbons, such as benzene, toluene and nitrobenzene; amides, such as formamide, N,N-dimethylformamide,
N,N-dimethylacetamide, N-methyl-2-pyrrolidinone and hexamethylphosphoric triamide; nitriles, such as acetonitrile and benzonitrile; sulfoxides, such as dimethyl suloxide and sulfolane; and ketones, such as acetone and diethylketone. Of these solvents, tetrahydrofuran is preferred.

The reaction is carried out in the presence of an azide agent such as diphenylphosphorylazide (DPPA).

In this reaction, a base can accelerate the reaction. The reaction may be carried out with or without a base. There is likewise no particular restriction on the nature of the bases used, and any base commonly used in reactions of this type may equally be used here. Examples of such bases include: organic amines, such as triethylamine, tripropyllamine, tributylamine, dicyclohexylamine, N,N-diisopropylethylamine, N-methylpiperdine, N-methylmorpholine, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 1,5-diazabicyclo[4.3.0]non-5-ene (DBN). Of these, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about -20 °C to about 150 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 10 minutes to about 24 hours will usually suffice.

(Step B5)

, In this step, the compound (IIa) is prepared by the hydrogenation of the azide moiety of the compound of formula (XVI).

The reaction is normally and preferably effected in the presence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: ethers, such as diethyl ether, disopropyl ether, tetrahydrofuran and dioxane; esters, such as ethyl acetate; amides, such as formamide, N,N-dimethylformamide, N,N-dimethylacetamide, N-methyl-2-pyrrolidinone and hexamethylphosphoric triamide; and alcohols, such as methanol, ethanol, propanol, 2-propanol and butanol. Of these solvents, methanol is preferred.

The reaction is carried out under hydrogen atmosphere and in the presence of a catalyst. There is likewise no particular restriction on the nature of the catalysts used, and any catalysts commonly used in reaction of this type may equally be used here. Examples of such catalysts include: palladium on carbon, palladium hydroxide, platinum and Raney nickel. Of these catalysts, palladium on carbon is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 0 °C to about 80 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected
under the preferred conditions outlined above, a period of from about 10 minutes to about 8 hours will usually suffice.

**Method C**

This illustrates the preparation of compounds of formula (Mlb) wherein B is CH₂.

**Reaction Scheme C**

In Reaction Scheme C, R⁰⁺¹ and R⁰⁺² independently represent a C₁-₆ alkyl group.

(Step C1)

In this step, the compound of formula (XVIII) is prepared by halogenation of the methyl group of the compound of formula (XVII).

The reaction is normally and preferably effected in the presence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: halogenated hydrocarbons, such as dichloromethane, chloroform, carbon tetrachloride and 1,2-dichloroethane; ethers, such as diethyl ether, diisopropyl ether, tetrahydrofuran and dioxane; nitriles, such as acetonitrile and benzonitrile; and sulfoxides, such as dimethyl sulfoxide and sulfolane; or mixed solvents thereof. Of these, carbon tetrachloride or 1,2-dichloroethane is preferred.

The reaction is carried out in the presence of a halogenating agent. There is likewise no particular restriction on the nature of the halogenating agents used, and any halogenating agent commonly used in reactions of this type may equally be used here. Examples of such halogenating agents include: succinimides, such as N-bromosuccinimide (NBS), N-chlorosuccinimide (NCS); bromine. Of these, NBS is preferred.

Reagents, such as benzoyl peroxide and 2,2’-azobis(isobutyronitrile) (AIBN) may be employed for this step. Of these, benzoyl peroxide is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to
carry out the reaction at a temperature of from about 0 °C to about 100 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 30 minutes to about 24 hours will usually suffice.

(Step C2)

In this step, the compound of formula (XX) is prepared by ether formation reaction of the compound of formula (XVIII) with the compound of formula (XIX), which is commercially available.

The reaction is normally and preferably effected in the presence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: ethers, such as diethyl ether, disopropyl ether, tetrahydrofuran and dioxane; aromatic hydrocarbons, such as benzene, toluene and nitrobenzene; amides, such as formamide, \(N_N\)-dimethylformamide, \(N_N\)-dimethylacetamide and hexamethylphosphoric triamide; nitriles, such as acetonitrile and benzonitrile; and sulfoxides, such as dimethyl sulfoxide and sulfolane; or mixed solvents thereof. Of these, \(N_N\)-dimethylformamide or tetrahydrofuran is preferred.

The reaction is carried out in the presence of a base. There is likewise no particular restriction on the nature of the bases used, and any base commonly used in reactions of this type may equally be used here. Examples of such bases include: alkali metal hydroxides, such as lithium hydroxide, sodium hydroxide and potassium hydroxide; alkali metal hydrides, such as lithium hydride, sodium hydride and potassium hydride; alkali metal alkoxides, such as sodium methoxide, sodium ethoxide and potassium tert-butoxide; alkali metal amides, such as lithium amide, sodium amide, potassium amide, lithium disopropyl amide, potassium bis(isopropyl) amide, sodium bis(isopropyl) amide, lithium bis(trimethylsilyl)amidate and potassium bis(trimethylsilyl)amidate. Of these, sodium hydride is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 20 °C to about 150 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 60 minutes to about 48 hours, will usually suffice.

(Step C3)

In this step, the compound of formula (XXI) is prepared by cyclization (Dieckmann Condensation) of the compound of formula (XX).

The reaction is normally and preferably effected in the presence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: ethers, such as diethyl ether, disopropyl ether, tetrahydrofuran and dioxane; aromatic hydrocarbons, such as benzene, toluene, xylene and nitrobenzene; and alcohols, such as
methanol, ethanol, propanol, 2-propanol and butanol; or mixed solvents thereof. Of these, toluene is preferred.

The reaction is carried out in the presence of a base. There is likewise no particular restriction on the nature of the bases used, and any base commonly used in reactions of this type may equally be used here. Examples of such bases include: alkali metal, such as lithium and sodium; alkali metal hydrides, such as lithium hydride, sodium hydride and potassium hydride; alkali metal alkoxides, such as sodium methoxide, sodium ethoxide and potassium tert-butoxide; alkali metal amides, such as lithium amide, sodium amide, potassium amide, lithium diisopropyl amide, potassium diisopropyl amide, sodium diisopropyl amide, lithium bis(trimethylsilyl)amide and potassium bis(trimethylsilyl)amide. Of these, potassium tert-butoxide and sodium are preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 0 °C to about 150 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 30 minutes to about 24 hours, will usually suffice.

(Step C4)

In this step, the compound of formula (XXVI) is prepared by decarboxylation of the compound of formula (XXI).

The reaction is normally and preferably effected in the presence of solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or the reagents involved and that it can dissolve reagents, at least to some extent. Examples of suitable solvents include: ethers, such as diethyl ether, diisopropyl ether, tetrahydrofuran and dioxane; amides, such as formamide, \( \Lambda, \Lambda \)-dimethylformamide, \( \Lambda, \Lambda \)-dimethylacetamide, \( \Lambda, \Lambda \)-methyl-2-pyrrolidinone, and hexamethylphosphoric triamide; alcohols, such as methanol, ethanol, propanol, 2-propanol, ethylene glycol and butanol; nitriles, such as acetonitrile and benzonitrile; sulfoxides, such as dimethyl sulfoxide and sulfolane; and water; or mixed solvents thereof. Of these, ethanol and water are preferred.

The reaction may be carried out in the presence of a base. There is likewise no particular restriction on the nature of the bases used, and any base commonly used in reactions of this type may equally be used here. Examples of such bases include: alkali metal hydrides, such as lithium hydride, sodium hydride and potassium hydride; alkali metal carbonates, such as lithium carbonate, sodium carbonate and potassium carbonate. Of these, sodium hydride is preferred.

The reaction may be carried out in the presence of an acid. There is likewise no particular restriction on the nature of the acids used, and any acid commonly used in reactions of this type may equally be used here. Examples of such acids include: carboxylic acids, such as acetic acid or propionic acid; and acids, such as hydrochloric acid, sulfuric acid, or hydrobromic acid. Of these, hydrochloric acid is preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction
temperature is not critical to the invention. The preferred reaction temperature will depend upon such factors as the nature of the solvent, and the starting materials. However, in general, it is convenient to carry out the reaction at a temperature of from about 20 °C to about 120 °C. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the starting materials and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from about 60 minutes to about 48 hours, will usually suffice.

(Step C5)

In this step, the compound of formula (XXVII) is prepared by reduction of the compound of formula (XXVI). The reaction may be carried out under the same condition as described in Step B3 of Method B.

(Step C6)

In this step, the compound of formula (XXVIII) is prepared by the azide displacement of the hydroxy group of the compound of formula (XXVII). The reaction may be carried out under the same condition as described in Step B4 of Method B.

(Step C7)

In this step, the compound of formula (1Mb) is prepared by reduction of the compound of formula (XXVIII). The reaction may be carried out under the same condition as described in Step B5 of Method B.

(Step C8)

In this step, the compound of formula (XXIV) is prepared by ether formation reaction of the compound of formula (XXII) with the compound of formula (XXIII), which is commercially available. The reaction may be carried out under the same condition as described in Step C2 of Method C.

(Step C9)

In this step, the compound of formula (XXV) is prepared by hydrolysis of the compound of formula (XXIV). The reaction may be carried out under the same condition as described in Step A4 of Method A.

(Step C10)

In this step, the compound of formula (XVI) is prepared by cyclization (C10a) of the compound of formula (XXV) or by formation of acid halide (C10b) followed by Friedel Crafts reaction (C10c) of the compound of formula (XXV). The reaction may be carried out under the same condition as described in Step B2 of Method B.

The preparation/isolation of individual enantiomers can be prepared by conventional techniques, such as chiral synthesis from a suitable optically pure precursor which may be prepared according to the Method C or resolution of the racemate (or the racemate of a salt or derivative) using, for example, chiral high-pressure liquid chromatography (HPLC) and supercritical fluid chromatography (SFC).

Alternatively, a method of optical resolution of a racemate (or a racemic precursor) can be appropriately selected from conventional procedures, for example, preferential crystallization, or resolution of diastereomeric salts between a basic moiety of the compound of formula (I) and a suitable optically active acid such as tartaric acid.

The compounds of formula (I), and the intermediates in the above-mentioned preparation
methods can be isolated and purified by conventional procedures, such as distillation, recrystallization or chromatographic purification.

Compounds of the invention intended for pharmaceutical use may be administered as crystalline or amorphous products. They may be obtained, for example, as solid plugs, powders, or films by methods such as precipitation, crystallization, freeze-drying, spray drying, or evaporative drying. Microwave or radio frequency drying may be used for this purpose.

They may be administered alone or in combination with one or more other compounds of the invention or in combination with one or more other drugs (or as any combination thereof). Generally, they will be administered as a pharmaceutical composition or formulation in association with one or more pharmaceutically acceptable carriers or excipients. The term "carrier" or "excipient" is used herein to describe any ingredient other than the compound(s) of the invention. The choice of carrier or excipient will to a large extent depend on factors such as the particular mode of administration, the effect of the excipient on solubility and stability, and the nature of the dosage form.

Pharmaceutical compositions suitable for the delivery of compounds of the present invention and methods for their preparation will be readily apparent to those skilled in the art. Such compositions and methods for their preparation may be found, for example, in 'Remington's Pharmaceutical Sciences', 19th Edition (Mack Publishing Company, 1995).

