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CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
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		TOTAL :	\$ 135,000.00

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**** CONSIGNACION ****

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CLARKE MODET Y CD. COLOMB	CONSIGNACION	BANCO POPULAR	050-00110-6	53267311	26/11/2007	28,480,000.00

**** CONCEPTO ****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01	SOLICITUDES	
		82 INVOCACION DE UNA PRIORIDAD EN NU	135,000.00
		TOTAL :	\$ 135,000.00

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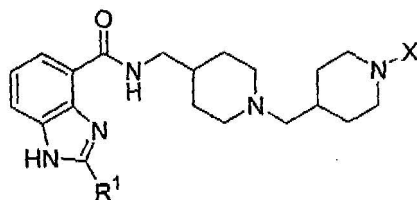
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(54) Title: BENZIMIDAZOLE-CARBOXAMIDE COMPOUNDS AS 5-HT₄ RECEPTOR AGONISTS

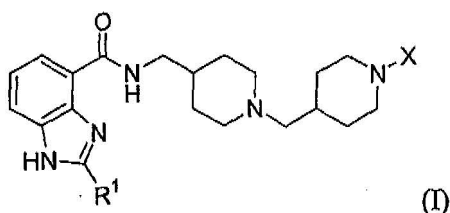


(I)

(57) Abstract: The invention relates to benzimidazole-carboxamide 5-HT₄ receptor agonist compounds of formula (I), wherein R¹ and X are as defined in the specification, or a pharmaceutically acceptable salt or solvate or stereoisomer thereof. The invention also relates to pharmaceutical compositions comprising such compounds, methods of using such compounds to treat diseases associated with 5-HT₄ receptor activity, and processes and intermediates useful for preparing such compounds. The invention further relates to crystalline forms of a compound of formula (I).

ABSTRACT

5 The invention relates to benzimidazole-carboxamide 5-HT₄ receptor agonist compounds of formula (I)



wherein R¹ and X are as defined in the specification, or a pharmaceutically acceptable salt or solvate or stereoisomer thereof. The invention also relates to pharmaceutical
10 compositions comprising such compounds, methods of using such compounds to treat diseases associated with 5-HT₄ receptor activity, and processes and intermediates useful for preparing such compounds. The invention further relates to crystalline forms of a compound of formula (I).

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BENZIMIDAZOLE-CARBOXAMIDE COMPOUNDS AS 5-HT₄ RECEPTOR AGONISTS

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BACKGROUND OF THE INVENTION

Field of the Invention

The invention is directed to benzimidazole-carboxamide compounds which are useful as 5-HT₄ receptor agonists. The invention is also directed to pharmaceutical compositions comprising such compounds, methods of using such compounds for treating or preventing medical conditions mediated by 5-HT₄ receptor activity, and processes and intermediates useful for preparing such compounds.

State of the Art

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that is widely distributed throughout the body, both in the central nervous system and in peripheral systems. At least seven subtypes of serotonin receptors have been identified and the interaction of serotonin with these different receptors is linked to a wide variety of physiological functions. There has been, therefore, substantial interest in developing therapeutic agents that target specific 5-HT receptor subtypes.

In particular, characterization of 5-HT₄ receptors and identification of pharmaceutical agents that interact with them has been the focus of significant recent activity. (See, for example, the review by Langlois and Fischmeister, *J. Med. Chem.* **2003**, *46*, 319-344.) 5-HT₄ receptor agonists are useful for the treatment of disorders of reduced motility of the gastrointestinal tract. Such disorders include irritable bowel syndrome (IBS), chronic constipation, functional dyspepsia, delayed gastric emptying,

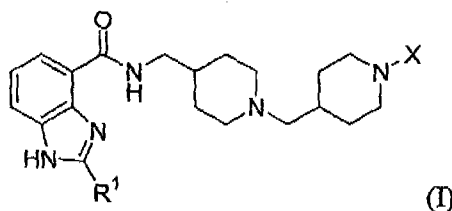
gastroesophageal reflux disease (GERD), gastroparesis, post-operative ileus, intestinal pseudo-obstruction, and drug-induced delayed transit. In addition, it has been suggested that some 5-HT₄ receptor agonist compounds may be used in the treatment of central nervous system disorders including cognitive disorders, behavioral disorders, mood disorders, and disorders of control of autonomic function.

Despite the broad utility of pharmaceutical agents modulating 5-HT₄ receptor activity, few 5-HT₄ receptor agonist compounds are in clinical use at present. Accordingly, there is a need for new 5-HT₄ receptor agonists that achieve their desired effects with minimal side effects. Preferred agents may possess, among other properties, improved selectivity, potency, pharmacokinetic properties, and/or duration of action.

SUMMARY OF THE INVENTION

The invention provides novel compounds that possess 5-HT₄ receptor agonist activity. Among other properties, compounds of the invention have been found to be potent and selective 5-HT₄ receptor agonists. In addition, preferred compounds of the invention have been found to exhibit favorable pharmacokinetic properties in an animal model which are predictive of good bioavailability upon oral administration.

Accordingly, the invention provides a compound of formula (I):



wherein:

R¹ is C₃₋₅alkyl, optionally substituted with -OH; and

X is selected from

(a) -C(O)OR² wherein R² is C₁₋₄alkyl or -(CH₂)_n-phenyl wherein n is 0 or 1;

(b) -C(O)R³ wherein R³ is selected from:

phenyl, optionally substituted with 1, 2, or 3 substituents selected from C₁₋₄alkyl, halo, C₁₋₄alkoxy, -CF₃, -OCF₃, -OCHF₂, and -CN, C₁₋₅alkyl, C₄₋₅cycloalkyl, and

-(CH₂)_m-A wherein *m* is 0 or 1 and A is selected from amino, furanyl, thiophenyl, morpholinyl, tetrahydrofuranyl, pyridinyl, naphthalenyl, pyrrolyl, thiomorpholinyl, pyrrolidinyl, piperidinyl, oxoazetidiny, thiazolidinyl, 1,1-dioxo isothiazolidinyl, and 2,4-

5 dimethylisoxazolyl;

(c) -C(O)NR⁴R⁵ wherein R⁴ is hydrogen or C₁₋₃alkyl, and R⁵ is phenyl optionally substituted with 1, 2, or 3 substituents selected from C₁₋₄alkyl, halo, C₁₋₄alkoxy, -CF₃, -OCF₃, and -OCHF₂;

10 (d) -C(O)C(R⁶R⁷)R⁸ wherein R⁶ is hydrogen or C₁₋₃alkyl and R⁷ is hydrogen, -OH, or C₁₋₃alkyl; or R⁶ and R⁷ taken together form oxo or -(CH₂)₂-; and R⁸ is phenyl or cyclohexyl, wherein phenyl or cyclohexyl are optionally substituted with 1, 2, or 3 substituents selected from C₁₋₄alkyl, halo, C₁₋₄alkoxy, -CF₃, -OCF₃, -OCHF₂, and -CN;

15 (e) -C(O)C(HR⁹)OR¹⁰ wherein R⁹ is hydrogen or C₁₋₃alkyl, and R¹⁰ is phenyl optionally substituted with 1, 2, or 3 substituents selected from C₁₋₄alkyl, halo, C₁₋₄alkoxy, -CF₃, -OCF₃, and -OCHF₂; and

20 (f) -S(O)₂R¹¹ wherein R¹¹ is selected from C₁₋₃alkyl, -CH₂-phenyl, furanyl, thiophenyl, morpholinyl, tetrahydrofuranyl, pyridinyl, naphthalenyl, pyrrolyl, thiomorpholinyl, pyrrolidinyl, piperidinyl, oxoazetidiny, thiazolidinyl, 1,1-dioxo isothiazolidinyl, 2,4-dimethylisoxazolyl, and phenyl optionally substituted with 1, 2, or 3 substituents selected from C₁₋₄alkyl, halo, C₁₋₄alkoxy, -CF₃, -OCF₃, -OCHF₂, and -CN;

or a pharmaceutically-acceptable salt or solvate or stereoisomer thereof.

25 The invention also provides a pharmaceutical composition comprising a compound of the invention and a pharmaceutically-acceptable carrier.

In another aspect, the invention provides a particular compound of formula (I) in crystalline free base form. Crystalline 4-(4-{{(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)amino]methyl}-piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester has been found to have a melting temperature in the range of about 145 °C to about 30 155 °C, typically between about 146 and about 148 °C, a degradation temperature above about 240 °C, and to exhibit weight changes of less than about 0.25 % when exposed to a range of relative humidity between about 2 % and about 90 % at room temperature. Additional crystalline forms of 4-(4-{{(2-isopropyl-1*H*-benzoimidazole-4-

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carbonyl)amino]methyl}-piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester are also provided in further aspects of the invention.

In a method aspect, the invention provides a method of treating a disease or condition associated with 5-HT₄ receptor activity, e.g. a disorder of reduced motility of the gastrointestinal tract, the method comprising administering to the mammal, a therapeutically effective amount of a compound of the invention.

Further, the invention provides a method of treating a disease or condition associated with 5-HT₄ receptor activity in a mammal, the method comprising administering to the mammal, a therapeutically effective amount of a pharmaceutical composition of the invention.

The compounds of the invention can also be used as research tools, i.e. to study biological systems or samples, or for studying the activity of other chemical compounds. Accordingly, in another of its method aspects, the invention provides a method of using a compound of formula (I), or a pharmaceutically acceptable salt or solvate or stereoisomer thereof, as a research tool for studying a biological system or sample or for discovering new 5-HT₄ receptor agonists, the method comprising contacting a biological system or sample with a compound of the invention and determining the effects caused by the compound on the biological system or sample.

In separate and distinct aspects, the invention also provides synthetic processes and intermediates described herein, which are useful for preparing compounds of the invention.

The invention also provides a compound of the invention as described herein for use in medical therapy, as well as the use of a compound of the invention in the manufacture of a formulation or medicament for treating a disease or condition associated with 5-HT₄ receptor activity, e.g. a disorder of reduced motility of the gastrointestinal tract, in a mammal.

BRIEF DESCRIPTION OF THE DRAWINGS

Various aspects of the present invention are illustrated by reference to the accompanying drawings.

Figure 1 shows a powder x-ray diffraction (PXRD) pattern of crystalline 4-(4-[(2-isopropyl-1H-benzoimidazole-4-carbonyl)amino]methyl)-piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester (Form I).

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Figure 2 shows a differential scanning calorimetry (DSC) trace (top trace, right vertical axis) and a thermal gravimetric analysis (TGA) trace (bottom trace, left vertical axis) for crystalline 4-(4-[(2-isopropyl-1H-benzimidazole-4-carbonyl)amino]methyl)-piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester (Form I).

5 Figure 3 shows a dynamic moisture sorption (DMS) isotherm for crystalline 4-(4-[(2-isopropyl-1H-benzimidazole-4-carbonyl)amino]methyl)-piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester (Form I).

Figure 4 shows a differential scanning calorimetry (DSC) trace (top trace, right vertical axis) and a thermal gravimetric analysis (TGA) trace (bottom trace, left vertical axis) for crystalline 4-(4-[(2-isopropyl-1H-benzimidazole-4-carbonyl)amino]methyl)-piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester (Form II).

Figure 5 shows a powder x-ray diffraction (PXRD) pattern of crystalline 4-(4-[(2-isopropyl-1H-benzimidazole-4-carbonyl)amino]methyl)-piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester (Form III).

15 Figure 6 shows a differential scanning calorimetry (DSC) trace (top trace, right vertical axis) and a thermal gravimetric analysis (TGA) trace (bottom trace, left vertical axis) for crystalline 4-(4-[(2-isopropyl-1H-benzimidazole-4-carbonyl)amino]methyl)-piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester (Form III).