ORAL ADMINISTRATION

The compounds of the invention may be administered orally. Oral administration may involve swallowing, so that the compound enters the gastrointestinal tract, or buccal or sublingual administration may be employed by which the compound enters the blood stream directly from the mouth.

Formulations suitable for oral administration include solid formulations such as, for example, tablets, capsules containing particulates, liquids, or powders, lozenges (including liquid-filled), chews, multi- and nano-particulates, gels, solid solution, liposome, films (including muco-adhesive), ovules, sprays and liquid formulations.

Liquid formulations include, for example, suspensions, solutions, syrups and elixirs. Such formulations may be employed as fillers in soft or hard capsules and typically comprise a carrier, for example, water, ethanol, polyethylene glycol, propylene glycol, methylcellulose, or a suitable oil, and one or more emulsifying agents and/or suspending agents. Liquid formulations may also be prepared by the reconstitution of a solid, for example, from a sachet.

The compounds of the invention may also be used in fast-dissolving, fast-disintegrating dosage forms such as those described in Expert Opinion in Therapeutic Patents, H (6), 981-986 by Liang and Chen (2001).

For tablet dosage forms, depending on dose, the drug may make up from about 1 wt% to about 80 wt% of the dosage form, more typically from about 5 wt% to about 60 wt% of the dosage form. In addition to the drug, tablets generally contain a disintegrant. Examples of disintegrants include sodium starch glycolate, sodium carboxymethyl cellulose, calcium carboxymethyl cellulose, croscarmellose sodium, crospovidone, polyvinylpyrrolidone, methyl cellulose, microcrystalline cellulose, lower alkyl-substituted hydroxypropyl cellulose, starch, pregelatinised starch and sodium alginate. Generally, the
disintegrant will comprise from about 1 wt% to about 25 wt%, preferably from about 5 wt% to about 20 wt% of the dosage form.

Binders are generally used to impart cohesive qualities to a tablet formulation. Suitable binders include microcrystalline cellulose, gelatin, sugars, polyethylene glycol, natural and synthetic gums, polyvinylpyrrolidone, pregelatinised starch, hydroxypropyl cellulose and hydroxypropyl methylcellulose. Tablets may also contain diluents, such as lactose (monohydrate, spray-dried monohydrate, anhydrous and the like), mannitol, xylitol, dextrose, sucrose, sorbitol, microcrystalline cellulose, starch and dibasic calcium phosphate dihydrate.

Tablets may also optionally comprise surface-active agents, such as sodium lauryl sulfate and polysorbate 80, and glidants such as silicon dioxide and talc. When present, surface active agents may comprise from about 0.2 wt% to about 5 wt% of the tablet, and glidants may comprise from about 0.2 wt% to about 1 wt% of the tablet.

Tablets also generally contain lubricants such as magnesium stearate, calcium stearate, zinc stearate, sodium stearyl fumarate, and mixtures of magnesium stearate with sodium lauryl sulphate.

Lubricants generally comprise from about 0.25 wt% to about 10 wt%, preferably from about 0.5 wt% to about 3 wt% of the tablet.

Other possible ingredients include anti-oxidants, colourants, flavouring agents, preservatives and taste-masking agents.

Exemplary tablets contain up to about 80% drug, from about 10 wt% to about 90 wt% binder, from about 0 wt% to about 85 wt% diluent, from about 2 wt% to about 10 wt% disintegrant, and from about 0.25 wt% to about 10 wt% lubricant.

Tablet blends may be compressed directly or by roller to form tablets. Tablet blends or portions of blends may alternatively be wet-, dry-, or melt-granulated, melt congealed, or extruded before tableting. The final formulation may comprise one or more layers and may be coated or uncoated; it may even be encapsulated.


Solid formulations for oral administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

Suitable modified release formulations for the purposes of the invention are described in US Patent No. 6,1 06,864. Details of other suitable release technologies such as high energy dispersions and osmotic and coated particles are to be found in Verma et al, Pharmaceutical Technology On-line, 25(2), 1-14 (2001). The use of chewing gum to achieve controlled release is described in WO00/35298.

PARENTERAL ADMINISTRATION

The compounds of the invention may also be administered directly into the blood stream, into muscle, or into an internal organ. Suitable means for parenteral administration include intravenous, intraarterial, intraperitoneal, intrathecal, intraventricular, intraurethral, intrasternal, intracranial, intramuscular and subcutaneous. Suitable devices for parenteral administration include needle (including
microneedle) injectors, needle-free injectors and infusion techniques.

Parenteral formulations are typically aqueous solutions which may contain excipients such as salts, carbohydrates and buffering agents (preferably to a pH of from about 3 to about 9), but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water.

The preparation of parenteral formulations under sterile conditions, for example, by lyophilisation, may readily be accomplished using standard pharmaceutical techniques well known to those skilled in the art.

The solubility of compounds of formula (I) used in the preparation of parenteral solutions may be increased by the use of appropriate formulation techniques, such as the incorporation of solubility-enhancing agents.

Formulations for parenteral administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release. Thus compounds of the invention may be formulated as a solid, semi-solid, or thixotropic liquid for administration as an implanted depot providing modified release of the active compound. Examples of such formulations include drug-coated stents and PGLA microspheres.

TOPICAL ADMINISTRATION

The compounds of the invention may also be administered topically to the skin or mucosa, that is, dermally or transdermally. Typical formulations for this purpose include gels, hydrogels, lotions, solutions, creams, ointments, dusting powders, dressings, foams, films, skin patches, wafers, implants, sponges, fibres, bandages and microemulsions. Liposomes may also be used. Typical carriers include alcohol, water, mineral oil, liquid petrolatum, white petrolatum, glycerin, polyethylene glycol and propylene glycol. Penetration enhancers may be incorporated - see, for example, J Pharm Sci, 88 (10), 955-958 by Finnin and Morgan (October 1999).

Other means of topical administration include delivery by electroporation, iontophoresis, phonophoresis, sonophoresis and microneedle or needle-free (e.g. Powderject™, Bioject™, etc.) injection.

Formulations for topical administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

INHALED/INTRANASAL ADMINISTRATION

The compounds of the invention can also be administered intranasally or by inhalation, typically in the form of a dry powder (either alone, as a mixture, for example, in a dry blend with lactose, or as a mixed component particle, for example, mixed with phospholipids, such as phosphatidylcholine) from a dry powder inhaler or as an aerosol spray from a pressurized container, pump, spray, atomiser (preferably an atomiser using electrohydrodynamics to produce a fine mist), or nebuliser, with or without the use of a suitable propellant, such as 1,1,1,2-tetrafluoroethane or 1,1,1,2,3,3,3-heptafluoropropane. For intranasal use, the powder may comprise a bioadhesive agent, for example, chitosan or cyclodextrin.

The pressurized container, pump, spray, atomizer, or nebuliser contains a solution or suspension
of the compound(s) of the invention comprising, for example, ethanol, aqueous ethanol, or a suitable alternative agent for dispersing, solubilising, or extending release of the active, a propellant(s) as solvent and an optional surfactant, such as sorbitan trioleate, oleic acid, or an oligolactic acid.

Prior to use in a dry powder or suspension formulation, the drug product is micronised to a size suitable for delivery by inhalation (typically less than 5 microns). This may be achieved by any appropriate comminuting method, such as spiral jet milling, fluid bed jet milling, supercritical fluid processing to form nanoparticles, high pressure homogenization, or spray drying.

Capsules (made, for example, from gelatin or HPMC), blisters and cartridges for use in an inhaler or insufflator may be formulated to contain a powder mix of the compound of the invention, a suitable powder base such as lactose or starch and a performance modifier such as β-leucine, mannitol, or magnesium stearate. The lactose may be anhydrous or in the form of the monohydrate, preferably the latter. Other suitable excipients include dextran, glucose, maltose, sorbitol, xylitol, fructose, sucrose and trehalose.

A suitable solution formulation for use in an atomiser using electrohydrodynamics to produce a fine mist may contain from about 1µg to about 20mg of the compound of the invention per actuation and the actuation volume may vary from about 1µl to about 100µl. A typical formulation may comprise a compound of formula (I), propylene glycol, sterile water, ethanol and sodium chloride. Alternative solvents which may be used instead of propylene glycol include glycerol and polyethylene glycol.

Suitable flavors, such as menthol and levomenthol, or sweeteners, such as saccharin or saccharin sodium, may be added to those formulations of the invention intended for inhaled/intranasal administration. Formulations for inhaled/intranasal administration may be formulated to be immediate and/or modified release using, for example, poly(DL-lactic-coglycolic acid (PGLA). Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

In the case of dry powder inhalers and aerosols, the dosage unit is determined by means of a valve which delivers a metered amount. Units in accordance with the invention are typically arranged to administer a metered dose or "puff" containing from about 1 to about 100 µg of the compound of formula (I). The overall daily dose will typically be in the range about 50 µg to about 20 mg which may be administered in a single dose or, more usually, as divided doses throughout the day.

RECTAL/INTRAVAGINAL ADMINISTRATION

The compounds of the invention may be administered rectally or vaginally, for example, in the form of a suppository, pessary, or enema. Cocoa butter is a traditional suppository base, but various alternatives may be used as appropriate.

Formulations for rectal/vaginal administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

OTHER TECHNOLOGIES

The compounds of the invention may be combined with soluble macromolecular entities, such as cyclodextrin and suitable derivatives thereof or polyethylene glycol-containing polymers, in order to
improve their solubility, dissolution rate, taste-masking, bioavailability and/or stability for use in any of the aforementioned modes of administration.

Drug-cyclodextrin complexes, for example, are found to be generally useful for most dosage forms and administration routes. Both inclusion and non-inclusion complexes may be used. As an alternative to direct complexation with the drug, the cyclodextrin may be used as an auxiliary additive, i.e. as a carrier, diluent, or solubiliser. Most commonly used for these purposes are alpha-, beta- and gamma-cyclodextrins, examples of which may be found in WO91/11172, WO94/02518 and WO98/55148.

KIT-OF-PARTS

Inasmuch as it may be desirable to administer a combination of active compounds, for example, for the purpose of treating a particular disease or condition, it is within the scope of the present invention that two or more pharmaceutical compositions, at least one of which contains a compound in accordance with the invention, may conveniently be combined in the form of a kit suitable for co-administration of the compositions.

Thus the kit of the invention comprises two or more separate pharmaceutical compositions, at least one of which contains a compound of formula (I) in accordance with the invention, and means for separately retaining said compositions, such as a container, divided bottle, or divided foil packet. An example of such a kit is the familiar blister pack used for the packaging of tablets, capsules and the like.

The kit of the invention is particularly suitable for administering different dosage forms, for example, oral and parenteral, for administering the separate compositions at different dosage intervals, or for titrating the separate compositions against one another. To assist compliance, the kit typically comprises directions for administration and may be provided with a so-called memory aid.

DOSAGE

For administration to human patients, the total daily dose of the compounds of the invention is typically in the range of about 0.05 mg to about 500 mg depending, of course, on the mode of administration, preferred in the range of about 0.1 mg to about 400 mg and more preferred in the range of about 0.5 mg to about 300 mg. For example, oral administration may require a total daily dose of from about 1 mg to about 300 mg, while an intravenous dose may only require from about 0.5 mg to about 100 mg. The total daily dose may be administered in single or divided doses.

These dosages are based on an average human subject having a weight of about 65 kg to about 70 kg. The physician will readily be able to determine doses for subjects whose weight falls outside this range, such as infants and the elderly.