20 DETAILED DESCRIPTION OF THE INVENTION

The invention provides novel benzimidazole-carboxamide 5-HT₄ receptor agonists of formula (I), or pharmaceutically-acceptable salts or solvates or stereoisomers thereof. The following substituents and values are intended to provide representative examples of various aspects of this invention. These representative values are intended to further
25 define such aspects and are not intended to exclude other values or limit the scope of the invention.

In a specific aspect of the invention, R¹ is C₃₋₅alkyl, optionally substituted with -OH.

In another specific aspect, R¹ is C₃₋₅alkyl.

30 In other specific aspects, R¹ is C₃₋₄alkyl; or R¹ is isopropyl or *tert*-butyl.

In another specific aspect, R¹ is isopropyl.

In yet other specific aspects, R¹ is 1-hydroxy-1-methylethyl, or 2-hydroxy-1-methylethyl.

(a)

In a specific aspect, X is $-C(O)OR^2$ wherein R^2 is C_{1-4} alkyl or $-(CH_2)_n$ -phenyl wherein n is 0 or 1.

In another specific aspect, X is $-C(O)OR^2$ wherein R^2 is C_{1-3} alkyl or phenyl.

In other specific aspects, X is $-C(O)OR^2$ wherein R^2 is methyl or phenyl, or
5 wherein R^2 is methyl.

In a specific aspect, X is $-C(O)R^3$ wherein R^3 is selected from phenyl, optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, $-OCHF_2$, and $-CN$; C_{1-5} alkyl; C_{4-5} cycloalkyl; and $-(CH_2)_m$ -A wherein m is 0 or 1 and A is selected from amino, furanyl, thiophenyl, morpholinyl, tetrahydrofuranyl,
10 pyridinyl, naphthalenyl, pyrrolyl, thiomorpholinyl, pyrrolidinyl, piperidinyl, oxoazetidiny, thiazolidinyl, 1,1-dioxo isothiazolidinyl, and 2,4-dimethylisoxazolyl.

In another specific aspect, X is $-C(O)R^3$ wherein R^3 is phenyl, optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, $-OCHF_2$, and $-CN$.

15 In another specific aspect, X is $-C(O)R^3$ wherein R^3 is C_{1-5} alkyl or C_{4-5} cycloalkyl.

In another specific aspect, X is $-C(O)R^3$ wherein R^3 is $-(CH_2)_m$ -A wherein m is 0 and A is selected from amino, furanyl, thiophenyl, morpholinyl, tetrahydrofuranyl, pyridinyl, naphthalenyl, pyrrolyl, thiomorpholinyl, pyrrolidinyl, piperidinyl, oxoazetidiny, thiazolidinyl, 1,1-dioxo isothiazolidinyl, and 2,4-dimethylisoxazolyl.

20 In another specific aspect, X is $-C(O)R^3$ wherein R^3 is phenyl, optionally substituted with 1 or 2 substituents selected from C_{1-4} alkyl, halo, and $-CF_3$; furanyl; or thiophenyl.

In yet other specific aspects, X is $-C(O)R^3$ wherein R^3 is phenyl optionally substituted with 1 or 2 substituents selected from methyl, chloro, fluoro, and $-CF_3$; or R^3
25 is furan-2-yl or thiophen-2-yl.

In a specific aspect, X is $-C(O)NR^4R^5$ wherein R^4 is hydrogen or C_{1-3} alkyl, and R^5 is phenyl optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, and $-OCHF_2$.

In another specific aspect, X is $-C(O)NR^4R^5$ wherein R^4 is hydrogen.

30 In another specific aspect, X is $-C(O)NR^4R^5$ wherein R^4 is hydrogen and R^5 is phenyl optionally substituted with 1 or 2 substituents selected from C_{1-4} alkyl and halo.

In other specific aspects, X is $-C(O)NR^4R^5$ wherein R^4 is hydrogen and R^5 is phenyl optionally substituted with 1 halo, or with one fluoro or chloro.

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in a specific aspect, X is $-C(O)C(R^6R^7)R^8$ wherein R^6 is hydrogen or C_{1-3} alkyl; R^7 is hydrogen, -OH, or C_{1-3} alkyl; or R^6 and R^7 taken together form oxo or $-(CH_2)_2$; and R^8 is phenyl or cyclohexyl, wherein phenyl or cyclohexyl are optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, $-OCHF_2$, and -CN.

In another specific aspect, X is $-C(O)C(R^6R^7)R^8$ wherein R^6 is hydrogen.

In another specific aspect, X is $-C(O)C(R^6R^7)R^8$ wherein R^8 is phenyl optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, $-OCHF_2$, and -CN.

10 In another specific aspect, X is $-C(O)C(R^6R^7)R^8$ wherein R^8 is cyclohexyl optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, $-OCHF_2$, and -CN.

In yet other specific aspects, X is $-C(O)C(R^6R^7)R^8$ wherein R^6 is hydrogen and R^7 is hydrogen, -OH, or methyl; or R^6 and R^7 taken together form oxo or $-(CH_2)_2$; and R^8 is phenyl or cyclohexyl, wherein phenyl or cyclohexyl are optionally substituted with 1 or 2 substituents selected from C_{1-4} alkyl and halo; or R^8 is phenyl or cyclohexyl, wherein phenyl or cyclohexyl are optionally substituted with 1 or 2 substituents selected from methyl, fluoro, and chloro.

20 In a specific aspect, X is $-C(O)C(HR^9)OR^{10}$ wherein R^9 is hydrogen or C_{1-3} alkyl, and R^{10} is phenyl optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, and $-OCHF_2$.

In another specific aspect, X is $-C(O)C(HR^9)OR^{10}$ wherein R^9 is hydrogen or methyl.

25 In other specific aspects, X is $-C(O)C(HR^9)OR^{10}$ wherein R^9 is hydrogen or methyl and R^{10} is phenyl optionally substituted with 1 or 2 substituents selected from C_{1-4} alkyl and halo, or phenyl optionally substituted with 1 or 2 substituents selected from methyl, fluoro, and chloro.

30 In a specific aspect, X is $-S(O)_2R^{11}$ wherein R^{11} is selected from C_{1-3} alkyl, $-CH_2$ -phenyl, furanyl, thiophenyl, morpholinyl, tetrahydrofuranyl, pyridinyl, naphthalenyl, pyrrolyl, thiomorpholinyl, pyrrolidinyl, piperidinyl, oxoazetidiny, thiazolidinyl, 1,1-dioxo isothiazolidinyl, 2,4-dimethylisoxazolyl, and phenyl optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, $-OCHF_2$, and -CN.

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In another specific aspect, X is $-S(O)_2R^{11}$ wherein R^{11} is C_{1-3} alkyl, 2,4-dimethylisoxazolyl, or phenyl, optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, $-OCHF_2$, and $-CN$.

In other specific aspects, X is $-S(O)_2R^{11}$ wherein R^{11} is methyl or phenyl
5 optionally substituted with 1 or 2 substituents selected from C_{1-4} alkyl and halo; or with 1 or 2 substituents selected from methyl, fluoro, and chloro.

In one aspect, the invention provides a compound of formula (I) wherein
 R^1 is C_{3-4} alkyl; and

X is selected from:

- 10 (a) $-C(O)OR^2$ wherein R^2 is C_{1-3} alkyl or phenyl;
(b) $-C(O)R^3$ wherein R^3 is phenyl, optionally substituted with 1 or 2 substituents selected from C_{1-4} alkyl, halo, and $-CF_3$; furanyl; or thiophenyl;
(c) $-C(O)NR^4R^5$ wherein R^4 is hydrogen and R^5 is phenyl optionally substituted with 1 or 2 substituents selected from C_{1-4} alkyl and halo;
15 (d) $-C(O)C(R^6R^7)R^8$ wherein R^6 is hydrogen and R^7 is hydrogen, $-OH$, or methyl; or R^6 and R^7 taken together form oxo or $-(CH_2)_2-$; and R^8 is phenyl or cyclohexyl, wherein phenyl or cyclohexyl are optionally substituted with 1 or 2 substituents selected from C_{1-4} alkyl and halo;
(e) $-C(O)C(HR^9)OR^{10}$ wherein R^9 is hydrogen or methyl and R^{10} is phenyl
20 optionally substituted with 1 or 2 substituents selected from C_{1-4} alkyl and halo; and
(f) $-S(O)_2R^{11}$ wherein R^{11} is methyl or phenyl, optionally substituted with 1 or 2 substituents selected from C_{1-4} alkyl and halo.

The invention further provides a compound of formula (I) wherein:

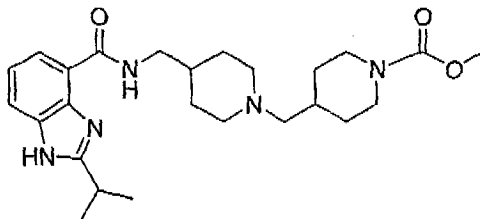
25 R^1 is isopropyl or *tert*-butyl; and

X is selected from:

- (a) $-C(O)OR^2$ wherein R^2 is methyl or phenyl;
(b) $-C(O)R^3$ wherein R^3 is phenyl, optionally substituted with 1 or 2 substituents selected from methyl, chloro, fluoro, and $-CF_3$; furan-2-yl; or
30 thiophen-2-yl; and
(c) $-C(O)NR^4R^5$ wherein R^4 is hydrogen and R^5 is phenyl optionally substituted with 1 fluoro or chloro.

In still other specific aspects, the invention provides the compounds listed in the Examples and in Tables I to IX below.

The chemical naming convention used herein is illustrated for the compound of Example 1:



5 which is designated 4-(4-{{(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)amino}methyl}-piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester, according to the AutoNom software, provided by MDL Information Systems, GmbH (Frankfurt, Germany). The fused ring structure "benzoimidazole" is alternatively named
10 "benzimidazole". The two terms are equivalent as used herein.

As exemplified by particular compounds listed in the tables below, the compounds of the invention may contain a chiral center. Accordingly, the invention includes racemic mixtures, pure stereoisomers, and stereoisomer-enriched mixtures of such isomers, unless
15 otherwise indicated. When a particular stereoisomer is shown, it will be understood by those skilled in the art, that minor amounts of other stereoisomers may be present in the compositions of the invention unless otherwise indicated, provided that any utility of the composition as a whole is not eliminated by the presence of such other isomers.

In one aspect, the invention provides a compound selected from:

- 20 4-(4-{{(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)-amino}methyl}piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester;
- 4-(4-{{(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)-amino}-methyl}piperidin-1-ylmethyl)piperidine-1-carboxylic acid phenyl ester;
- 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(2-chlorobenzoyl)piperidin-4-ylmethyl] piperidin-4-ylmethyl} amide;
- 25 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(2,4-difluorobenzoyl)piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide;
- 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(furan-2-carbonyl)piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide;

2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(thiophene-2-carbonyl)piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide;

2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(2-fluoro-5-trifluoromethylbenzoyl)piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide;

5 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(2-fluoro-phenylcarbamoyl)piperidin-4-ylmethyl]piperidin-4-ylmethyl} -amide;

4-(4-[[2-*tert*-butyl-1*H*-benzoimidazole-4-carbonyl]-amino]-methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester;

2-*tert*-butyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(2-fluoro-benzoyl)-piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide;

2-*tert*-butyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(3-methyl-benzoyl)-piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide; and

2-*tert*-butyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(4-fluorobenzoyl)-piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide.

15

Definitions

When describing the compounds, compositions and methods of the invention, the following terms have the following meanings, unless otherwise indicated.