COMBINATIONS

As discussed above, a compound of the invention exhibits acid pump inhibitory activity. An acid pump antagonist of the present invention may be usefully combined with another pharmacologically active compound, or with two or more other pharmacologically active compounds, particularly in the treatment of gastroesophageal reflux disease. For example, an acid pump antagonist, particularly a compound of the formula (I), or a pharmaceutically acceptable salt thereof, as defined above, may be administered simultaneously, sequentially or separately in combination with one or more agents selected from:
(i) histamine H₂ receptor antagonists, e.g. ranitidine, lansoprazole, nizatidine, cimetidine, famotidine and oxatidine;
(ii) proton pump inhibitors, e.g. omeprazole, esomeprazole, pantoprazole, rabeprazole, tenatoprazole, ilaprazole and lansoprazole;
(iii) oral antacid mixtures, e.g. Maalox®, Aludrox® and Gaviscon®;
(iv) mucosal protective agents, e.g. polaprezinc, ebcabut sodium, rebamipide, teprenone, cetroxate, sucralate, chloropryline-copper and plaunotol;
(v) anti-gastric agents, e.g. Anti-gastrin vaccine, itriglumide and Z-360;
(vi) 5-HT₃ antagonists, e.g. dolasetron, palonosetron, aholsetron, azasetron, ramosetron, mitrazapine, gransetron, tropisetron, E-3620, ondansetron and indisetron;
(vii) 5-HT₄ agonists, e.g. tegaserod, mosapride, cinapride and oxitriptane;
(viii) laxatives, e.g. Trifyba®, Fybo®gell®, Konysyl®, Isogel®, Regulan®, Celevac® and Normacol®;
(ix) GABA_A agonists, e.g. baclofen and AZD-3355;
(x) GABAB antagonists, e.g. GAS-360 and SGS-742;
(xii) calcium channel blockers, e.g. arandipine, lacidipine, faludipine, azelnidipine, clindipine, lomerizine, dilizazem, gallopam, efonidipine, nisoldipine, amlodipine, lercanidipine, bevantolol, nicardipine, isradipine, benidipine, verapamil, nitrendipine, barnadipine, propafenone, manidipine, bepridil, nifedipine, nilvadipine, nimodipine and fasudil;
(xii) dopamine antagonists, e.g. metoclopramide, domperidone and levosulpiride;
(xiii) Tachykinin (NK) antagonists, particularly NK-3, NK-2 and NK-1 antagonists, e.g. napadutant, saredutant, talnetant, (αR,9R)-7-[[3,5-bis(trifluoromethyl)benzyl]-8,9,1 0.11-tetrahydro-9-methyl-5- (4-methylphenyl)-7H-[1.4]diazocino[2,1-g][1,7]naphthidine-6-1 3-dione (TAK-637), 5-[[2R,3S]-2- (1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy-3-(4-fluorophenyl)-4-morpholinyl[methyl]-1 ,2-dihydro-3 H-1,2,4-thazol-3-one (MK-869), lanepitant, dapitant and 3-[[2-methoxy-5-(trifluoromethoxy)phenyl] methylamino]-2-phenyl-piperidine (2S,3S);
(xiv) Helicobacter pylori infection agents, e.g. clarithromicyn, roxithromycin, rokitamycin, flurithromycin, telithromycin, amoxicillin, ampicillin, temocillin, bacampicillin, azasplacicin, sulfamcin, piperecinilin, lenamicilin, tetracycline, metronidazole, bithmuth citrate and bithmuth subsalicylate;
(xv) nitric oxide synthase inhibitors, e.g. GW-274150, tilarginine, P54, guanidioethyldisulfide and nitrofuriprofen;
(xvi) vanilloid receptor 1 antagonists, e.g. AMG-517 and GW-705498;
(xvii) muscarinic receptor antagonists, e.g. trosipim, solifenacin, tolterodine, tiotropium, cimetropium, oxitropium, ipratropium, tiquizium, dalifenacin and imidafencin;
(xviii) calmodulin antagonists, e.g. squalamine and DY-9760;
(xix) potassium channel agonists, e.g. pinacidil, tilsolol, nicorandil, NS-8 and retigabine;
(xx) beta-1 agonists, e.g. dobutamine, denopamine, xamoterol, denopamine, docarpamine and xamoterol;
(xxi) beta-2 agonists, e.g. salbutamol; terbutaline, arformoterol, meluadrine, mabuterol, ritodrine, fenoterol, clenbuterol, formoterol, procaterol, tulobuterol, pirbuterol, bambuterol, tulobuterol, dopexamine and levosalbutamol;


Method for assessing biological activities

The acid pump inhibitory activity and other biological activities of the compounds of this invention were determined by the following procedures. Symbols have their usual meanings: mL (milliliter(s)), µL (microliter(s)), Kg (kilogram(s)), g (gram(s)), mg (milligram(s)), µg (microgram(s)), pmol (pico molar(s)), mmol (milli molar(s)), M (molar mass (m⁹/mol)), mM (mili molar mass), µM (micro molar mass), quant. (quantitative yield), nm (nanometer(s)), min (minute(s)), Cat# (catalog number), mV (millivolt(s)), ms (millisecond(s)), i.p. (intraperitoneal).

Preparation of gastric vesicles from fresh porcine stomachs

The porcine gastric vesicles for Porcine gastric H⁺/K⁺-ATPase inhibition assays were prepared from mucous membrane in fresh porcine stomachs by homogenization with a tight-fitted polytetrafluoroethylene (Teflon®) homogenizer in 0.25 M sucrose at 4 °C. The crude pellet was removed with centrifugation at 20,000 g for 30 min. Then supernatant was centrifuged at 100,000 g for 30 min. The resulting pellet was re-suspended in 0.25 M sucrose, and then subjected to density gradient centrifugation at 132,000 g for 90 min. The gastric vesicles were collected from interface on 0.25 M sucrose layer containing 7% Ficoll™ PM400(Amersham Biosciences). This procedure was performed in a cold room.

Ion-leaky Porcine gastric H⁺/K⁺-ATPase inhibition

Ion-leaky porcine gastric H⁺/K⁺-ATPase inhibition was measured according to the modified
method described in Biochemical Pharmacology, 1988, 37, 2231-2236.

The isolated vesicles were lyophilized, and then kept in deep-freezer until use. For enzyme assay, lyophilized vesicles were reconstituted with 3 mM MgSO₄ containing 40 mM Bis-tris (pH 6.4 at 37°C).

Enzyme reaction was performed incubating 5 mM KCl, 3 mM Na₂ATP, 3 mM MgSO₄ and 1.0 µg of reconstituted vesicles for 30 minutes at 37°C in a final 60 µl of reaction mixture (40 mM Bis-tris, pH 6.4) with or without the test compound. Enzyme reaction was stopped by adding 10% sodium dodecyl sulphate (SDS). Released inorganic phosphate from ATP was detected by incubation with mixture of 1 part of 35 mM ammonium molybdate tetrahydrate in 15 mM Zinc acetate hydrate and 4 parts of 10% ascorbic acid (pH 5.0), resulting in phosphomolybdate, which has optical density at 750 nm. All example compounds showed potent inhibitory activity.

**Ion-tight porcine gastric H⁺/K⁺-ATPase inhibition**

Ion-tight porcine gastric H⁺/K⁺-ATPase inhibition was measured according to the modified method described in Biochemical Pharmacology, 1988, 37, 2231-2236.

The isolated vesicles were kept in deep-freezer until use. For enzyme assay, vesicles were diluted with 3 mM MgSO₄ containing 5 mM Tris (pH 7.4 at 37°C).

Enzyme reaction was performed incubating 150 mM KCl, 3 mM Na₂ATP, 3 ml/l MgSO₄, 15 µM valinomycin and 3.0 µg of vesicles for 30 minutes at 37°C in a final 60 µl of reaction mixture (5mM Tris, pH 7.4) with or without the test compound. Enzyme reaction was stopped by adding 10% SDS. Released inorganic phosphate from ATP was detected by incubating with mixture of 1 part of 35 mM ammonium molybdate tetrahydrate in 15 mM Zinc acetate hydrate and 4 parts of 10% ascorbic acid (pH 5.0), resulting in phosphomolybdate, which has optical density at 750 nm.

The results of IC₅₀ values of the inhibitory activity for the compounds of following examples are shown in Table 1.

**Table 1.**

<table>
<thead>
<tr>
<th>Example No.</th>
<th>IC₅₀ (µM)</th>
<th>Example No.</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>0.31</td>
<td>5-1</td>
<td>0.30</td>
</tr>
<tr>
<td>2-2</td>
<td>0.29</td>
<td>5-2</td>
<td>0.18</td>
</tr>
<tr>
<td>2-3</td>
<td>0.55</td>
<td>5-3</td>
<td>0.35</td>
</tr>
<tr>
<td>4-1</td>
<td>0.39</td>
<td>6-1</td>
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<td>4-2</td>
<td>0.42</td>
<td>6-2</td>
<td>0.31</td>
</tr>
<tr>
<td>4-3</td>
<td>0.83</td>
<td>6-3</td>
<td>0.48</td>
</tr>
</tbody>
</table>

All the tested compounds showed acid pump antagonistic activity.

**Canine kidney Na⁺/K⁺-ATPase inhibition**

The powdered canine kidney Na7K⁺-ATPase (Sigma) was reconstituted with 3 mM MgSO₄ containing 40 mM Tris (pH 7.4 at 37°C). Enzyme reaction was performed incubating 100 mM NaCl, 2 mM KCl, 3 mM Na₂ATP, 3 mM MgSO₄ and 12 µg of enzyme for 30 minutes at 37°C in a final 60 µl of reaction mixture (40 mM Tris, pH 7.4) with or without the test compound. Enzyme reaction was stopped by adding 10% SDS. Released inorganic phosphate from ATP was detected by incubating with mixture of 1 part of 35 mM ammonium molybdate tetrahydrate in 15 mM Zinc acetate hydrate and 4 parts of 10% ascorbic acid...
(pH 5.0), resulting in phosphomolybdate, which has optical density at 750 nm.

**Inhibition of acid secretion in the gastric lumen-perfused rat**

Acid secretion in the gastric lumen-perfused rat was measured according to Watanabe et al. [Watanabe et al., J. Physiol. (Paris) 2000; 94: 111-116]. Male Sprague-Dawley rats, 8 weeks old, deprived of food for 18 hours before the experiment with free access to water, were anesthetized with urethane (1.4 g/kg, i.p.) and tracheotomized. After a middle abdominal incision, a dual polyethylene cannula was inserted into the forestomach and the stomach was perfused with saline (37 °C, pH 5.0) at a rate of 1 ml/min. The acid output in the perfusate was determined at 5 minutes interval by titration with 0.02 M NaOH to pH 5.0. After the determination of basal acid secretion for 30 min, the acid secretion was stimulated by a continuous intravenous infusion of pentagastrin (16 μg/kg/h). The test compounds were administered by an intravenous bolus injection or intraduodenal administration after the stimulated acid secretion reached a plateau phase. The acid secretion was monitored after the administration.

The activity was evaluated either inhibition of total acid secretion from 0 hours to 1.5 or 3.5 hours after administration or the maximum inhibition after administration.

The compound of Example 2 and 4 showed a good inhibitory activity.

**Inhibition of gastric acid secretion in the Heidenhain pouch dog**

Male Beagle dogs weighing 7 - 15 kg with Heidenhain pouch [Heidenhain R, Arch Ges Physiol. 1879; 19: 148-167] were used. The animals were allowed to recover from surgery for at least three weeks before the experiments. The animals were kept at a 12 hour light-dark rhythm, housed singly. They received standard food once daily at 11:00 a.m. and tap water ad libitum, and were fasted overnight prior to the experiment, with free access to water. Gastric juice samples were collected throughout the experiment by gravity drainage every 15 min. Acidity in the gastric juice was measured by titration to the end point of pH 7.0. Acid secretion was stimulated by a continuous intravenous infusion of histamine (80 μg/kg/h). Oral or intravenous bolus administration of the test compounds was done 90 minutes after commencement of the histamine infusion. The acid secretion was monitored after the administration. The activity was evaluated by the maximum inhibition relative to the corresponding control value.

**Human dofetilide binding**

Human ether a-go-go related gene (HERG) transfected HEK293S cells were prepared and grown in-house. Cell paste of HEK-293 cells expressing the HERG product can be suspended in 10-fold volume of 50 mM Tris buffer adjusted at pH 7.5 at 25 °C with 2 M HCl containing 1 mM MgCl₂, 10 mM KCl. The cells were homogenized using a Polytron homogenizer (at the maximum power for 20 seconds) and centrifuged at 48,000 g for 20 minutes at 4°C. The pellet was resuspended, homogenized and centrifuged once more in the same manner. The resultant supernatant was discarded and the final pellet was resuspended (10-fold volume of 50 mM Tris buffer) and homogenized at the maximum power for 20 seconds. The membrane homogenate was aliquoted and stored at -80°C until use. An aliquot was used for protein concentration determination using a Protein Assay Rapid Kit (wako) and Spectra max plate reader (Wallac). All the manipulation, stock solution and eequalsDiment were keDt on ice at all times. For
saturation assays, experiments were conducted in a total volume of 200 µl. Saturation was determined by incubating 36 µl of [3H]-dofetilide, and 160 µl of membrane homogenates (20-30 µg protein per well) for 60 minutes at room temperature in the absence or presence of 10µM dofetilide at final concentrations (4 µl) for total or nonspecific binding, respectively. All incubations were terminated by rapid vacuum filtration over PEI soaked glass fiber filter papers using Skatron cell harvester followed by two washes with 50 mM Tris buffer (pH 7.4 at 25 °C). Receptor-bound radioactivity was quantified by liquid scintillation counting using Packard LS counter.

For the competition assay, compounds were diluted in 96 well polypropylene plates as 4-point dilutions in semi-log format. All dilutions were performed in DMSO first and then transferred into 50 mM Tris buffer (pH 7.4 at 25 °C) containing 1 mM MgCl₂, 10 mM KCl so that the final DMSO concentration became equal to 1%. Compounds were dispensed in triplicate in assay plates (4 µl). Total binding and nonspecific binding wells were set up in 6 wells as vehicle and 10 µM dofetilide at final concentration, respectively. The radioligand was prepared at 5.6x final concentration and this solution was added to each well (36 µl). The assay was initiated by addition of YSi poly-L-lysine SPA beads (50 µl, 1 mg/well) and membranes (110 µl, 20 µg/well). Incubation was continued for 60 minutes at room temperature. Plates were incubated for a further 3 hours at room temperature for beads to settle. Receptor-bound radioactivity was quantified by counting Wallac MicroBeta plate counter.

**PAMPA (parallel artificial membrane permeation assay)**

Experiments were performed in 96-well acceptor and donor plates. Such 96-well system was described in J. Med. Chem., 1996, 41, 1007. 4% phosphatidylcholine and 1% stearic acid in dodecane were used as artificial membrane material. The acceptor plate (96 well hydrophobic filter plate (MAIP N45, Millipore)) was prepared by adding 5 µL of artificial membrane material on the top of the filter and the plate was filled with 250 µL of 2-(N-morpholino)ethanesulfonic acid (MES) buffered Hank’s balanced salt solution (HBSS) (pH 6.5). The donor plate (Transport Receiver plate (MATRNPS50, Millipore)) was filled with 300 µL of MES buffered HBSS (pH 6.5) containing 10 µM of the test compounds. The acceptor plate was placed onto the donor plate to form a “sandwich” and was incubated at 30° C for 2.5 hours. After the incubation period, acceptor, donor and initial donor solution (reference) were analyzed via LC-MS/MS. Data were reported as the effective permeability value in cm X 106/sec and the membrane retention value.

**Half-life in human liver microsomes (HLM)**

Test compounds (1 µM) were incubated with 1 mM MgCl₂, 1 mM NADP⁺, 5 mM isocitric acid, 1U/mL isocitric dehydrogenase and 0.8 mg/mL HLM in 100 mM potassium phosphate buffer (pH 7.4) at 37°C on a number of 384-well plates. At several time points, a plate was removed from the incubator and the reaction was terminated with two incubation volumes of acetonitrile. The compound concentration in supernatant was measured by LC/MS/MS system. The intrinsic clearance value was calculated using following equations:

\[
Cl_{in} \ (\text{ul/min/mg protein}) = k \times \text{incubation volume} \\
\text{Protein concentration}
\]

Where, \( k = - \) slope of ln(concentration) vs. time (min⁻¹)
**hERG patch clamp assay**

To determine the potential of compounds to inhibit the hERG channel, the cloned counterpart of the rapidly inactivating delayed rectifier potassium current (IKr).

HEK293 cells stably expressing the hERG channel were used in whole-cell patch clamp electrophysiology studies at ambient temperature (26.5-28.5°C). The methodology for stable transfection of this channel in HEK293 cells can be found elsewhere (Zhou et al 1998, Biophysical Journal, 74, pp230-241). The solutions used for experimentation were standard extracellular solution of the following composition (mM): NaCl, 137; KCl, 4; CaCl2, 1.8; MgCl2, 1; Glucose, 10; HEPES, 10; pH 7.4 ± 0.05 with NaOH/HCl; and standard intracellular solution of the following composition (mM): KCl, 130; MgCl2, 1; HEPES, 10; EGTA, 5; MgATP, 5; pH 7.2 ± 0.05 with KOH. The voltage protocol applied was designed to activate the hERG channel and allow the measurement of drug block of the channel and is as follows. First the membrane potential was stepped from a holding potential of -80mV to +30mV for 1s. This was followed by a descending voltage ramp at a rate of 0.5mV/ms back to holding potential of -80mV and the peak outward current observed during the repolarizing ramp was measured. This protocol was evoked repeatedly every 4 seconds (0.25Hz). After establishing a stable baseline period in the presence of vehicle (0.1% v/v DMSO), four increasing concentrations of test compound were then bath-applied sequentially until the response reached steady-state or 10 minutes (whichever occurred first). 10 micromol/L dofetilide was used at the end of each experiment as an internal positive control and to define maximum block.

**Bioavailability in rat**

Adult rats of the Sprague-Dawley strain were used. One to two days prior to the experiments all rats were prepared by cannulation of the right jugular vein under anesthesia. The cannula was exteriorized at the nape of the neck. Blood samples (0.2-0.3 mL) were drawn from the jugular vein at intervals up to 24 hours after intravenous or oral administrations of the test compound. The samples were frozen until analysis. Bioavailability was assessed by calculating the quotient between the area under plasma concentration curve (AUC) following oral administration or intravenous administration.

**Bioavailability in dog**

Adult Beagle dogs were used. Blood samples (0.2-0.5 mL) were drawn from the cephalic vein at intervals up to 24 hours after intravenous or oral administrations of the test compound. The samples were frozen until analysis. Bioavailability was assessed by calculating the quotient between the area under plasma concentration curve (AUC) following oral administration or intravenous administration.

**Plasma protein binding**

Plasma protein binding of the test compound (1 µM) was measured by the method of equilibrium dialysis using 96-well plate type equipment. Spectra-Por®, regenerated cellulose membranes (molecular weight cut-off 12,000-14,000, 22 mm x 120 mm) were soaked for over night in distilled water, then for 20 minutes in 30% ethanol, and finally for 15 minutes in dialysis buffer (Dulbecco's phosphate buffered saline,
pH 7.4). Frozen plasma of human, Sprague-Dawley rats, and Beagle dogs were used. The dialysis equipment was assembled and added 150 µL of compound-fortified plasma to one side of each well and 150 µL of dialysis buffer to the other side of each well. After 4 hours incubation at 37 °C for 150 r.p.m, aliquots of plasma and buffer were sampled. The compound in plasma and buffer were extracted with 300 µL of acetonitrile containing internal standard compounds for analysis. The concentration of the compound was determined with LC/MS/MS analysis.

The fraction of the compound unbound was calculated by the following equation:

\[ f_u = 1 - \left(\frac{[\text{plasma}]_{eq} - [\text{buffer}]_{eq}}{[\text{plasma}]_{eq}}\right) \]

wherein [plasma]_{eq} and [buffer]_{eq} are the concentrations of the compound in plasma and buffer, respectively.

**Aqueous solubility**

Aqueous solubility in the mediums (a)-(c) was determined by following method:

Whatman mini-UniPrep chambers (Clifton, NJ, USA) containing more than 0.5 mg of compound and 0.5 mL of each medium were shaken overnight (over 8 hours) at room temperature. All samples were filtered through a 0.45 µm Polyvinylidene Difluoride (PVDF) membrane into the Whatman mini-UniPrep plunger before analysis. The filtrates were assayed by HPLC.

<medium>(a) Simulated gastric fluid with no enzyme (SGN) at pH 1.2: Dissolve 2.0 g of NaCl in 7.0 mL of 10 M HCl and sufficient water to make 1000 mL; (b) Phosphate buffer saline (PBS) at pH 6.5: Dissolve 6.35 g of KH₂PO₄, 2.84 g of Na₂HPO₄ and 5.50 g of NaCl in sufficient water to make 1000 mL, adjusting the pH to 6.5; (c) 3.94 mg of sodium taurocholate (NaTC) and 1.06 mg of 1-palmitoyl-2-oleyl-L-phosphatidylcholine (POPC) in 1 mL of PBS (pH 6.5).

**Estimation of hepatic clearance using the metabolic stability in human hepatocytes**

Tested compounds (1 µM) were incubated statically with hepatocytes from human at 37 °C in a 95 % air/ 5 % CO₂ with target cell density of 0.5 x 10⁶ cells/ml and a total volume of 50 µL. Incubation was stopped at each time point by the addition of ice-cold acetonitrile (ACN). Aliquots of samples were mixed with 10 % ACN containing an internal standard for LC/MS/MS analysis. After samples were sonicated for 10 minutes, samples were centrifuged at 2,000 rpm for 15 minutes, and then the supernatant was transferred to the other plates for analysis. The compound concentrations in supernatant were measured by LC/MS/MS system.

The disappearance rates of tested compounds were obtained by plotting the common logarithm of the peak area ratio of compounds / internal standard versus time. The slope of the line of best fit through the points yielded the rate of metabolism (kᵤ). This value was scaled to hepatocellularity, liver and body weight into account to give an intrinsic clearance value (CLᵢᵢᵢ) in ml/min/kg as illustrated in Equation 1. Hepatic clearance (CLᵢₚ) was predicted from this intrinsic clearance value using the parallel tube model as shown in Equation 2. The predicted clearance divided by the hepatic blood flow (Qₚ) afforded the extraction ratio (Eᵢₚ) (Equation 3).