The term "alkyl" means a monovalent saturated hydrocarbon group which may be linear or branched or combinations thereof. Unless otherwise defined, such alkyl groups typically contain from 1 to 10 carbon atoms. Representative alkyl groups include, by way of example, methyl (Me), ethyl, *n*-propyl (*n*-Pr), isopropyl (iPr), *n*-butyl (*n*-Bu), *sec*-butyl, isobutyl, *tert*-butyl (tBu), *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, *n*-decyl and the like.

25 The term "alkoxy" means a monovalent group -O-alkyl, where alkyl is defined as above. Representative alkoxy groups include, by way of example, methoxy, ethoxy, propoxy, butoxy, and the like.

The term "cycloalkyl" means a monovalent saturated carbocyclic group which may be monocyclic or multicyclic. Unless otherwise defined, such cycloalkyl groups typically contain from 3 to 10 carbon atoms. Representative cycloalkyl groups include, by way of example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and the like.

The term "halo" means fluoro, chloro, bromo or iodo.

The term "oxo" means a double-bonded oxygen atom (=O).

The term "compound" means a compound that was synthetically prepared or produced in any other way, such as by metabolism.

The term "therapeutically effective amount" means an amount sufficient to effect
5 treatment when administered to a patient in need of treatment.

The term "treatment" as used herein means the treatment of a disease, disorder, or medical condition in a patient, such as a mammal (particularly a human) which includes:

- (a) preventing the disease, disorder, or medical condition from occurring, i.e., prophylactic treatment of a patient;
- 10 (b) ameliorating the disease, disorder, or medical condition, i.e., eliminating or causing regression of the disease, disorder, or medical condition in a patient;
- (c) suppressing the disease, disorder, or medical condition, i.e., slowing or arresting the development of the disease, disorder, or medical condition in
15 a patient; or
- (d) alleviating the symptoms of the disease, disorder, or medical condition in a patient.

The term "pharmaceutically-acceptable salt" means a salt prepared from an acid or base which is acceptable for administration to a patient, such as a mammal. Such salts
20 can be derived from pharmaceutically-acceptable inorganic or organic acids and from pharmaceutically-acceptable bases. Typically, pharmaceutically-acceptable salts of compounds of the present invention are prepared from acids.

Salts derived from pharmaceutically-acceptable acids include, but are not limited to, acetic, adipic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethanesulfonic,
25 fumaric, gluconic, glutamic, hydrobromic, hydrochloric, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pantothenic, phosphoric, succinic, sulfuric, tartaric, p-toluenesulfonic, xinafoic (1-hydroxy-2-naphthoic acid), naphthalene-1,5-disulfonic acid and the like.

The term "solvate" means a complex or aggregate formed by one or more
30 molecules of a solute, i.e. a compound of the invention or a pharmaceutically-acceptable salt thereof, and one or more molecules of a solvent. Such solvates are typically crystalline solids having a substantially fixed molar ratio of solute and solvent. Representative solvents include by way of example, water, methanol, ethanol,

isopropanol, acetic acid, and the like. When the solvent is water, the solvate formed is a hydrate.

It will be appreciated that the term "or a pharmaceutically-acceptable salt or solvate of stereoisomer thereof" is intended to include all permutations of salts, solvates and stereoisomers, such as a solvate of a pharmaceutically-acceptable salt of a stereoisomer of a compound of formula (I).

The term "amino-protecting group" means a protecting group suitable for preventing undesired reactions at an amino nitrogen. Representative amino-protecting groups include, but are not limited to, formyl; acyl groups, for example alkanoyl groups, such as acetyl; alkoxycarbonyl groups, such as *tert*-butoxycarbonyl (Boc); arylmethoxycarbonyl groups, such as benzyloxycarbonyl (Cbz) and 9-fluorenylmethoxycarbonyl (Fmoc); arylmethyl groups, such as benzyl (Bn), trityl (Tr), and 1,1-di-(4'-methoxyphenyl)methyl; silyl groups, such as trimethylsilyl (TMS) and *tert*-butyldimethylsilyl (TBDMS); and the like.

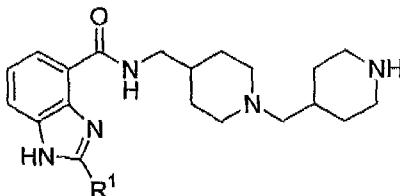
General Synthetic Procedures

Compounds of the invention can be prepared from readily available starting materials using the following general methods and procedures. Although a particular aspect of the present invention is illustrated in the schemes below, those skilled in the art will recognize that all aspects of the present invention can be prepared using the methods described herein or by using other methods, reagents and starting materials known to those skilled in the art. It will also be appreciated that where typical or preferred process conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given, other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

Additionally, as will be apparent to those skilled in the art, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. The choice of a suitable protecting group for a particular functional group, as well as suitable conditions for protection and deprotection, are well known in the art. For example, numerous protecting groups, and their introduction and removal, are described

in T. W. Greene and G. M. Wuts, *Protecting Groups in Organic Synthesis*, Third Edition, Wiley, New York, 1999, and references cited therein.

In one method of synthesis, compounds of formula (I) are prepared by reacting a piperidinylmethyl-piperidinylmethyl intermediate of formula (II):



(II)

with a reagent of formula (III):

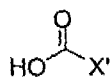


(III)

- 10 where L is a leaving group, for example a halo, such as chloro, or an acyloxy, sulfonic ester, or oxysuccinimide, and R¹ and X are defined as in formula (I).

The reaction is typically conducted by contacting intermediate (II) with between about 1 and about 1.5 equivalents of intermediate (III) in a polar aprotic diluent, such as dichloromethane, in the presence of at least one equivalent of an amine base, such as *N,N*-diisopropylethylamine. Suitable inert diluents for this process and those described below, also include *N,N*-dimethylformamide, trichloromethane, 1,1,2,2-tetrachloroethane, tetrahydrofuran, and the like. Suitable amine bases for the processes of the present invention also include triethylamine, pyridine, and the like. The reaction is typically conducted at a temperature in the range of about 0 °C to about 30 °C for about a quarter
 15
 20 hour to about 2 hours, or until the reaction is substantially complete. Exemplary reagents L—X in which L is chloro include methyl chloroformate, phenyl chloroformate, chlorobenzoyl chloride, and methanesulfonyl chloride.

In an alternative method of synthesis, compounds of formula (I) in which X is selected from -C(O)R³, -C(O)C(R⁶R⁷)R⁸, and -C(O)C(HR⁹)OR¹⁰ can be prepared by the
 25 amide coupling reaction of an intermediate of formula (II) with a carboxylic acid of formula (IV):

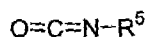


(IV)

In formula (IV), X' represents R³, C(R⁶R⁷)R⁸, or C(HR⁹)OR¹⁰, such that -C(O)X' corresponds to X, as set forth above formula (IV). In the amide coupling reaction of intermediate (II), between about 1 and about 1.5 equivalents of the carboxylic acid (IV) are first contacted with between 1 and about 1.5 equivalents of a coupling agent such as O-(7-axabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) in a polar aprotic solvent such as dimethylformamide or those discussed above. The acid mixture is then contacted with intermediate (II) in the presence of between about 2 and about 4 equivalents of an amine base, for example, N,N-diisopropylethylamine. The reaction is typically conducted at a temperature in the range of about 0 °C to about 30 °C for about a quarter hour to about 2 hours, or until the reaction is substantially complete.

Suitable alternative coupling agents include N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), 1,1'-carbonyldiimidazole (CDI), 1,3 dicyclohexylcarbodiimide (DCC), and benzotriazol-1-yloxytripyrrolidino-phosphonium hexafluorophosphate (PyBop). The coupling agents may be combined with promoting agents, for example, 1-hydroxy-7-azabenzotriazole (HOAt), hydroxybenzotriazole (HOBt), or 1,4-diazabicyclo[2,2,2]octane (DABCO).

In yet another alternative process, compounds of formula (I) in which X is -C(O)NHR⁵ can be prepared by reacting an intermediate of formula (II) with an isocyanate of the form:



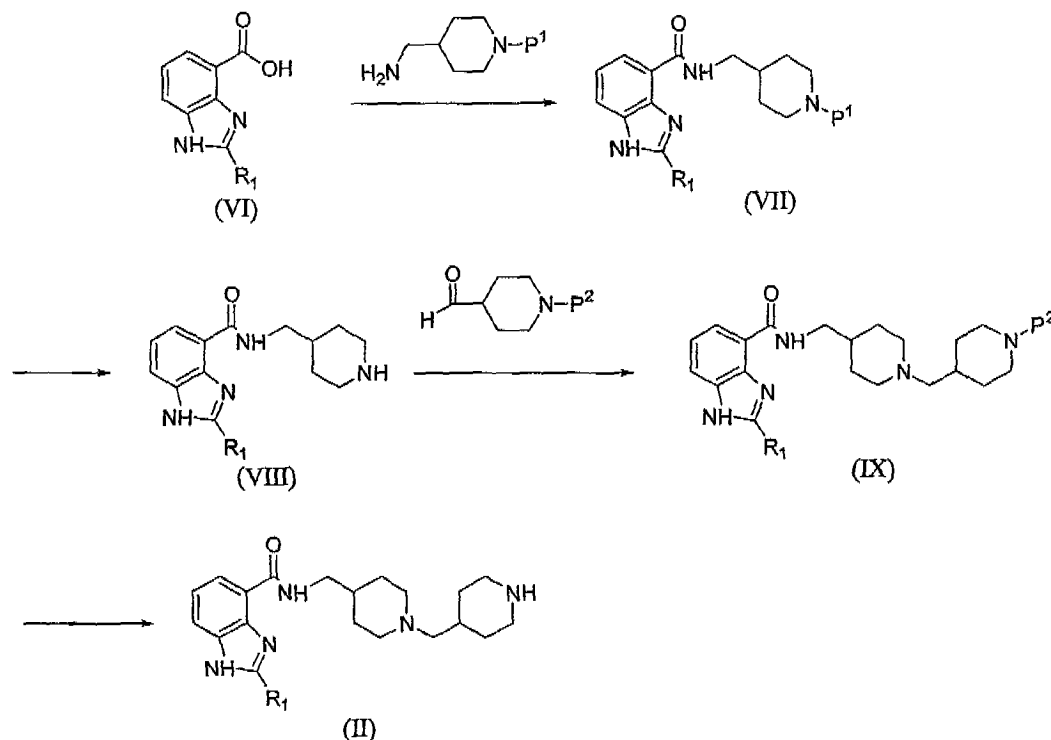
(V)

The reaction is typically conducted by contacting intermediate (II) with between about 1 and about 1.5 equivalents of intermediate (V) in a polar aprotic diluent in the presence of at between about 2 and about 4 equivalents of an amine base. The reaction is typically conducted at a temperature in the range of about 0 °C to about 30 °C for about a quarter hour to about 24 hours, or until the reaction is substantially complete.

The product of formula (I) is isolated and purified by conventional procedures. For example, the product can be concentrated to dryness under reduced pressure and the residue purified by HPLC chromatography.

The piperidinylmethyl-piperidinylmethyl intermediates of formula (II) are prepared from readily available starting materials by the procedure illustrated in Scheme A.

Scheme A



- 5 where P^1 and P^2 independently represent an amino protecting group, such as *tert*-butoxycarbonyl (Boc).

First, a carboxylic acid of formula (VI) is reacted with a protected aminomethyl piperidine to form a protected intermediate of formula (VII). This reaction is typically conducted by contacting (VI) with between about 1 and about 2 equivalents of protected aminomethylpiperidine in a polar aprotic diluent, in the presence of an amide coupling agent described above, for example *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) combined with hydroxybenzotriazole (HOBt) or 1,1'-carbonyldiimidazole (CDI) combined with 1,4-diazabicyclo[2,2,2]octane (DABCO). The reaction is typically conducted at a temperature in the range of about 0 °C to about 60 °C for between about 1 and about 24 hours or until the reaction is substantially complete.

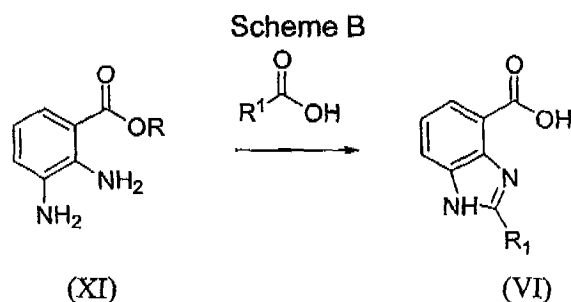
The protecting group P^1 is removed from intermediate (VII) by conventional means to provide intermediate (VIII). For example, when Boc is used as the protecting

group, it may be removed by treatment with an acid, such as trifluoroacetic acid or hydrochloric acid.

An intermediate of formula (IX) is then formed by the reductive amination of intermediate (VIII) with a protected piperidine-carboxaldehyde. This reaction is typically conducted by contacting (VIII) with between about 1 and about 2 equivalents of the protected piperidine-carboxaldehyde in an inert diluent in the presence of between about 1 and about 2 equivalents of a reducing agent. Optionally, about one equivalent of a weak acid, such as acetic acid can be included to accelerate the reaction. The reaction may be conducted at a temperature between about 0 °C and about 30 °C, typically between about 20 °C and about 30 °C, for about 0.25 to about 2 hours, or until the reaction is substantially complete.

Suitable inert diluents include dichloromethane, trichloromethane, 1,1,2,2-tetrachloroethane, and the like. Typical reducing agents include sodium triacetoxyborohydride, sodium borohydride, and sodium cyanoborohydride. The product (IX) is isolated by standard procedures. When the amine (VIII) is supplied as an acid salt, typically between about 1 and about 3 equivalents of an amine base, such as *N,N*-diisopropylethylamine, is included in the reaction. Finally, the protecting group P² is removed from intermediate (IX) by conventional procedures to provide the piperidinylmethyl-piperidinylmethyl intermediate (II).

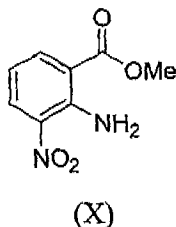
A carboxylic acid of formula (VI) can be prepared from a diaminobenzoic acid or ester by the process illustrated in Scheme B:



where R represents methyl or hydrogen. Intermediate (XI) is reacted with a carboxylic acid R¹C(O)OH to form the acid intermediate (VI). This reaction is typically conducted by contacting the acid or ester (XI) with between about 2 and about 4 equivalents of the carboxylic acid R¹C(O)OH in an aqueous acidic solution. The reaction is typically conducted at a temperature in the range of about 80 °C to about 100 °C for about 12 to

about 72 hours. The pH of the solution is then raised by the addition of base, such as sodium hydroxide, and the product isolated by conventional means.

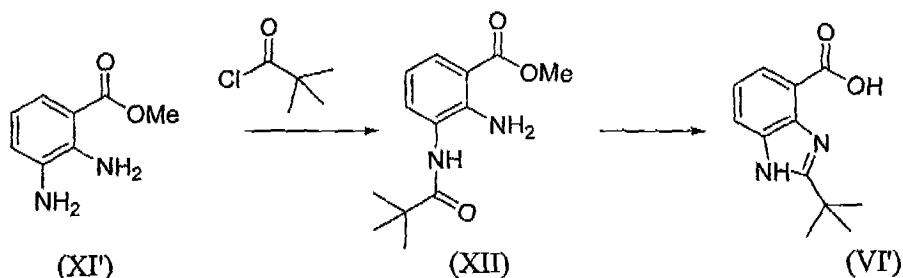
A convenient process for providing intermediate (XI) as the methyl ester uses 2-amino-3-nitrobenzoic acid methyl ester (X):



as the starting material. Typically, 2-amino-3-nitrobenzoic acid methyl ester (X) is dissolved in a polar diluent and reduced by exposure to a hydrogen atmosphere in the presence of a transition metal catalyst to provide the diaminobenzoic acid methyl ester (XI). The reaction is typically conducted at ambient temperature for about 12 to about 72 hours.

When the substituent R^1 is sterically bulky, as for example, when R^1 is *tert*-butyl, *tert*-butylbenzimidazole carboxylic acid (VI) can be prepared by the conversion of methyl ester (XI') according to a two-step process, as illustrated, for example, in Scheme C:

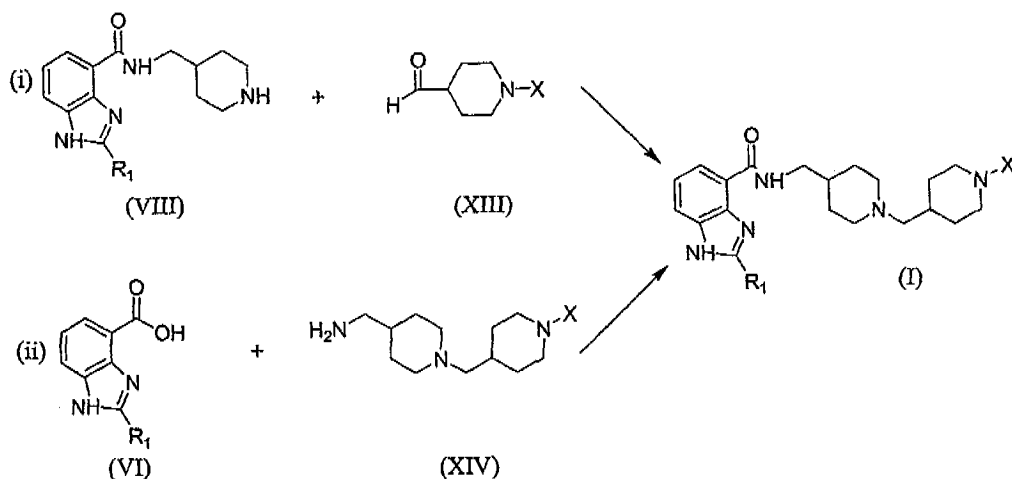
Scheme C



As described in detail in Preparation 3 below, the methyl ester (XI') is first reacted with 2,2-dimethylpropionyl chloride to provide the intermediate (XII) which is refluxed in a strong acid solution, typically, for between about 12 and about 72 hours, to provide the *tert*-butylbenzimidazole carboxylic acid (VI).

In alternative methods of synthesis, compounds of formula (I) can be prepared according to the process routes illustrated in Scheme D using the reductive amination and other reactions described above, and/or using alternative reactions well known to those skilled in the art.

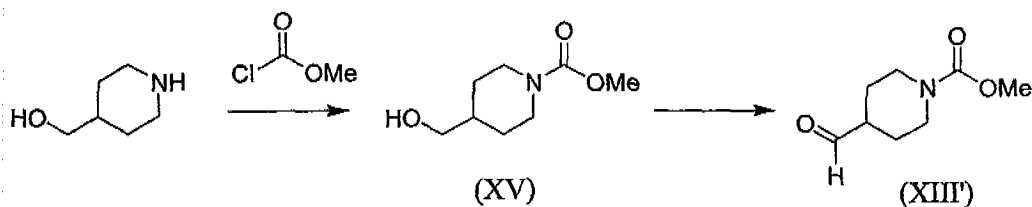
Scheme D



As shown in process route (i), an intermediate of formula (VIII) is reacted with an intermediate of formula (XIII) to provide a compound of formula (I). The reaction is typically performed under the conditions described above for the reaction of amine (VIII) with the protected piperidine-carboxaldehyde in Scheme A.

Intermediate (XIII) can be prepared by the reaction of 4-hydroxymethylpiperidine with a reagent L—X, of formula (III), followed by oxidation of the resulting intermediate. For example, for the particular case of X is —C(O)OCH₃, intermediate (XIII) can be prepared as shown in Scheme E.

Scheme E



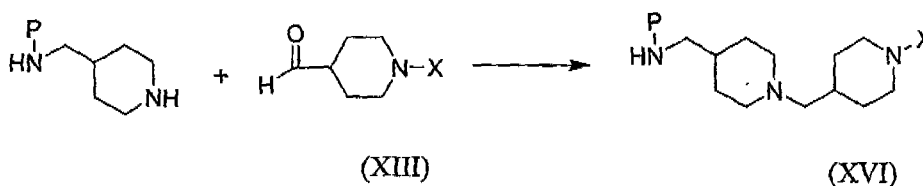
First 4-hydroxymethylpiperidine is reacted with methylchloroformate to form the hydroxymethylpiperidine intermediate (XV). The reaction is typically conducted by contacting 4-hydroxymethylpiperidine in an aqueous solution with between about 3 and about 5 equivalents of methylchloroformate in the presence of between about 3 and about 5 equivalents of base. The reaction is typically conducted at a temperature in the range of about 0 °C to about 30 °C for about 12 to about 72 hours or until the reaction is substantially complete. Intermediate (XV) is then oxidized to form the formylpiperidinyl intermediate (XIII'). The oxidation reaction typically makes use of an oxidation reagent such as a combination of oxalyl chloride and dimethylsulfoxide (Swern oxidation), a

chromate reagent, such as pyridinium chlorochromate, or an oxidizing agent, such as sodium hypochlorite, together with a catalyst such as 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO).

The preparation of a compound of formula (I) according to process route (i) of Scheme D using intermediate (XIII') is described in Examples 214 and 216 below.

A compound of formula (I) can also be prepared by reacting a carboxylic acid of formula (VI) with an intermediate of formula (XIV), as shown in process route (ii). As illustrated in Scheme F, intermediate (XIV) can be prepared by the reaction of a protected aminomethylpiperidine with intermediate (XIII):

Scheme F



where P is an amino-protecting group, to provide a protected intermediate of formula (XVI), followed by a deprotection step. The preparation of compounds according to process route (ii) is described in Preparation 4 and in Examples 14 and 15 below.

The reagents $L-X$ (III), $X'C(O)OH$ (IV), $O=C=N=R^5$ (V) and $R^1C(O)OH$ are available commercially or are readily prepared by standard procedures from common starting materials.

Further details regarding specific reaction conditions and other procedures for preparing representative compounds of the invention or intermediates thereto are described in the examples below.

Accordingly, in a method aspect, the invention provides a process for preparing a compound of formula (I), or a salt or stereoisomer thereof, the process comprising (a) reacting a compound of formula (II) with compound of the formula (III); (b) reacting a compound formula (VIII) with a compound of formula (XIII); or (c) reacting a compound of formula (VI) with a compound of formula (XIV) to provide a compound of formula (I), or a salt or stereoisomer thereof.

In an additional method aspect, the invention provides a process for preparing a compound of formula (I) wherein X is selected from $-C(O)R^3$, $-C(O)C(R^6R^7)R^8$, and $-C(O)C(HR^9)OR^{10}$, or a salt or stereoisomer thereof, the process comprising reacting a compound of formula (II) with a compound of formula (IV), wherein X' represents R^3 ,

$C(R^6R^7)R^8$, or $C(HR^9)OR^{10}$, to provide a compound of formula (I), or a salt or stereoisomer thereof.

The invention further provides a compound of formula (II), or a salt or stereoisomer or protected derivative thereof, wherein R^1 is defined as in formula (I).