Equation 1: \[ k_u \times (g \text{ liver/kg body weight}) \times (ml \text{ incubation/ number of cells in incubation}) \times (\text{cells/g liver}) \]
Equation 2: \[ \text{CL}_h = \text{Q}_h \times \left\{ 1 - \exp \left( -\frac{\text{CL}_{\text{int}}}{\text{Q}_h} \right) \right\} \]
Equation 3: \[ E_h = \frac{\text{CL}_h}{\text{Q}_h} \]

Wherein, "g liver weight /kg body weight" is 2.1, "Cells /g liver" is 1.2 \times 10^8, "ml incubation/ number of cells in incubation" is 2.0 \times 10^5, and \( \text{Q}_h \) is 20 ml/min/kg.

Supposing that hepatic metabolism is the main route of drug elimination, systemic exposure (AUC\(_{\text{po}}\)) after oral administration is calculated using Equation 4.

Equation 4: \[ \text{AUC}_{\text{po}} = \text{Dose} \times \left( 1 - E_h \right) / \text{CL}_h \]

RMS (Reactive Metabolite-Screening)

Tested compounds (100 \( \mu \)M) were incubated with 5 mM glutathione (GSH) and human liver microsomes (HL\(^{101}: 2.0\) mg/mL) in 100 mM phosphate buffer (pH 7.4). The reaction was initiated by addition of cofactors (3.3 mM glucose 6-phosphate, 3.3 mM MgCl\(_2\), 10 unit/mL glucose 6-phosphate dehydrogenase, 1.3 mM NADP\(^+\) and 0.93 mM NADH). The final incubation volume was 1.5 mL. After 2 hr incubation at 37 \(^\circ\)C, the reaction was stopped by addition of 1mL of 0.2 M monochloroacetic acid (MCA) and centrifuged at 3000 rpm for 15 min. The supernatant was attempted to solid phase extraction (isolute C\text{18 SPE, International Sorbent Technology}). This included removing proteins, washing 3 times with 1mL of 0.2 M MCA and eluting twice with 2.5 mL of methanol. Following solvent dry-up, the residues were dissolved in 150 \( \mu \)L of 10% methanol containing 2 mM ammonium acetate and 0.027% formic acid and filtrated using centrifugal filter device (Ultrafree MC, Millipore). Analyses were measured by LC/MS/MS systems.

Examples

The following examples are provided for the purpose of further illustration only and are not intended to be limitations on the disclosed invention. Unless stated on otherwise in the following examples, general experimental conditions are as follows: all operations were carried out at room or ambient temperature, that is, in the range of 18-25 \(^\circ\)C; evaporation of solvent was carried out using a rotary evaporator under reduced pressure with a bath temperature of up to 60 \(^\circ\)C; reactions were monitored by thin layer chromatography (TLC) and reaction times are given for illustration only; melting points (mp) given are uncorrected (polymorphism may result in different melting points); the structure and purity of all isolated compounds were assured by at least one of the following techniques: TLC (Merck silica gel 60 F\text{254}, precoated TLC plates or Merck NH\text{2} gel (an amine coated silica gel) F\text{2543} precoated TLC plates), mass spectrometry, nuclear magnetic resonance spectra (NMR), infrared absorption spectra (IR) or microanalysis. Yields are given for illustrative purposes only. Flash column chromatography was carried out using Biotage KP-SIL (40-63 \( \mu \)m), Biotage KP-NH (an amine coated silica gel) (40-75 \( \mu \)m) or Wako silica gel 300HG (40-60 \( \mu \)m). Preparative TLC was carried out using Merck silica gel 60 F\text{254} precoated TLC plates (0.5 or 1.0 \( \text{mm} \) thickness). All Mass data was obtained in Low-resolution mass spectral data (ESI) using ZMD\textsuperscript{TM} or ZQ\textsuperscript{TM} (Waters) and mass spectrometer. NMR data were determined at 270 MHz (JEOL JNM-LA 270 spectrometer) or 300 MHz (JEOL JNM-LA300 spectrometer) using deuterated chloroform (99.8%) or dimethylsulfoxide (99.9%) as solvent unless indicated otherwise, relative
to tetramethylsilane (TMS) as internal standard in parts per million (ppm); conventional abbreviations used are: s = singlet, d = doublet, m = multiplet, dd = doublet of doublet, sep = septet. br.s = broad singlet, br.d = broad doublet, etc. IR spectra were measured by a Fourier transform infrared spectrophotometer (Shimazu FTIR-8300). Optical rotations were measured using a P-1020 Digital Polarimeter (Japan Spectroscopic CO, Ltd.). Microwave irradiation was carried out using Initiator 60 (Biotage).

Example 1
B-rtFJ-Diflouro-S^-dihydro- 2H-chromen^-vDaminoi-M.  N2.trimethylimidazori .2-apyrazine- β-carbo

\[ \text{Xamide} \]

STEP 1: 5.7-Difluorochroman-4-ol

To a stirred solution of 5.7-difluoro-2,3-dihydro-4H-chromen-4-one (2.00 g, 11 mmol, US 2005038032) in methanol (30 mL) was added sodium borohydride (0.49 g, 13 mmol) at 0 °C and the mixture was stirred at room temperature for 20 hours. After the mixture was evaporated in vacuo, the residue was treated with water (20 mL) and extracted with ethyl acetate (30 mL x 2). The combined extracts were washed with brine, dried over magnesium sulfate, and concentrated in vacuo to afford the title compound as a white solid (2.00 g, 97%).

\(^1\)H NMR (CDCl\(_3\), 270 MHz) δ: 6.50-6.33 (m, 2H), 5.07-4.95 (m, 1H), 4.36-4.18 (m, 2H), 2.16-1.94 (m, 2H) ppm. (-OH was not observed.)

STEP 2: 4-Azido-5.7-difluorochromane

To a mixture of 5.7-difluorochroman-4-ol (5.00 g, 26.9 mmol, STEP 1) and diphenylphosphoryl azide (6.95 ml, 32.2 mmol) in tetrahydrofuran (50 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (5.22 ml, 34.9 mmol) dropwise. The reaction mixture was stirred at room temperature for 5 hours. The reaction mixture was concentrated in vacuo and the residue was partitioned between ethyl acetate (300 mL) and water (200 mL). The organic layer was washed with saturated aqueous ammonium chloride solution (50 mL) and brine (50 mL). The organic layer was dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (n-hexane / ethyl acetate = 9 / 1 as eluent) to afford the title compound as colorless oil (5.09 g, 90%).

\(^1\)H NMR (CDCl\(_3\), 270 MHz) δ: 6.52-6.39 (m, 2H), 4.93-4.86 (m, 1H), 4.38-4.28 (m, 1H), 4.26-4.08 (m, 1H), 2.07-1.99 (m, 2H) ppm.

STEP 3: 5.7-Difluorochroman-4-amine
A mixture of 4-azido-5,7-difluorochromane (5.05 g, 23.9 mmol, STEP 2) and 5% Pd-C (200 mg) in methanol (200 ml) was stirred under H₂ atmosphere (3 atm) at room temperature for 5 hours. The mixture was filtrated through a pad of Celite. The filtrate was concentrated in vacuo to afford the title compound as colorless oil (4.40 g, 99%).

1H NMR (CDCl₃, 270 MHz) δ: 6.44-6.33 (m, 2H), 4.33-4.21 (m, 3H), 2.16-1.97 (m, 2H) ppm. (NH₂ was not observed.)

STEP 4: 5-Bromo-Λ²-(5,7-difluoro-3,4-dihydro-2/-/-chromen-4-yl)pyrazine-2,3-diamine

A mixture of 5,7-difluorochroman-4-amine (4.39 g, 23.7 mmol, STEP 3), 3,5-dibromopyrazin-2-amine (3.00 g, 11.9 mmol) and triethylamine (3.31 ml, 23.7 mmol) in N-methylpyrrolidinone (8 mL) in a sealed tube was irradiated in a microwave-oven for 1.5 hours. After cooled to room temperature, the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (50 mL x 3). The organic layer was washed with saturated aqueous ammonium chloride solution (30 mL), dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (π-hexane / ethyl acetate = 9 / 1 to 4 / 1 as eluent) to afford the title compound as orange oil (2.90 g, 68%).

1H NMR (CDCl₃, 270 MHz) δ: 7.54 (s, 1H), 6.50-6.38 (m, 2H), 5.33-5.24 (m, 1H), 4.43-4.30 (m, 2H), 4.24-4.06 (m, 3H), 2.42-2.31 (m, 1H), 2.14-2.00 (m, 1H) ppm.

MS (ESI): 358 (M+H)+, 356 (M-H)-

STEP 5: 6-Bromo-Λ²-(5,7-difluoro-3,4-dihydro-2/-/-chromen-4-yl)-2-methylimidazorí-2-alpyrazin-8-amine

To a solution of 5-bromo-Λ²-(5,7-difluoro-3,4-dihydro-2/-/-chromen-4-yl)pyrazine-2,3-diamine (2.88 g, 8.06 mmol, STEP 4) in 1,4-dioxane (40 mL) was added bromoacetone (1.35 g, 8.90 mmol) at room temperature. The reaction mixture was refluxed for 16 hours. After cooled to room temperature, the mixture was poured into ice-saturated sodium hydroxide carbonate solution (100 mL), and extracted with dichloromethane (200 mL). The organic layer was dried over magnesium sulfate and concentrated in vacuo. The residue was purified by column chromatography on silica gel (n-hexane / ethyl acetate = 3 / 1 as eluent) to afford the title compound as a brown solid (2.68 g, 84%).

1H NMR (CDCl₃, 270 MHz) δ: 7.55 (s, 1H), 7.21 (s, 1H), 6.43-6.36 (m, 2H), 6.15-6.13 (m, 1H), 5.47 (br.s, 1H), 4.37-4.17 (m, 2H), 2.45-2.38 (m, 1H), 2.38 (s, 3H), 2.18-2.04 (m, 1H) ppm.

MS (ESI): 395 (M+H)+, 393 (M-H)-

STEP 6: 8-[(5,7-Difluoro-3,4-dihydro-2/-/-chromen-4-yl)aminol- Λ²-2-trimethylimidazo Λ₂,2-alpyrazine-6-c arboxamide

A mixture of 6-bromo-Λ²-(5,7-difluoro-3,4-dihydro-2/-/-chromen-4-yl)-2-methylimidazo[1,2-a]pyrazin-8-amine (1.50 g, 3.80 mmol, STEP 5) and tetraakis(triphenylphosphine)palladium(0) (877 mg, 0.76 mmol) in 2M dimethylamine tetrahydrofuran solution (60 mL) was stirred at 65 °C under carbon monoxide gas (1 atm) for 16 hours. After cooled to room temperature, the mixture was diluted with ethyl acetate (200 mL).

The organic fraction was washed with water (100 mL), dried over magnesium sulfate, and concentrated in vacuo.

...
vacuo. The residue was purified by column chromatography on silica gel (n-hexane / ethyl acetate = 50 / 1 to 1 / 2 as eluent) to afford a mixture of the title compound and triphenylphosphine oxide (2.03 g, crude) as slight yellow syrup. This was used for the next step without further purification.

Rf: 0.23 (hexane / ethyl acetate = 1 / 2)

MS (ESI): 388 (M+H)+, 386 (M-H)-

Example 2
**8-[(5,7-Difluoro-3,4-dihydro-2H-chromen-4-yl)amino]-3-(hydroxymethyl)-N,N,2-trimethylimidazo[1,2-a]pyrazine-6-carboxamide**

**STEP 1:** 8-[(5,7-Difluoro-3,4-dihydro-2H-chromen-4-yl)amino]-3-(hydroxymethyl)-N,N,2-trimethylimidazo[1,2-a]pyrazine-6-carboxamide (example 2-1)

A mixture of 8-[(5,7-difluoro-3,4-dihydro-2H-chromen-4-yl)amino]-N,N,2-trimethylimidazo[1,2-a]pyrazine-6-carboxamide (1.47 g, 3.79 mmol, STEP 6 of Example 1), formaldehyde 37 wt.% in water (9.24 g, 114 mmol), acetic acid (1.09 mL, 19.0 mmol), and sodium acetate (1.56 g, 19.0 mmol) in acetonitrile (6 mL) was heated at 100 °C for 16 hours. After cooled to room temperature, saturated sodium hydrogen carbonate solution (50 mL) was added to the reaction mixture and extracted with dichloromethane (100 mL x 2). The combined extracts were dried over magnesium sulfate, and evaporated in vacuo. The residue was purified by column chromatography on silica gel (dichloromethane / methanol = 30 / 1 to 10 / 1 as eluent) to afford the title compound as a white solid (955 mg, 60%).

1H NMR (CDCl₃, 270 MHz) δ: 8.07 (s, 1H), 6.47-6.33 (m, 2H), 6.20-6.13 (m, 1H), 5.57-5.50 (m, 1H), 4.88 (d, J = 5.3 Hz, 2H), 4.41-4.18 (m, 2H), 3.26 (s, 3H), 3.14 (s, 3H), 2.60-2.52 (m, 1H), 2.41 (m, 3H), 2.37-2.25 (m, 1H), 2.02-2.19 (m, 1H) ppm.

MS (ESI): 418 (M+H)+, 416 (M-H)-

**STEP 2:** (+)-8-[(5,7-Difluoro-3,4-dihydro-2H-chromen-4-yl)amino]-N,N,2-trimethylimidazo[1,2-a]pyrazine-6-carboxamide and (-)-8-[(5,7-Difluoro-3,4-dihydro-2H-chromen-4-yl)amino]-N,N,2-trimethylimidazo[1,2-a]pyrazine-6-carboxamide

The fraction-1 (59 mg) and fraction-2 (61 mg) were prepared from racemic 8-[(5,7-difluoro-3,4-dihydro-2H-chromen-4-yl)amino]-3-(hydroxymethyl)-N,N,2-trimethylimidazo[1,2-a]pyrazine-6-carboxamide (130 mg, STEP 1) by HPLC as follows.

Isolation condition

Column: CHIRALCEL OJ-H (20 mm x 250 mm, DAICEL)
Mobile phase: n-Hexane / Ethanol / Diethylamine (95 / 5 / 0.1)
Flow rate: 18.9 mL/min

(-)-8-[(5,7-Difluoro-3,4-dihydro-2H-chromen-4-yl)aminol-3-(hydroxymethyl)-N,N,2-trimethylimidazo-2-alpyrazine-6-carboxamide (fraction-1) (example 2-2)

1H NMR: spectrum data were identical with those of the racemate
optical rotation: \([\alpha]_D^{23} = -84^\circ\) (c = 1.01, Methanol)
retention time: 13 min

 (+)-8-(5,7-Difluoro-3,4-dihydro-2H-chromen-4-yl)aminol-3-(hydroxymethyl)-N,N,2-trimethylimidazo-2-alpyrazine-6-carboxamide (fraction-2) (example 2-3)

1H NMR: spectrum data were identical with those of the racemate
optical rotation: \([\alpha]_D^{23} = +86^\circ\) (c = 1.01, Methanol)
retention time: 19 min

Example 3

\(\Lambda,\Lambda^-\)-Trimethyl-S-ftS-methyl-S,4-dihydro-2H-chromen^-v-Daminoimidazoi_2-alpyrazine-6-carboxamide

STEP 1: 4-Azido-5-methylchromane

The title compound was prepared in 84% yield (4.86 g, colorless oil) from 5-methylchroman-4-ol (5.00 g, 30.5 mmol, Tetrahedron Asym., 1997, 8, 3059.) by the same manner in STEP 2 of Example 1.

1H NMR (CDCl₃, 300 MHz) δ: 7.14 (dd, J = 8.8 and 7.3 Hz, 1H), 6.81 (d, J = 7.3 Hz, 1H), 6.73 (d, J = 8.8 Hz, 1H), 4.64 (br.s, 1H), 4.36-4.27 (m, 1H), 4.23-4.08 (m, 1H), 2.59 (s, 3H), 2.21-2.10 (m, 2H) ppm.

STEP 2: 5-Methylchroman-4-amine

The title compound was prepared in 99% yield (4.14 g, white needles) from 4-azido-5-methylchromane (4.86 g, 25.7 mmol, STEP 1) by the same manner in STEP 3 of Example 1.

1H NMR (CDCl₃, 300 MHz) δ: 7.06 (dd, J = 8.1 and 7.3 Hz, 1H), 6.75 (d, J = 7.3 Hz, 1H), 6.70 (d, J = 8.1 Hz, 1H), 4.29-4.23 (m, 2H), 4.12 (br.s, 1H), 2.40 (s, 3H), 2.14-2.03 (m, 1H), 1.87-1.80 (m, 1H), 1.44 (br.s, 2H) ppm.

STEP 3: 5-Bromo-\(\Lambda,\Lambda^-\)-(5-methyl-3,4-dihydro-2H-chromen-4-yl)pyrazine-2,3-diamine

The title compound was prepared in 74% yield (2.95 g, yellow syrup) from 5-methylchroman-4-amine (3.87 g, 25.7 mmol, STEP 2) and 3,5-dibromopyrazin-2-amine (3.00 g, 11.9 mmol) by the same manner in STEP 4 of Example 1.
**Example 4**

3-(Hydroxymethyl)-N,N,N'-2-trimethyl-8-fl(5-methyl-3,4-dihydro-2H-chromen-4-yl)aminimidazol1,2-alpyrazin-8-amine

The title compound was prepared in 71% yield (2.43 g, white solid) from 3-(Hydroxymethyl)-N,N,N'-2-trimethyl-8-fl(5-methyl-3,4-dihydro-2H-chromen-4-yl)aminimidazol1,2-alpyrazin-8-amine (2.43 g, crude, STEP 5 of Example 3) by the same manner in STEP 1 of Example 2.

**Example 5**

6-Bromo-2-methyl- N-(5-methyl-3,4-dihydro-2H-chromen-4-yl)imidazo1,2-alpyrazin-8-amine

The title compound was prepared in 69% yield (2.27 g, slight brown solid) from S-bromo-N3-{5-methyl-S3-dihydro4H-chromen4-yOpyrazine4-S-diamine (2.95 g, 8.80 mmol, STEP 3) by the same manner in STEP 5 of Example 1.

**Example 6**

N,N,N',2-Trimethyl-8-fl(5-methyl-3,4-dihydro-2H-chromen-4-yl)aminimidazo1,2-alpyrazin-8-amine

A mixture of the title compound and thphenylphosphine oxide was prepared (2.43 g, crude, slight yellow syrup) from 6-bromo-2-methyl- N-(5-methyl-3,4-dihydro-2H-chromen-4-yl)imidazo1,2-alpyrazin-8-amine (1.50 g, 4.02 mmol, STEP 4) by the same manner in STEP 6 of Example 1. 

**Example 7**

3-(Hydroxymethyl)-N,N,N'-2-trimethyl-8-fl(5-methyl-3,4-dihydro-2H-chromen-4-yl)aminimidazol1,2-alpyrazin-8-amine

The title compound was prepared in 71% (2 steps) yield (1.13 g, white solid) from 3-(Hydroxymethyl)-N,N,N'-2-trimethyl-8-fl(5-methyl-3,4-dihydro-2H-chromen-4-yl)aminimidazol1,2-alpyrazin-8-amine (2.43 g, crude, STEP 5 of Example 3) by the same manner in STEP 1 of Example 2.

**Example 8**

6-Bromo-2-methyl- N-(5-methyl-3,4-dihydro-2H-chromen-4-yl)imidazo1,2-alpyrazin-8-amine

The title compound was prepared in 69% yield (2.27 g, slight brown solid) from S-bromo-N3-{5-methyl-S3-dihydro4H-chromen4-yOpyrazine4-S-diamine (2.95 g, 8.80 mmol, STEP 3) by the same manner in STEP 5 of Example 1.

**Example 9**

N,N,N',2-Trimethyl-8-fl(5-methyl-3,4-dihydro-2H-chromen-4-yl)aminimidazo1,2-alpyrazin-8-amine

A mixture of the title compound and thphenylphosphine oxide was prepared (2.43 g, crude, slight yellow syrup) from 6-bromo-2-methyl- N-(5-methyl-3,4-dihydro-2H-chromen-4-yl)imidazo1,2-alpyrazin-8-amine (1.50 g, 4.02 mmol, STEP 4) by the same manner in STEP 6 of Example 1. 

**Example 10**

3-(Hydroxymethyl)-N,N,N'-2-trimethyl-8-fl(5-methyl-3,4-dihydro-2H-chromen-4-yl)aminimidazol1,2-alpyrazin-8-amine

The title compound was prepared in 71% (2 steps) yield (1.13 g, white solid) from 3-(Hydroxymethyl)-N,N,N'-2-trimethyl-8-fl(5-methyl-3,4-dihydro-2H-chromen-4-yl)aminimidazol1,2-alpyrazin-8-amine (2.43 g, crude, STEP 5 of Example 3) by the same manner in STEP 1 of Example 2.
MS (ESI) m/z: 396 (M+H)⁺, 394 (M-H)⁻.

**STEP 2**: (SH-)-3-(Hydroxymethyl)-\( N,N \)-2-trimethyl-8-\( N,N \)-2-trimethyl-8-(5-methyl-3,4-dihydro-2H-chromen-4-yl)aminoimidazo[1,2-alpyrazine-6-carboxamide and (S)-3-(Hydroxymethyl)-\( N,N \)-2-trimethyl-8-(5-methyl-3,4-dihydro-2H-chromen-4-yl)aminoimidazo[1,2-alpyrazine-6-carboxamide (example 4-2) were prepared from racemic 3-(hydroxymethyl)-N,N,2-trimethyl-8-(5-methyl-3,4-dihydro-2H-chromen-4-yl)aminoimidazo[1,2-alpyrazine-6-carboxamide (200 mg, STEP 1) by HPLC as follows.

**Isolation condition**

**Column**: CHIRALCEL OJ-H (20 mm x 250 mm, DAICEL)

**Mobile phase**: n-Hexane / Ethanol / Diethylamine (85 / 15 / 0.1)

**Flow rate**: 18.9 mL/min

**(S)-(-)-3-(Hydroxymethyl)-\( N,N \)-2-trimethyl-8-(5-methyl-3,4-dihydro-2H-chromen-4-yl)aminoimidazo[1,2-alpyrazine-6-carboxamide (example 4-2)**

**1H NMR**: spectrum data were identical with those of the racemate

**melting point**: 187.0 °C

**optical rotation**: \([\alpha]_D^{22} = -66° \quad (c = 1.01, \text{Methanol})

**retention time**: 7 min

**(S)-(+)-3-(Hydroxymethyl)-\( N,N \)-2-trimethyl-8-(5-methyl-3,4-dihydro-2H-chromen-4-yl)aminoimidazo[1,2-alpyrazine-6-carboxamide (example 4-3)**

**1H NMR**: spectrum data were identical with those of the racemate

**melting point**: 186.4 °C

**optical rotation**: \([\alpha]_D^{23} = +67° \quad (c = 1.01, \text{Methanol})

**retention time**: 12 min

**Example 5**

8 furyl(5,7-Difluoro-3,4-dihydro-2H-chromen-4-yl)amino-\( N,N \)-2,3-tetramethylimidazolium-β-carboxamide

**STEP 1**: 6-Bromo-\( N,N \)-(5,7-difluoro-3,4-dihydro-2H-chromen-4-yl)-2,3-dimethylimidazolium-2-alpyrazin-8-amine

To a solution of 5-bromo-\( N,N \)-(5,7-difluoro-3,4-dihydro-2H-chromen-4-yl)pyrazine-2,3-diamine (100 g, 2.80 mmol, STEP 4 of Example 1) in 1,4-dioxane (10 mL) was added 3-bromo-2-butanone (1.4 g,
9.0 mmol) in a sealed tube was irradiated in a microwave-oven for 0.5 hour. After cooled to room
temperature, the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (50 mL x 3).
The organic layer was washed with saturated aqueous ammonium chloride solution (30 mL), dried
over magnesium sulfate, and concentrated in vacuo. The residue was purified by column
chromatography on silica gel (n-hexane / ethyl acetate = 5 / 1 to 2 / 1 as eluent) to afford the title
compound as orange oil (0.5 g, 44%).