5

Crystalline Forms

In another aspect, the invention provides 4-(4-[(2-isopropyl-1H-benzimidazole-4-carbonyl)amino]methyl)-piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester in crystalline freebase form or a solvate thereof. Three distinguishable forms of
10 crystalline 4-(4-[(2-isopropyl-1H-benzimidazole-4-carbonyl)amino]methyl)-piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester (hereinafter compound **1**) have been observed.

Crystalline Form I of the present invention is a crystalline freebase. Form I is characterized by a powder x-ray diffraction (PXRD) pattern having two or more
15 diffraction peaks at 2θ values selected from 15.08 ± 0.20 , 15.41 ± 0.20 , 19.00 ± 0.20 , 19.70 ± 0.20 , and 23.68 ± 0.20 . In particular, Form I is characterized by a powder x-ray diffraction pattern having diffraction peaks at 2θ values of 19.00 ± 0.20 and 19.70 ± 0.20 .

As is well known in the field of powder x-ray diffraction, peak positions of PXRD spectra are relatively less sensitive to experimental details, such as details of sample
20 preparation and instrument geometry, than are the relative peak heights. Thus, in one aspect, the crystalline Form I is characterized by a powder x-ray diffraction pattern in which the peak positions are substantially in accordance with those shown in Figure 1.

Form I has been further characterized by x-ray diffraction analysis of crystal structure, providing the following lattice parameters: unit cell is orthorhombic with
25 dimensions $a = 16.9053 \text{ \AA}$, $b = 9.5172 \text{ \AA}$, $c = 15.4659 \text{ \AA}$; space group is $Pna2_1$; calculated density is 1.22 g/cm^3 . The resulting molecular structure confirms the chemical composition is that of compound **1** and that the asymmetric unit does not contain water or other solvent molecules. Powder x-ray diffraction peaks predicted from the derived atomic positions are in excellent agreement with observed results.

30 Crystalline Form I of the present invention is also characterized by high temperature thermal stability as evidenced by its differential scanning calorimetry (DSC) profile which exhibits a peak in endothermic heat flow in the range of about 145°C to about 155°C , typically between about 146 and 148°C as illustrated in Figure 2, which

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may be identified as a melting point. Furthermore, the thermal gravimetric analysis (TGA) profile shows no significant weight change event below the onset of degradation which is above about 240 °C.

In yet another aspect, the present crystalline form is characterized by its infrared
5 absorption spectrum which shows significant absorption bands at about 766, 1097, 1251, 1413, 1449, 1579, 1609, 1640, and 1696 cm⁻¹.

The present crystalline form has been demonstrated to have a reversible
sorption/desorption profile with an exceptionally low level of hygroscopicity (i.e., less
than about 0.25 % weight gain in the humidity range of 2 % relative humidity to 90 %
10 relative humidity at room temperature) as shown in Figure 3.

Additionally, crystalline Form I of compound 1 has been found to be stable upon
exposure to elevated temperature and humidity. After storage for three months at 40 °C
and 75 % relative humidity, there were no detectable changes in the DSC, TGA, or PXRD
profiles, in the chemical purity as determined by HPLC analysis, and no observable
15 changes in visual appearance. There were also no changes detectable by DSC, TGA, or
PXRD after milling particles of Form I from a volume-based mean particle size of about
470 microns to a volume-based mean particle size of about 15, about 22, or about 29
microns.

Crystalline Form II of compound 1 is characterized by the DSC and TGA profiles
20 of Figure 4. TGA analysis shows an onset of weight loss in the temperature range of
about 95 to about 105 °C, typically at about 100 °C, in a step profile consistent with water
or solvent loss, while the DSC profile exhibits a peak in endothermic heat flow at
between about 143 and about 145 °C, concurrent with the melting event. The PXRD
pattern of Form II is indistinguishable from that of Form I. While positive identification
25 has not been made, the TGA profile of Form II is consistent with the interpretation of
Form II as a solvate.

The third crystalline form of the invention has been identified as a monohydrate.
Form III is characterized by a powder x-ray diffraction (PXRD) pattern having two or
more diffraction peaks at 2θ values selected from 9.14±0.20, 12.41±0.20, 12.74±0.20,
30 17.75±0.20, 18.47±0.20, 20.63±0.20, 21.13±0.20, and 27.05±0.20, as illustrated in
Figure 5. In particular, Form III is characterized by a powder x-ray diffraction pattern
having diffraction peaks at 2θ values of 9.14±0.20 and 20.63±0.20.

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The DSC and TGA profiles of Form III, shown in Figure 6, demonstrate the material undergoes a step profile weight loss with onset in the temperature range of about 65 to about 75 °C, typically at about 70 °C, and a peak in endothermic heat flow at a temperature between about 90 and about 100 °C, consistent with loss of the monohydrate water and subsequent melting. Form III has been further characterized by x-ray diffraction analysis of crystal structure, providing the following lattice parameters: unit cell is monoclinic with dimensions $a = 14.8101 \text{ \AA}$, $b = 9.9985 \text{ \AA}$, $c = 17.9222 \text{ \AA}$; $\beta = 106.3020^\circ$, space group is $P2_1/n$; calculated density is 1.23 g/cm^3 . The resulting molecular structure confirms the chemical composition is that of compound 1 and that the assymmetric unit contains a single water molecule.

The separate crystalline forms of the present invention may be reproducibly obtained by the following procedures. Crystalline Form I may be prepared by dispersing compound 1 in an inert diluent selected from acetonitrile, ether, cyclohexane, and ethyl acetate in a proportion of between about 15 mg and about 25 mg compound 1 per milliliter of diluent to form a mixture, and allowing the mixture to evaporate at ambient temperature, resulting in crystal formation.

Alternatively, Form I may be obtained by a solvent exchange process from crude compound 1 in solution, without first isolating amorphous compound 1, as described in Example 216 below. Typically the reaction to prepare compound 1 is carried out in a polar aprotic diluent, such as dichloromethane, in which the compound is highly soluble. To prepare crystalline Form I, acetonitrile is added while vacuum distilling the crude reaction product to remove the dichloromethane. A mixture having between about 1 and about 200 mg compound 1 per milliliter of acetonitrile, typically between about 50 and about 125 mg compound 1 per milliliter of acetonitrile, is prepared from the residue remaining after distillation and heated to a temperature sufficient to dissolve the residue, for example a temperature of about 75 °C. The mixture is then cooled to a temperature of no more than about 20 °C to provide crystalline Form I, which is isolated by conventional procedures. In an exemplary process, the mixture is cooled until nucleation occurs, typically at a temperature of between about 55 and about 65 °C and held at that temperature for about an hour. It is then cooled at a rate of between about 0.25 and about 0.4 °C per minute to a temperature of about 20 °C. To increase the yield of crystalline Form I, the mixture may be further cooled at a rate of between about 0.5 and about 0.75 °C per minute to a temperature of between about 0 and about 5 °C.

To prepare Form II, amorphous compound 1 is dispersed in hexane at ambient temperature to a final concentration of about 10 mg/mL and the resulting mixture is sonicated. After about 24 hours at ambient temperature, crystalline solids of Form II are obtained.

- 5 Form III is prepared by dissolving amorphous amorphous compound 1 in a 1:1 ethanol: water solvent mixture at ambient temperature to a final concentration of about 20 mg/mL and sonicating the solution for about 30 seconds. The solution is allowed to partially evaporate in an uncapped vial. After about 24 hours, crystalline solids of Form III are obtained.

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Pharmaceutical Compositions

- The benzimidazole-carboxamide compounds of the invention are typically administered to a patient in the form of a pharmaceutical composition. Such pharmaceutical compositions may be administered to the patient by any acceptable route of administration including, but not limited to, oral, rectal, vaginal, nasal, inhaled, topical (including transdermal) and parenteral modes of administration.

- Accordingly, in one of its compositions aspects, the invention is directed to a pharmaceutical composition comprising a pharmaceutically-acceptable carrier or excipient and a therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or solvate thereof. Optionally, such pharmaceutical compositions may contain other therapeutic and/or formulating agents if desired.

- The pharmaceutical compositions of the invention typically contain a therapeutically effective amount of a compound of the present invention or a pharmaceutically-acceptable salt thereof. Typically, such pharmaceutical compositions will contain from about 0.1 to about 95% by weight of the active agent; including from about 5 to about 70% by weight; and from about 10 to about 60% by weight of the active agent.

- Any conventional carrier or excipient may be used in the pharmaceutical compositions of the invention. The choice of a particular carrier or excipient, or combinations of carriers or excipients, will depend on the mode of administration being used to treat a particular patient or type of medical condition or disease state. In this regard, the preparation of a suitable pharmaceutical composition for a particular mode of administration is well within the scope of those skilled in the pharmaceutical arts.

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Additionally, the ingredients for such compositions are commercially-available from, for example, Sigma, P.O. Box 14508, St. Louis, MO 63178. By way of further illustration, conventional formulation techniques are described in *Remington: The Science and Practice of Pharmacy*, 20th Edition, Lippincott Williams & White, Baltimore, Maryland (2000); and H.C. Ansel et al., *Pharmaceutical Dosage Forms and Drug Delivery Systems*, 7th Edition, Lippincott Williams & White, Baltimore, Maryland (1999).

Representative examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, the following: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, such as microcrystalline cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical compositions.

The pharmaceutical compositions of the invention are typically prepared by thoroughly and intimately mixing or blending a compound of the invention with a pharmaceutically-acceptable carrier and one or more optional ingredients. If necessary or desired, the resulting uniformly blended mixture can then be shaped or loaded into tablets, capsules, pills and the like using conventional procedures and equipment.

The pharmaceutical compositions of the invention are preferably packaged in a unit dosage form. The term "unit dosage form" refers to a physically discrete unit suitable for dosing a patient, i.e., each unit containing a predetermined quantity of active agent calculated to produce the desired therapeutic effect either alone or in combination with one or more additional units. For example, such unit dosage forms may be capsules, tablets, pills, and the like.

In a preferred embodiment, the pharmaceutical compositions of the invention are suitable for oral administration. Suitable pharmaceutical compositions for oral administration may be in the form of capsules, tablets, pills, lozenges, cachets, dragees,

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powders, granules; or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion; or as an elixir or syrup; and the like; each containing a predetermined amount of a compound of the present invention as an active ingredient.

5 When intended for oral administration in a solid dosage form (i.e., as capsules, tablets, pills and the like), the pharmaceutical compositions of the invention will typically comprise a compound of the present invention as the active ingredient and one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate. Optionally or alternatively, such solid dosage forms may also comprise: (1) fillers or
10 extenders, such as starches, microcrystalline cellulose, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and/or sodium carbonate; (5) solution retarding agents, such
15 as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as cetyl alcohol and/or glycerol monostearate; (8) absorbents, such as kaolin and/or bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and/or mixtures thereof; (10) coloring agents; and (11) buffering agents.

20 Release agents, wetting agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the pharmaceutical compositions of the invention. Examples of pharmaceutically-acceptable antioxidants include: (1) water-soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfate sodium sulfite and the like; (2) oil-
25 soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal-chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like. Coating agents for tablets, capsules, pills and like, include those used for enteric coatings, such as cellulose acetate
30 phthalate (CAP), polyvinyl acetate phthalate (PVAP), hydroxypropyl methylcellulose phthalate, methacrylic acid-methacrylic acid ester copolymers, cellulose acetate trimellitate (CAT), carboxymethyl ethyl cellulose (CMEC), hydroxypropyl methyl cellulose acetate succinate (HPMCAS), and the like.

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If desired, the pharmaceutical compositions of the present invention may also be formulated to provide slow or controlled release of the active ingredient using, by way of example, hydroxypropyl methyl cellulose in varying proportions; or other polymer matrices, liposomes and/or microspheres.

5 In addition, the pharmaceutical compositions of the present invention may optionally contain opacifying agents and may be formulated so that they release the active ingredient only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-
10 encapsulated form, if appropriate, with one or more of the above-described excipients.

Suitable liquid dosage forms for oral administration include, by way of illustration, pharmaceutically-acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. Such liquid dosage forms typically comprise the active ingredient and an inert diluent, such as, for example, water or other solvents, solubilizing
15 agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (esp., cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Suspensions, in addition to the active ingredient, may contain suspending agents such as,
20 for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Alternatively, the pharmaceutical compositions of the invention are formulated for administration by inhalation. Suitable pharmaceutical compositions for administration by
25 inhalation will typically be in the form of an aerosol or a powder. Such compositions are generally administered using well-known delivery devices, such as a metered-dose inhaler, a dry powder inhaler, a nebulizer or a similar delivery device.

When administered by inhalation using a pressurized container, the pharmaceutical compositions of the invention will typically comprise the active ingredient
30 and a suitable propellant, such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas.

Additionally, the pharmaceutical composition may be in the form of a capsule or cartridge (made, for example, from gelatin) comprising a compound of the invention and

a powder suitable for use in a powder inhaler. Suitable powder bases include, by way of example, lactose or starch.

The compounds of the invention can also be administered transdermally using known transdermal delivery systems and excipients. For example, a compound of the invention can be admixed with permeation enhancers, such as propylene glycol, polyethylene glycol monolaurate, azacycloalkan-2-ones and the like, and incorporated into a patch or similar delivery system. Additional excipients including gelling agents, emulsifiers and buffers, may be used in such transdermal compositions if desired.

The following formulations illustrate representative pharmaceutical compositions of the present invention:

Formulation Example A

Hard gelatin capsules for oral administration are prepared as follows:

Ingredients	Amount
Compound of the invention	50 mg
Lactose (spray-dried)	200 mg
Magnesium stearate	10 mg

Representative Procedure: The ingredients are thoroughly blended and then loaded into a hard gelatin capsule (260 mg of composition per capsule).

Formulation Example B

Hard gelatin capsules for oral administration are prepared as follows:

Ingredients	Amount
Compound of the invention	20 mg
Starch	89 mg
Microcrystalline cellulose	89 mg
Magnesium stearate	2 mg

Representative Procedure: The ingredients are thoroughly blended and then passed through a No. 45 mesh U.S. sieve and loaded into a hard gelatin capsule (200 mg of composition per capsule).

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Formulation Example C

Capsules for oral administration are prepared as follows:

5	Ingredients	Amount
	Compound of the invention	10 mg
	Polyoxyethylene sorbitan monooleate	50 mg
10	Starch powder	250 mg

Representative Procedure: The ingredients are thoroughly blended and then loaded into a gelatin capsule (310 mg of composition per capsule).

Formulation Example D

15 Tablets for oral administration are prepared as follows:

	Ingredients	Amount
	Compound of the invention	5 mg
	Starch	50 mg
20	Microcrystalline cellulose	35 mg
	Polyvinylpyrrolidone (10 wt. % in water)	4 mg
	Sodium carboxymethyl starch	4.5 mg
	Magnesium stearate	0.5 mg
25	Talc	1 mg

Representative Procedure: The active ingredient, starch and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resulting powders, and this mixture is then
30 passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50-60°C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate and talc (previously passed through a No. 60 mesh U.S. sieve) are then added to the granules. After mixing, the mixture is compressed on a tablet machine to afford a tablet weighing 100 mg.

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Formulation Example E

Tablets for oral administration are prepared as follows:

	Ingredients	Amount
5	Compound of the invention	25 mg
	Microcrystalline cellulose	400 mg
	Silicon dioxide fumed	10 mg
10	Stearic acid	5 mg

Representative Procedure: The ingredients are thoroughly blended and then compressed to form tablets (440 mg of composition per tablet).

Formulation Example F

15 Single-scored tablets for oral administration are prepared as follows:

	Ingredients	Amount
	Compound of the invention	15 mg
	Cornstarch	50 mg
20	Croscarmellose sodium	25 mg
	Lactose	120 mg
	Magnesium stearate	5 mg

25 Representative Procedure: The ingredients are thoroughly blended and compressed to form a single-scored tablet (215 mg of compositions per tablet).

Formulation Example G

A suspension for oral administration is prepared as follows:

	Ingredients	Amount
30	Compound of the invention	0.1 g
	Fumaric acid	0.5 g
	Sodium chloride	2.0 g
35	Methyl paraben	0.15 g
	Propyl paraben	0.05 g
	Granulated sugar	25.5 g
	Sorbitol (70% solution)	12.85 g
	Veegum k (Vanderbilt Co.)	1.0 g
40	Flavoring	0.035 mL
	Colorings	0.5 mg
	Distilled water	q.s. to 100 mL

Representative Procedure: The ingredients are mixed to form a suspension containing 10 mg of active ingredient per 10 mL of suspension.

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Formulation Example H

A dry powder for administration by inhalation is prepared as follows:

Ingredients	Amount
Compound of the invention	1.0 mg
Lactose	25 mg

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Representative Procedure: The active ingredient is micronized and then blended with lactose. This blended mixture is then loaded into a gelatin inhalation cartridge. The contents of the cartridge are administered using a powder inhaler.

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Formulation Example I

A dry powder for administration by inhalation in a metered dose inhaler is prepared as follows:

Representative Procedure: A suspension containing 5 wt. % of a compound of the invention and 0.1 wt. % lecithin is prepared by dispersing 10 g of active compound as micronized particles with mean size less than 10 μm in a solution formed from 0.2 g of lecithin dissolved in 200 mL of demineralized water. The suspension is spray dried and the resulting material is micronized to particles having a mean diameter less than 1.5 μm . The particles are loaded into cartridges with pressurized 1,1,1,2-tetrafluoroethane.

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Formulation Example J

An injectable formulation is prepared as follows:

Ingredients	Amount
Compound of the invention	0.2 g
Sodium acetate buffer solution (0.4 M)	40 mL
HCl (0.5 N) or NaOH (0.5 N)	q.s. to pH 4
Water (distilled, sterile)	q.s. to 20 mL

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Representative Procedure: The above ingredients are blended and the pH is adjusted to 4 ± 0.5 using 0.5 N HCl or 0.5 N NaOH.

Formulation Example K

5 Capsules for oral administration are prepared as follows:

Ingredients	Amount
Compound of the Invention	4.05 mg
Microcrystalline cellulose (Avicel PH 103)	259.2 mg
10 Magnesium stearate	0.75 mg

Representative Procedure: The ingredients are thoroughly blended and then loaded into a gelatin capsule (Size #1, White, Opaque) (264 mg of composition per capsule).

Formulation Example L

Capsules for oral administration are prepared as follows:

Ingredients	Amount
Compound of the Invention	8.2 mg
Microcrystalline cellulose (Avicel PH 103)	139.05 mg
25 Magnesium stearate	0.75 mg

Representative Procedure: The ingredients are thoroughly blended and then loaded into a gelatin capsule (Size #1, White, Opaque) (148 mg of composition per capsule).

30 It will be understood that any form of the compounds of the invention, (i.e. free base, pharmaceutical salt, or solvate) that is suitable for the particular mode of administration, can be used in the pharmaceutical compositions discussed above.

Utility

35 The benzimidazole-carboxamide compounds of the invention are 5-HT₄ receptor agonists and therefore are expected to be useful for treating medical conditions mediated by 5-HT₄ receptors or associated with 5-HT₄ receptor activity, i.e. medical conditions which are ameliorated by treatment with a 5-HT₄ receptor agonist. Such medical

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conditions include, but are not limited to, irritable bowel syndrome (IBS), chronic constipation, functional dyspepsia, delayed gastric emptying, gastroesophageal reflux disease (GERD), gastroparesis, post-operative ileus, intestinal pseudo-obstruction, and drug-induced delayed transit. In addition, it has been suggested that some 5-HT₄ receptor
5 agonist compounds may be used in the treatment of central nervous system disorders including cognitive disorders, behavioral disorders, mood disorders, and disorders of control of autonomic function.

In particular, the compounds of the invention increase motility of the gastrointestinal (GI) tract and thus are expected to be useful for treating disorders of the
10 GI tract caused by reduced motility in mammals, including humans. Such GI motility disorders include, by way of illustration, chronic constipation, constipation-predominant irritable bowel syndrome (C-IBS), diabetic and idiopathic gastroparesis, and functional dyspepsia.

In one aspect, therefore, the invention provides a method of increasing motility of
15 the gastrointestinal tract in a mammal, the method comprising administering to the mammal a therapeutically effective amount of a pharmaceutical composition comprising a pharmaceutically-acceptable carrier and a compound of the invention.

When used to treat disorders of reduced motility of the GI tract or other conditions mediated by 5-HT₄ receptors, the compounds of the invention will typically be
20 administered orally in a single daily dose or in multiple doses per day, although other forms of administration may be used. The amount of active agent administered per dose or the total amount administered per day will typically be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered and its relative activity, the
25 age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

Suitable doses for treating disorders of reduced motility of the GI tract or other disorders mediated by 5-HT₄ receptors will range from about 0.0007 to about
20 mg/kg/day of active agent, including from about 0.0007 to about 1 mg/kg/day. For an
30 average 70 kg human, this would amount to from about 0.05 to about 70 mg per day of active agent.

In one aspect of the invention, the compounds of the invention are used to treat chronic constipation. When used to treat chronic constipation, the compounds of the

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invention will typically be administered orally in a single daily dose or in multiple doses per day. Preferably, the dose for treating chronic constipation will range from about 0.05 to about 70 mg per day.

In another aspect of the invention, the compounds of the invention are used to treat
5 irritable bowel syndrome. When used to treat constipation-predominant irritable bowel syndrome, the compounds of the invention will typically be administered orally in a single daily dose or in multiple doses per day. Preferably, the dose for treating constipation-predominant irritable bowel syndrome will range from about 0.05 to about 70 mg per day.

In another aspect of the invention, the compounds of the invention are used to treat
10 diabetic gastroparesis. When used to treat diabetic gastroparesis, the compounds of the invention will typically be administered orally in a single daily dose or in multiple doses per day. Preferably, the dose for treating diabetic gastroparesis will range from about 0.05 to about 70 mg per day.

In yet another aspect of the invention, the compounds of the invention are used to
15 treat functional dyspepsia. When used to treat functional dyspepsia, the compounds of the invention will typically be administered orally in a single daily dose or in multiple doses per day. Preferably, the dose for treating functional dyspepsia will range from about 0.05 to about 70 mg per day.

The invention also provides a method of treating a mammal having a disease or
20 condition associated with 5-HT₄ receptor activity, the method comprising administering to the mammal a therapeutically effective amount of a compound of the invention or of a pharmaceutical composition comprising a compound of the invention.

As described above, compounds of the invention are 5-HT₄ receptor agonists. The invention further provides, therefore, a method of agonizing a 5-HT₄ receptor in a
25 mammal, the method comprising administering a compound of the invention to the mammal. In addition, the compounds of the invention are also useful as research tools for investigating or studying biological systems or samples having 5-HT₄ receptors, or for discovering new 5-HT₄ receptor agonists. Moreover, since compounds of the invention exhibit binding selectivity for 5-HT₄ receptors as compared with binding to receptors of
30 other 5-HT subtypes, particularly 5-HT₃ receptors, such compounds are particularly useful for studying the effects of selective agonism of 5-HT₄ receptors in a biological system or sample. Any suitable biological system or sample having 5-HT₄ receptors may be employed in such studies which may be conducted either *in vitro* or *in vivo*.