\( ^1 \)H NMR (CDCl\( _3 \), 270 MHz) \( \delta \): 7.82 (s, 1 H), 6.44-6.36 (m, 3H), 6.01 (m, 1 H), 5.54 (br.s, 1 H), 4.48-4.24 (m, 1 H) ppm.

MS (ESI): 410 (M+H)+

STEP 2: 8-(5,7-difluorochroman-4-ylamino)-2,3-dimethylimidazo[1,2-a]pyrazin-8-amihe (0.5 g, 1.22 mmol, STEP 1), palladium acetate (16 mg, 0.072 mmol), 1,1′-bis(diphenylphosphino)ferrocene (74 mg, 0.13 mmol) and triethylamine (378 mg, 3.74 mmol) in dimethylfor
mamid (5 mL) and methanol (5 mL) was stirred at 65 °C under carbon monoxide gas (1 atm) f or 19 hours. After cooled to room temperature, the mixture was concentrated under reduced pres sure to the half volume. To the residue was added water (50 mL) and ethyl acetate (50 mL), the separated organic phase was washed with water (20 mL) and brine (20 mL), dried over magnesium sulfate, filtered, concentrated to give a greenish precipitate. The precipitate was dissolved in me thanol (5 mL) and tetrahydrofuran (5 mL) and then 2M sodium hydroxide solution (0.63 mL) was s tirred at 50 °C for 1 h. Then, the volatile components were removed under reduced pressure. The resultant was suspended in water (10 mL) and the solution was neutralized by 2M hydrogen chlori de solution. The resulting precipitate was collected by Kiriyama funnel (rinsed with 20 mL of diethy l ether) to afford the title compound as a colorless powder (87%)

\( ^1 \)H NMR (CDCl\( _3 \), 270 MHz) \( \delta \): 8.23 (s, 1 H), 6.40-6.10 (m, 3H), 5.48 (br.s, 1 H), 4.40-4.15 (m, 2H), 2.50-2.32 (m, 1H), 2.42 (s, 3H), 2.38 (s, 3H), 2.25-2.17 (m, 1H) ppm. The protons of COOH and NH were not found.

MS (ESI): 375 (M+H)+, 373 (M-H)+.

STEP 3: S-rflSy-Difluoro-S,4-dihdro*H-chromen^vDaminol^-N.A^a,S-tetramethylimidazolf^-alpyrazine-
6-carboxamide (example 5-1)

To a solution of 8-(5,7-difluorochroman-4-ylamino)-2,3-dimethylimidazo[1,2-a]pyrazine-6-carboxylic acid (273 mg, 0.73 mmol, STEP 2) and triethylamine (88 mg, 0.83 mmol) was added isobutyl chloroformate at -10 °C. The reaction mixture was stirred at -10 °C for 30 min. Then, 2M dimethylamine tetrahydrofuran solution (10 mL) was added to the mixture. The resulting mixture was stirred at room temperature for 30 min. Then, the reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (50 mL x 3). The organic layer was washed with saturated aqueous ammonium chloride solution (30 mL), dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (n-hexane / ethyl acetate = 1 / 6 to 1 / 100 as eluent) to afford the title compound as colorless powder (263 mg, 90%).

\( ^1 \)H NMR (CDCl\( _3 \), 270 MHz) \( \delta \): 7.82 (s, 1 H), 6.44-6.36 (m, 3H), 6.01 (m, 1H), 5.54 (br.s, 1H), 4.48-4.24 (m,
2H), 3.29 (s, 3H), 3.15 (s, 3H), 2.37 (s, 3H), 2.36 (s, 3H), 2.33-2.29 (m, 1H), 2.16-2.06 (m, 1H) ppm.

MS (ESI): 402 (M+H)⁺, 400 (M-H).  

**STEP 4:** (-VS-KS,T-Difluoro-S,4-dihydro-2H-chromen-yl)aminol-A/- S-tetramethylimidazo π-alpyrazine-6-carboxamide and (+)-8-f(5,7-Difluoro-3,4-dihydro-2H-chromen-4-yl)aminol- N,N',2,3-tetramethylimidazo[1,2-a]pyrazine-6-carboxamide

The fraction-1 (122 mg) and fraction-2 (108 mg) were prepared from racemic δ^-SJ-Difluoro-S^-dihydro^-H-chromen^-ylJaminol-Λ/Λ^-S-tetramethylimidazoπ^-alpyrazine-e-carboxamide (260 mg, STEP 4) by HPLC as follows.

**Isolation condition**

Column: CHIRALPAK AD-H (20 mm x 250 mm, DAICEL)

Mobile phase: n-Hexane / IPA / Diethylamine (95 / 5 / 0.1)

Flow rate: 20 mL/min

**(-)-8-f(5,7-Difluoro-3,4-dihydro-2H-chromen-4-yl)aminol-N,N,2,3-tetramethylimidazo[1,2-a]pyrazine-6-carboxamide**

1H NMR: spectrum data were identical with those of the racemate

optical rotation: [α]D²⁴ = -79° (c = 0.097, Methanol)

retention time: 6.7 min

**(+)-8-f(5,7-Difluoro-3,4-dihydro-2H-chromen-4-yl)aminol-N,N,2,3-tetramethylimidazo[1,2-a]pyrazine-6-carboxamide**

1H NMR: spectrum data were identical with those of the racemate

optical rotation: [α]D²⁴ = +86° (c = 0.10, Methanol)

retention time: 9.0 min

**Example 6**

**N,N,2,3-tetramethyl-8-fr(4R)-5-methyl-3,4-dihydro-2H-chromen-4-ylamino>imidazori .2-a1pyrazine-6-carboxamide**

![Structure](image)

**STEP 1:** 6-bromo-2,3-dimethyl- N^-{(5-methyl-3,4-dihydro-2H-chromen-4-yl)imidazol}^-2-a1pyrazin-8-amine

The title compound was prepared in 55% yield (578 mg, brown amorphous) from 5-bromo- N^-{(5-methyl-3,4-dihydro-2H-chromen-4-yl)pyrazine-2,3-diamine (910 mg, 2.71 mmol) by the same manner in STEP 1 of Example 5.

1H NMR (300 MHz, CHLOROFORM-d): 7.36 (1 H, s) 7.12 (1 H, t, J = 8.1 Hz) 6.69 - 6.81 (2 H, m) 6.00 - 6.12 (1 H, m) 5.27 - 5.38 (1 H, m) 4.24 - 4.36 (1 H, m) 4.10 - 4.24 (1 H, m) 2.37 - 2.47 (1 H, m) 2.33 (6 H, s)
2.23 (3 H, s) 2.08 - 2.24 (1 H, m) ppm.
MS (ESI): 387 (M + H)+ 385 (M - H)+.

STEP 2:
2,3-dimethyl-8-F-(5-methyl-3,4-dihydro-2H-chromen-4-yl)aminolimidazol[1,2-alPyrazine-6-carboxylic acid
The title compound was prepared in 76% yield (547 mg, greenish solid) from 5-bromo-2,3-dimethyl-N-(5-methyl-3,4-dihydro-2H-chromen-4-yl)imidazo[1,2-alpyrazin-8-amine (788 mg, 2.03 mmol) by the same manner in STEP 2 of Example 5.

1H NMR (300 MHz, CHLOROFORM-cO): 8.24 (1 H, s) 7.15 (1 H, t, J = 7.7 Hz) 6.77 (2 H, d, J = 8.1 Hz) 5.31 (1 H, br, s) 4.29 - 4.38 (1 H, m) 4.17 - 4.29 (1 H, m) 2.44 (3 H, s) 2.39 (3 H, s) 2.29 - 2.42 (1 H, m) 2.22 (3 H, s) 2.13 - 2.30 (1 H, m) ppm. The protons of COOH and NH were not found.

MS (ESI): 353 (M + H)+ 351 (M - H)+.

STEP 3:
N,N,2,3-tetramethyl-8-[5-methyl-3,4-dihydro-2H-chromen-4-yl]aminolimidazo[1,2-alpyrazine-6-carboxamide (example 6-1)
The title compound was prepared in 53% yield (311 mg, pale yellow-white solid) from 2,3-dimethyl-8-[5-methyl-3,4-dihydro-2H-chromen-4-yl]aminolimidazo[1,2-alpyrazine-6-carboxylic acid (547 mg, 1.55 mmol) by the same manner in STEP 2 of Example 5.

1H NMR (300 MHz, CHLOROFORM-cO): 7.81 (1 H, s) 7.12 (1 H, t, J = 7.7 Hz) 6.67 - 6.82 (2 H, m) 6.02 (1 H, d, J = 6.6 Hz) 5.31 - 5.43 (1 H, m) 4.26 - 4.37 (1 H, m) 4.15 - 4.26 (1 H, m) 3.30 (3 H, s) 3.16 (3 H, s) 2.37 (3 H, s) 2.35 (3 H, s) 2.25 - 2.35 (1 H, m) 2.19 (3 H, s) 2.06 - 2.24 (1 H, m) ppm.

MS (ESI): 380 (M + H)+.

STEP 4:
(-)-N,N,2,3-tetramethyl-8-[5-methyl-3,4-dihydro-2H-chromen-4-yl]aminolimidazo[1,2-alpyrazine-6-carboxamide and
(+)-/N,N,2,3-tetramethyl-8-[5-methyl-3,4-dihydro-2H-chromen-4-yl]aminolimidazo[1,2-alpyrazine-6-carboxamide
The fraction-1 (119 mg) and fraction-2 (113 mg) were prepared from racemic N,N,2,3-tetramethyl-8-[5-methyl-3,4-dihydro-2H-chromen-4-yl]aminolimidazo[1,2-alpyrazine-6-carboxamide (288 mg, STEP 3) by HPLC as follows.

Isolation condition
Column: CHIRALPAK AD-H (20 mm x 250 mm, DAICEL)
Mobile phase: /Hexane / IPA / Diethylamine (95 / 5 / 0.1)
Flow rate: 20 mL/min

(-)-N,N,2,3-tetramethyl-8-[5-methyl-3,4-dihydro-2H-chromen-4-yl]aminolimidazo[1,2-alPyrazine-6-carboxamide (example 6-2)
$^1$H NMR: spectrum data were identical with those of the racemate
optical rotation: $[\alpha]_D^{22} = -71^\circ$ (c = 0.097, Methanol)
retention time: 12 min

(+) - N,N,N,N-tetramethyl-8-f(5-methyl-3,4-dihydro-2/-/-chromen-4-yl)aminolimidazo[1 _2-alpyrazine-6-carbox
amide (example 6-3)

$^1$H NMR: spectrum data were identical with those of the racemate
optical rotation: $[\alpha]_D^{23} = +73^\circ$ (c = 0.11 , Methanol)
retention time: 16 min

All publications, including but not limited to, issued patents, patent applications, and journal articles, cited in this application are each herein incorporated by reference in their entirety.

Although the invention has been described above with reference to the disclosed embodiments, those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention. It should be understood that various modifications could be made without departing from the spirit of the invention.
CLAIMS

1. A compound of the formula (I):

[Chemical structure diagram]

or a pharmaceutically acceptable salt thereof, wherein:

- \( A-B- \) represents \(-0-\text{CH}_2-\), \(-\text{GH}_2\text{O}\), \(-\text{S-CH}_2-\), or \(-\text{CH}_2\text{S}\);

\( R^1 \) represents a hydrogen atom or a \( \text{CrC}_6 \) alkyl group being unsubstituted or substituted with 1 to 2 substituents independently selected from the group consisting of a hydroxy group or a moiety convertible into a hydroxy group \textit{in vivo};

\( R^2 \) represents a \( \text{C}_1-\text{C}_6 \) alkyl group being unsubstituted or substituted with 1 to 2 substituents independently selected from the group consisting of a hydroxy group or a moiety convertible into a hydroxy group \textit{in vivo};

\( R^3 \) and \( R^4 \) independently represent a \( \text{C}_1-\text{C}_6 \) alkyl group or a \( \text{C}_3-\text{C}_7 \) cycloalkyl group, said \( \text{C}_1-\text{C}_6 \) alkyl group and said \( \text{C}_3-\text{C}_7 \) cycloalkyl group being unsubstituted or substituted with 1 to 3 substituents independently selected from the group consisting of a halogen atom, a hydroxy group, a \( \text{C}_1-\text{C}_6 \) alkoxy group and a \( \text{C}_3-\text{C}_7 \) cycloalkyl group; or \( R^3 \) and \( R^4 \) taken together with the nitrogen atom to which they are attached form a 4 to 7 membered heterocyclic group being unsubstituted or substituted with 1 to 3 substituents selected from the group consisting of a hydroxy group, a \( \text{C}_1-\text{C}_6 \) alkyl group, a \( \text{C}_1-\text{C}_6 \) alkoxy group and a hydroxy-\( \text{C}_1-\text{C}_6 \) alkyl group; and

\( R^5, R^6, R^7 \) and \( R^8 \) independently represent a hydrogen atom, a halogen atom or a \( \text{C}_1-\text{C}_6 \) alkyl group.

2. The compound or the pharmaceutically acceptable salt, as claimed in claim 1, wherein:

- \( A-B- \) represents \(-0-\text{CH}_2-\) or \(-\text{CH}_2\text{O}\);

\( R^1 \) and \( R^2 \) are independently a hydroxy-\( \text{C}_1-\text{C}_6 \) alkyl group, \( \text{C}_1-\text{C}_6 \) alkoxy-\( \text{C}_1-\text{C}_6 \) alkyl group, \( \text{C}_1-\text{C}_6 \) alkyl-carbonyl-oxy-\( \text{CrC}_6 \) alkyl group or a \( \text{C}_1-\text{C}_6 \) alkyl group;

\( R^3 \) and \( R^4 \) are independently a \( \text{C}_1-\text{C}_6 \) alkyl group or a \( \text{C}_3-\text{C}_7 \) cycloalkyl group, said \( \text{C}_1-\text{C}_6 \) alkyl group and said \( \text{C}_3-\text{C}_7 \) cycloalkyl group being unsubstituted or substituted with 1 to 3 substituents independently selected from the group consisting of a halogen atom, a hydroxy group, a \( \text{C}_1-\text{C}_6 \) alkoxy group and a \( \text{C}_3-\text{C}_7 \) cycloalkyl group; or \( R^3 \) and \( R^4 \) taken together with the nitrogen atom to which they are attached form an azetidinyl group, a pyrrolidinyl group, a morpholino group or a homomorpholino group, said azetidinyl group, said pyrrolidinyl group, said morpholino group and said homomorpholino group being unsubstituted or substituted with 1 to 3 substituents selected from the group consisting of a hydroxy group, a \( \text{C}_1-\text{C}_6 \) alkyl group, a \( \text{C}_1-\text{C}_6 \) alkoxy group and a hydroxy-\( \text{C}_1-\text{C}_6 \) alkyl group.
3. The compound or the pharmaceutically acceptable salt, as claimed in claim 1, wherein:
   - A-B- represents -CH₂-O-
   - R¹ is a hydroxymethyl group or a C₁₋₆ alkyl group;
   - R², R³ and R⁴ are independently a C₁₋₆ alkyl group;
   - R⁵ and R⁷ are independently a hydrogen atom, a halogen atom or a C₁₋₆ alkyl group; and
   - R⁶ and R⁸ are independently a hydrogen atom or a halogen atom.

4. The compound of claim 1, which is selected from:
   - (-)-8-{[5,7-Difluoro-3,4-dihydro-2/-/-chromen-4-yl]amino}-3-(hydroxymethyl)· N,N',2-trimethylimidazo[1,2-a]pyrazine-6-carboxamide;
   - (+)-8-{[5,7-Difluoro-3,4-dihydro-2/-/-chromen-4-yl]amino}-3-(hydroxymethyl)· N,N',2-trimethylimidazo[1,2-a]pyrazine-6-carboxamide;
   - (S)-(-)-3-(Hydroxymethyl)· N,N',2-trimethyl-8-{[5-methyl-3,4-dihydro-2H-chromen-4-yl]amino}imidazo[1,2-a]pyrazine-6-carboxamide;
   - (R)-(+)3-(Hydroxymethyl)· N,N',2-trimethyl-8-{[5-methyl-3,4-dihydro-2H-chromen-4-yl]amino}imidazo[1,2-a]pyrazine-6-carboxamide;
   or a pharmaceutically acceptable salt thereof.

5. A pharmaceutical composition comprising the compound or the pharmaceutically acceptable salt thereof as claimed in any one of claims 1 to 4, and a pharmaceutically acceptable carrier.

6. The pharmaceutical composition as claimed in claim 5 further comprising other pharmacologically active agent(s).

7. A method for the treatment of a condition mediated by acid pump inhibitory activity in a mammalian subject including a human, which comprises administering to a mammal in need of such treatment a therapeutically effective amount of the compound or the pharmaceutically acceptable salt thereof, as claimed in any one of claims 1 to 4.

8. The method as claimed in claim 7, wherein said condition is gastrointestinal disease, gastroesophageal disease, gastroesophageal reflux disease (GERD), laryngopharyngeal reflux disease, peptic ulcer, gastric ulcer, duodenal ulcer, NSAID-induced ulcers, gastritis, infection of Helicobacter pylori, dyspepsia, functional dyspepsia, Zollinger-Ellison syndrome, non-erosive reflux disease (NERD), visceral pain, cancer, heartburn, nausea, esophagitis, dysphagia, hypersalivation, airway disorders or asthma.

9. A use of the compound of formula (I) or the pharmaceutically acceptable salt thereof, as claimed in any one of claims 1 to 4, for the manufacture of a medicament for the treatment of a condition mediated by acid pump inhibitory activity.

10. The use as claimed in claim 9, wherein said condition is gastrointestinal disease,
gastroesophageal disease, gastroesophageal reflux disease (GERD), laryngopharyngeal reflux disease, peptic ulcer, gastric ulcer, duodenal ulcer, NSAID-induced ulcers, gastritis, infection of Helicobacter pylori, dyspepsia, functional dyspepsia, Zollinger-Ellison syndrome, non-erosive reflux disease (NERD), visceral pain, cancer, heartburn, nausea, esophagitis, dysphagia, hypersalivation, airway disorders or asthma.
A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D487/04 A61K31/4985 A61P1/00

B. CLASSIFICATION OF SUBJECT MATTER

INV. C07D Minimum A61K

Documentation searched

C07D A61K A61P

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Category</th>
<th>Citation of document with indication, where appropriate of the relevant passages</th>
<th>Relevant to claim</th>
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<td>Y</td>
<td>WO 2006/064339 A (PFIZER JAPAN INC [JP]; PFIZER [US]; JINNO MADOKA [JP]; SHIMOKAWA HIROH) 22 June 2006 (2006-06-22) page 1, lines 3-6 examples 1, 3, 6, 8, 10, 12, 14, 17, 18 claims 1-10</td>
<td>1-10</td>
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Further documents are listed in the continuation of Box C

See patent family annex

Date of the actual completion of the international search

7 April 2008

Date of mailing of the international search report

14/04/2008

Name and mailing address of the ISA/ European Patent Office, P B 5818 Patentlaan 2 NL - 2280 HV Rijswijk
Tel (+31-70) 340-2040 Tx 31 651 epo nl Fax (+31-70) 340-3016

Authorized officer

Marzi, Elena
### DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<td>Y</td>
<td>KAMINSKI J J ET AL: &quot;ANTIULCER AGENTS 2. GASTRIC ANTISECRETORY, CYTOPROTECTIVE, AND METABOLIC PROPERTIES OF SUBSTITUTED IMIDAZO1, 2-APYRIDINES AND ANALOGUES&quot; JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 30, no. 11, January 1987 (1987-01), pages 2031-2046, XP002008621 ISSN: 0022-2623 page 2034; examples 7,14; table III page 2035; examples 61,68; table III examples 40,67; table III examples 80,84; table III page 2036; table V page 2038; examples 61,68; table VI page 2038; examples 40,67; table VI examples 79,84; table VI page 2041, column II</td>
<td>1-10</td>
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<td>Y</td>
<td>WO 2004/074289 A (ALTANA PHARMA AG [DE]; ZIMMERMANN PETER OAN [DE]; BUHR WILM [DE]) 2 September 2004 (2004-09-02) cited in the application claims 1-11</td>
<td>1-10</td>
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<tr>
<td>Y</td>
<td>WO 03/018582 A (ASTRAZENECA AB [SE]; AMIN KOSRAT [SE]; DAHLSTROEM MIKAEI [SE]; NORDBER) 6 March 2003 (2003-03-06) page 1, lines 4-11 claims 1-18</td>
<td>1-10</td>
</tr>
<tr>
<td>Y</td>
<td>WO 99/55706 A (ASTRA AB [SE]; AMIN KOSRAT [SE]; DAHLSTROEM MICHAEL [SE]; NORDBERG PET) 4 November 1999 (1999-11-04) page 1, lines 5-14 claims 1-21</td>
<td>1-10</td>
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<td>P,Y</td>
<td>WO 2007/026218 A (PFIZER JAPAN INC [JP]; PFIZER [US]; MATSUMOTO YUKARI [JP]; SHIMOKAWA H) 8 March 2007 (2007-03-08) page 1, lines 3-7 claims 1-10</td>
<td>1-10</td>
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</table>
Continuation of Box II.1

Although claims 7-8 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.

Continuation of Box II.I

Claims Nos.: -

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy
### INTERNATIONAL SEARCH REPORT

**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:  
   
   see FURTHER INFORMATION sheet PCT/ISA/210

2. ☐ Claims Nos.:  
   - because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:  
   - because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III**  
Observations where unity of invention is lacking  
(Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all search able claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, 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.
## INTERNATIONAL SEARCH REPORT

**International application No**
PCT/IB2007/003835

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