Representative biological systems or samples suitable for such studies include, but are limited to, cells, cellular extracts, plasma membranes, tissue samples, mammals (such as mice, rats, guinea pigs, rabbits, dogs, pigs, etc.) and the like.

In this aspect of the invention, a biological system or sample comprising a 5-HT₄ receptor is contacted with a 5-HT₄ receptor-agonizing amount of a compound of the invention. The effects of agonizing the 5-HT₄ receptor are then determined using conventional procedures and equipment, such as radioligand binding assays and functional assays. Such functional assays include ligand-mediated changes in intracellular cyclic adenosine monophosphate (cAMP), ligand-mediated changes in activity of the enzyme adenylyl cyclase (which synthesizes cAMP), ligand-mediated changes in incorporation of analogs of guanosine triphosphate (GTP), such as [³⁵S]GTPγS (guanosine 5'-O-(γ-thio)triphosphate) or GTP-Eu, into isolated membranes via receptor catalyzed exchange of GTP analogs for GDP analogs, ligand-mediated changes in free intracellular calcium ions (measured, for example, with a fluorescence-linked imaging plate reader or FLIPR[®] from Molecular Devices, Inc.), and measurement of mitogen activated protein kinase (MAPK) activation. A compound of the invention may agonize or increase the activation of 5-HT₄ receptors in any of the functional assays listed above, or assays of a similar nature. A 5-HT₄ receptor-agonizing amount of a compound of the invention will typically range from about 1 nanomolar to about 500 nanomolar.

Additionally, the compounds of the invention can be used as research tools for discovering new 5-HT₄ receptor agonists. In this embodiment, 5-HT₄ receptor binding or functional data for a test compound or a group of test compounds is compared to the 5-HT₄ receptor binding or functional data for a compound of the invention to identify test compounds that have superior binding or functional activity, if any. This aspect of the invention includes, as separate embodiments, both the generation of comparison data (using the appropriate assays) and the analysis of the test data to identify test compounds of interest.

Among other properties, compounds of the invention have been found to be potent agonists of the 5-HT₄ receptor and to exhibit substantial selectivity for the 5-HT₄ receptor subtype over the 5-HT₃ receptor subtype in radioligand binding assays. Further, compounds of the invention of which particular mention was made have demonstrated superior pharmacokinetic properties in a rat model. Such compounds are thus expected to be highly bioavailable upon oral administration. In addition, these compounds have been

shown not to exhibit an unacceptable level of inhibition of the potassium ion current in ...
in vitro voltage-clamp model using isolated whole cells expressing the hERG cardiac
potassium channel. The voltage-clamp assay is an accepted pre-clinical method of
assessing the potential for pharmaceutical agents to change the pattern of cardiac
5 repolarization, specifically to cause, so-called QT prolongation, which has been
associated with cardiac arrhythmia. (Cavero *et al.*, *Opinion on Pharmacotherapy*, 2000, 1,
947-73, Fermini *et al.*, *Nature Reviews Drug Discovery*, 2003, 2, 439-447) Accordingly,
pharmaceutical compositions comprising compounds of the invention are expected to
have an acceptable cardiac profile.

10 There properties, as well as the utility of the compounds of the invention, can be
demonstrated using various *in vitro* and *in vivo* assays well-known to those skilled in the
art. Representative assays are described in further detail in the following examples.

15 EXAMPLES

The following synthetic and biological examples are offered to illustrate the
invention, and are not to be construed in any way as limiting the scope of the invention.
In the examples below, the following abbreviations have the following meanings unless
otherwise indicated. Abbreviations not defined below have their generally accepted
20 meanings.

	Boc	=	<i>tert</i> -butoxycarbonyl
	DMSO	=	dimethyl sulfoxide
	MeCN	=	acetonitrile
	TFA	=	trifluoroacetic acid
25	R _f	=	retention factor

Reagents and solvents were purchased from commercial suppliers (Aldrich, Fluka,
Sigma, etc.), and used without further purification. Reactions were run under nitrogen
atmosphere, unless noted otherwise. Progress of reaction mixtures was monitored by thin
30 layer chromatography (TLC), analytical high performance liquid chromatography (anal.
HPLC), and mass spectrometry, the details of which are given below and separately in
specific examples of reactions. Reaction mixtures were worked up as described
specifically in each reaction; commonly they were purified by extraction and other
purification methods such as temperature-, and solvent-dependent crystallization, and

precipitation. In addition, reaction mixtures were routinely purified by preparative HPLC. A general protocol is described below. Characterization of reaction products was routinely carried out by mass and ^1H -NMR spectrometry. For NMR measurement, samples were dissolved in deuterated solvent (CD_3OD , CDCl_3 , or $\text{DMSO}-d_6$), and ^1H -NMR spectra were acquired with a Varian Gemini 2000 instrument (300 MHz) under standard observation conditions. Mass spectrometric identification of compounds was performed by an electrospray ionization method (ESMS) with an Applied Biosystems (Foster City, CA) model API 150 EX instrument or an Agilent (Palo Alto, CA) model 1100 LC/MSD instrument.

10

Preparation 1: Synthesis of 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid (piperidin-4-ylmethyl)amide

a. Preparation of 2,3-diaminobenzoic acid methyl ester

To a nitrogen-saturated solution of 2-amino-3-nitrobenzoic acid methyl ester (Chess GmbH, 50 g, 0.26 mol) in absolute ethanol (800 mL) was added palladium hydroxide (Degussa, 20% w/w on carbon, 58.75% w/w water, 10 g). The slurry was degassed then shaken vigorously under hydrogen (4 atm) at room temperature for 48 h. The catalyst was filtered and the filtrate concentrated in vacuo to afford 2,3-diaminobenzoic acid methyl ester as a dark orange oil that solidified on standing (43 g, 0.26 mol, 100%). (m/z): $[\text{M}-\text{OCH}_3]^+$ calcd for $\text{C}_8\text{H}_{10}\text{N}_2\text{O}_2$ 135.05; found 135.3. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ (ppm) 3.74 (s, 3H), 4.80 (br s, 1H), 6.20 (br s, 1H), 6.38 (t, 1H), 6.70 (d, 1H), 7.06 (d, 1H).

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b. Preparation of 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid

A slurry of 2,3-diaminobenzoic acid methyl ester (21.5 g, 0.13 mol) and isobutyric acid (36.2 mL, 0.39 mol) in aqueous hydrochloric acid (4M, 210 mL) was stirred under reflux for 24 h to afford a homogenous solution. The solution was cooled to 10 °C and the pH raised to 3.5 using aqueous sodium hydroxide solution (4M, approx. 210 mL), while maintaining the temperature below 30 °C. The reaction mixture was stirred at room temperature for 2 h, cooled to 10 °C, and the resultant precipitate filtered off. The solid cake was transferred to a beaker and acetonitrile (300 mL) was added. The slurry was stirred at room temperature for 1 h then filtered to afford a grey solid. The solid was dried under vacuum to afford the title intermediate (23 g, 0.11 mol, 87%). (m/z): $[\text{M}+\text{H}]^+$

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calcd for $C_{11}H_{12}N_2O_2$ calcd. 205.09; found 205.3. 1H NMR (300 MHz, DMSO- d_6): δ (ppm) 1.27 (d, 6H), 3.39 (m, 1H), 7.29 (t, 1H), 7.78 (m, 2H).

c. Preparation of 4-{[(2-isopropyl-1H-benzoimidazole-4-carbonyl)amino]methyl}-piperidine-1-carboxylic acid tert-butyl ester

5 To a solution of 2-isopropyl-1H-benzoimidazole-4-carboxylic acid (9.0 g, 44.1 mmol) in anhydrous *N,N*-dimethylformamide (100 mL) was added 4-aminomethyl-piperidine-1-carboxylic acid *tert*-butyl ester (9.4 g, 44.1 mmol), followed by *N,N*-diisopropylethylamine (16.9 mL, 97.0 mmol). The solution was stirred for 15 min at room temperature prior to the addition of hydroxybenzotriazole (5.9 g, 44.1 mmol), *N*-Ethyl-*N*-
10 (3-dimethylaminopropyl)carbodiimide hydrochloride (8.4 g, 44.1 mmol), and additional *N,N*-dimethylformamide (50 mL). The reaction mixture was stirred at room temperature for 16 h, diluted with dichloromethane (300 mL), and washed sequentially with 1M aqueous phosphoric acid, 1M aqueous sodium hydroxide and brine. The solution was then dried over sodium sulfate and concentrated in vacuo to afford a brown oil which solidified
15 upon addition of hexanes. The solid was filtered to give the title intermediate as a beige solid (13.8 g, 36.0 mmol, 78%). (m/z): $[M+H]^+$ calcd for $C_{22}H_{32}N_4O_3$ 401.26; found 401.5; $[M-Boc+H]^+$ 301.5. Retention time (anal. HPLC: 2-90% MeCN/ H_2O over 6 min) = 3.7 min. 1H NMR (300 MHz, DMSO- d_6): δ (ppm) 1.20 (m, 2H), 1.37 (s, 9H), 1.37 (s, 6H), 1.72 (m, 1H), 1.75 (m, 2H), 2.73 (br s, 2H), 3.22 (septet, 1H), 3.36 (m, 2H), 3.95 (m,
20 2H), 7.26 (t, 1H), 7.63 (d, 1H), 7.79 (d, 1H), 10.11 (t, 1H).

d. Synthesis of 2-isopropyl-1H-benzoimidazole-4-carboxylic acid (piperidin-4-ylmethyl)amide

To a solution of 4-{[(2-isopropyl-1H-benzoimidazole-4-carbonyl)amino]methyl}-piperidine-1-carboxylic acid *tert*-butyl ester (10.8 g, 27.0 mmol) dissolved in
25 dichloromethane (50 mL) at 0 °C was slowly added trifluoroacetic acid (50 mL) in 5 mL portions. The solution was allowed to warm to room temperature, stirred for an additional 20 minutes then evaporated in vacuo. Excess trifluoroacetic acid was removed by co-evaporation with toluene. The residue was then dissolved in a minimal volume of dichloromethane and slowly added to diethyl ether (1 L) at 0 °C. The resulting slurry was
30 stirred for 2 h at room temperature then filtered to afford the bis-trifluoroacetate salt of the title compound as a light brown solid (12.7 g, 24.0 mmol, 89%). (m/z): $[M+H]^+$ calcd for $C_{17}H_{24}N_4O$ 301.21; found 301.5. Retention time (anal. HPLC: 2-50% MeCN/ H_2O over 6 min) = 1.65 min. 1H NMR (300 MHz, MeOD- d_3): δ (ppm) 1.59 (d, 6H), 1.60 (m, 1H),

2.03 (m, 2H), 2.04 (m, 1H), 3.00 (m, 2H), 3.43 (m, 2H), 3.45 (m, 2H), 3.63 (septet, 1H), 7.63 (t, 1H), 7.90 (d, 1H), 7.96 (d, 1H), 9.04 (t, 1H).

Preparation 2: Synthesis of 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid (1-piperidin-4-ylmethylpiperidin-4-ylmethyl)amide

5 a. Preparation of 4-(4-[(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)amino]methyl)-piperidin-1-ylmethyl)piperidine-1-carboxylic acid *tert*-butyl ester

To a suspension of 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid (piperidin-4-ylmethyl)amide bis-trifluoroacetate (6.84 g, 12.95 mmol) in dichloromethane (65 mL) at room temperature under nitrogen was added sequentially *N,N*-diisopropylethylamine (1.67 g, 2.25 mL), a solution of 1-(*tert*-butoxycarbonyl)piperidine-4-carboxaldehyde (3.16 g, 14.89 mmol) in dichloromethane (5 mL) and sodium triacetoxyborohydride (3.84 g, 18.13 mmol). The resulting mixture was stirred at room temperature for 1.5 h, then acidified to pH 1 with 1M aqueous hydrochloric acid. The aqueous layer was removed, and the organic layer extracted with 1M aqueous hydrochloric acid until no product remained in the organic phase. The combined aqueous layers were washed with dichloromethane, cooled to 0 °C and basified to pH 12 with sodium hydroxide pellets. The solution was then extracted with dichloromethane until no product remained in the aqueous phase, and the combined organic layers washed with brine, dried over sodium sulfate, filtered and concentrated to give the desired product as a brown oil (5.4 g, 10.8 mmol, 84 %) which was used without further purification. (*m/z*): [*M*+*H*]⁺ calcd for C₂₈H₄₃N₅O₃ 498.35; found 498.5.

b. Synthesis of 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid (1-piperidin-4-ylmethylpiperidin-4-ylmethyl)amide

The product of the previous step (5.4 g, 10.8 mmol) was dissolved in dichloromethane (40 mL) and cooled to 0 °C. Trifluoroacetic acid (30 mL) was added, and the solution was stirred at 0 °C for a further 0.5 h. The mixture was then concentrated and co-evaporated twice with dichloromethane in vacuo. The resulting residue was dissolved in dichloromethane (20 mL), cooled to 0 °C and basified with 20% w/w aqueous sodium hydroxide (50 mL). The solution was allowed to warm to room temperature over 10 minutes, then filtered. The solid was rinsed with acetonitrile and dried in vacuo to afford a light gray powder (3.09 g, 7.8 mmol, 72 %) which was used without further purification. (*m/z*): [*M*+*H*]⁺ calcd for C₂₃H₃₅N₅O 398.29; found 398.4.

Preparation 3: Synthesis of 2-*tert*-butyl-1*H*-benzoimidazole-4-carboxylic acid (1-piperidin-4-ylmethylpiperidin-4-ylmethyl)amide

a. Preparation of 2-amino-3-(2,2-dimethylpropionylamino)benzoic acid methyl ester

To a solution of 2,3-diaminobenzoic acid methyl ester (2.3 g, 13.8 mmol) in
5 pyridine (40 mL) at room temperature was added 2,2-dimethylpropionyl chloride (1.7 g, 14.0 mmol). The solution was stirred at for 16 h, evaporated, and the residue partitioned between ethyl acetate (100 mL) and 1M aqueous hydrochloric acid (100 mL). The organic phase was separated, washed with 1M aqueous hydrochloric acid (100 mL), dried over sodium sulfate and evaporated to afford the title compound as a dark oil (2.7 g,
10 10.8 mmol, 78 %) which was used without further purification. (*m/z*): [M+H]⁺ calcd for C₁₃H₁₈N₂O₃ 251.14; found 250.8

b. Preparation of 2-*tert*-butyl-1*H*-benzoimidazole-4-carboxylic acid

A slurry of the product of the previous step (2.7 g, 10.8 mmol) in 4M aqueous hydrochloric acid (100 mL) was stirred under reflux for 24 h to afford a homogenous
15 solution. The solvent was evaporated to afford the hydrochloride salt of the title intermediate as a brick-red solid (2.5 g, 9.8 mmol, 91 %). (*m/z*): [M+H]⁺ calcd for C₁₂H₁₄N₂O₂ 219.12; found 219.3. ¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) 1.45 (d, 9H), 3.39 (m, 1H), 7.91 (d, 1H), 7.95 (d, 1H).

c. Preparation of 4-{[(2-*tert*-butyl-1*H*-benzoimidazole-4-carbonyl)amino]methyl}-piperidine-1-carboxylic acid *tert*-butyl ester

To a solution of 2-*tert*-butyl-1*H*-benzoimidazole-4-carboxylic acid hydrochloride (1.11 g, 4.37 mmol) in anhydrous *N,N*-dimethylformamide (5 mL) was added 1,1'-carbonyldiimidazole (0.77g, 4.75 mmol). The solution was stirred at 50°C for 2 h, then 4-aminomethyl-piperidine-1-carboxylic acid *tert*-butyl ester (0.94 g, 4.39 mmol) was
25 added, followed by 1,4-diazabicyclo[2,2,2]octane (1.46 g, 13 mmol). The solution was stirred at 50°C for 16 h, allowed to cool and diluted with water (20 mL) and ethyl acetate (60 mL). The aqueous layer was removed, the organic layer washed with water (20 mL), dried over sodium sulfate and concentrated in vacuo to afford the title intermediate (1.32 g, 3.18 mmol, 73 %) which was used without further purification. (*m/z*): [M+H]⁺
30 calcd for C₂₃H₃₄N₄O₃ 415.27; found 415.5.

d. Preparation of 2-*tert*-butyl-1*H*-benzoimidazole-4-carboxylic acid (piperidin-4-ylmethyl)amide

4- $\{[(2\text{-}tert\text{-Butyl-1}H\text{-benzoimidazole-4-carbonyl)amino]methyl\}$ -piperidine-1-carboxylic acid *tert*-butyl ester (13.5 g, 32.6 mmol) was dissolved in 4N HCl in dioxane (200 mL) and stirred at room temperature for 0.5 h. The resulting solid was filtered to afford the bis hydrochloride salt of the title intermediate (11.3 g, 29.3 mmol, 89 %). (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{26}N_4O$ 315.22; found 315.3. 1H NMR (300 MHz, D_2O + MeOD- d_3): 1.54 (s, 8H), 1.96 (m, 4H), 2.91 (m, 4H), 3.31 (br s, 1H), 3.45 (d, 2H), 7.56 (t, 1H), 7.89-7.92 (m, 2H)

e. Preparation of 4-(4- $\{[(2\text{-}tert\text{-butyl-1}H\text{-benzoimidazole-4-carbonyl)amino]methyl\}$ -piperidin-1-ylmethyl)piperidine-1-carboxylic acid *tert*-butyl ester

To a suspension of the bis HCl salt of 2-*tert*-butyl-1*H*-benzoimidazole-4-carboxylic acid (piperidin-4-ylmethyl)amide (4.28 g, 11.06 mmol) in dichloromethane (55 mL) at room temperature was added *N,N*-diisopropylethylamine (1.71 g, 2.31 mL), 1-(*tert*-butoxycarbonyl)piperidine-4-carboxaldehyde (2.58 g, 12.17 mmol) and sodium triacetoxyborohydride (3.28 g, 15.48 mmol) sequentially. The resulting mixture was stirred at room temperature for 2 h then extracted with 1M aqueous hydrochloric acid. The combined aqueous layers were basified to pH 12 with sodium hydroxide pellets, then extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and evaporated. The resulting residue was dried under high vacuum to give a light brownish foam (4.9 g, 9.6 mmol, 87 %) which was used without further purification. (*m/z*): $[M+H]^+$ calcd for $C_{29}H_{45}N_5O_3$ 512.35, found: 512.4.

f. Synthesis of 2-*tert*-butyl-1*H*-benzoimidazole-4-carboxylic acid (1-piperidin-4-ylmethylpiperidin-4-ylmethyl)amide

Crude 4-(4- $\{[(2\text{-}tert\text{-butyl-1}H\text{-benzoimidazole-4-carbonyl)amino]methyl\}$ -piperidin-1-ylmethyl)piperidine-1-carboxylic acid *tert*-butyl ester, prepared as in the previous step (5.1 g, 10 mmol) was treated with a mixture of trifluoroacetic acid (40 mL) and dichloromethane (40 mL) at room temperature for 0.5 h. The mixture was concentrated in vacuo, redissolved in dichloromethane (25 mL) and basified with 1M aqueous sodium hydroxide (15 mL). The organic layer was removed, and the aqueous layer re-extracted with dichloromethane. The combined organic phases were dried over sodium sulfate, filtered, and concentrated in vacuo to afford the desired product as a

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brown foam (3.6 g, 8.8 mmol, 88 %). (*m/z*): [M+H]⁺ calcd for C₂₄H₃₇N₅O 412.31, found 412.6.

Preparation 4. Synthesis of 4-(4-aminomethylpiperidin-1-ylmethyl)-piperidine-1-carboxylic acid methyl ester

5 a. Preparation of 4-[4-(*tert*-butoxycarbonylamino-methyl)piperidin-1-ylmethyl]-piperidine-1-carboxylic acid methyl ester

To a solution of 4-*tert*-butoxycarbonylaminomethylpiperidine (3.62 g, 16.9 mmol) in dichloromethane (100 mL) was added 4-formylpiperidine-1-carboxylic acid methyl ester (2.89 g, 16.9 mmol) and acetic acid (0.96 mL). The mixture was stirred at room
10 temperature for 10 min prior to the addition of sodium triacetoxyborohydride (5.4 g, 25.5 mmol). The final mixture was stirred at room temperature for 1 h. The reaction was terminated by adding saturated sodium bicarbonate solution (50 mL). The mixture was extracted with dichloromethane (100 mL), and the organic layer was dried over MgSO₄. Evaporation of the organic solution afforded a pale yellow oily residue. It was purified by
15 flash silica column chromatography (CH₂Cl₂ to 5% MeOH/CH₂Cl₂), yielding the title intermediate (4.4 g). (*m/z*): [M+H]⁺ calcd for C₁₉H₃₅N₃O₄ 370.27; found 370.5.

b. Synthesis of 4-(4-aminomethylpiperidin-1-ylmethyl)-piperidine-1-carboxylic acid methyl ester

To a solution of 4-[4-(*tert*-butoxycarbonylamino-methyl)piperidin-1-ylmethyl]-
20 piperidine-1-carboxylic acid methyl ester (4.4 g, 10.8 mmol) in dichloromethane (20 mL) was added trifluoroacetic acid (20 mL). After stirring for 20 min at room temperature, the solution was evaporated in vacuo, yielding the bis-trifluoroacetate salt of the title compound as a pale yellow oil, which was used without further treatment. (*m/z*): [M+H]⁺ calcd for C₁₄H₂₇N₃O₂ 270.22; found 270.5. ¹H-NMR (CD₃OD) δ (ppm) 4.0 (br d, 2H), 3.6
25 (m, 5H), 2.9-2.7 (m, 6H), 2.1-1.9 (m, 2H), 1.7-1.5 (m, 6H), 1.2-1.0 (m, 4H).

Example 1: Synthesis of 4-(4-[(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)-amino]methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester

To a suspension of 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid (1-piperidin-4-ylmethylpiperidin-4-ylmethyl)amide (2.9 g, 7.3 mmol) in dichloromethane
30 (50 mL) was added *N,N*-diisopropylethylamine (1.05 mL, 7.3 mmol). The resulting solution was cooled to 0 °C, and methyl chloroformate (576 μL, 7.3 mmol) was added dropwise. The mixture was stirred at 0 °C for 1.5 h, quenched with acetic acid (1 mL) and evaporated in vacuo to afford a beige solid (4.8 g) which was purified via preparative

reverse phase HPLC [gradient of 5-10-25% (5-10% over 10min; 10-25% over 50 min); flow rate 15 mL/min; detection at 280 nm] to afford the bis trifluoroacetate salt of the title compound as a white solid (3.5 g, 5.1 mmol, 70 %). (*m/z*): [M+H]⁺ calcd for C₂₅H₃₇N₅O₃ 456.30; found 456.3. Retention time (anal. HPLC: 2-50% MeCN/H₂O over 6 min) = 3.06 min.

Example 2: Synthesis of 4-(4-[(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)-amino]-methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid phenyl ester

To a solution of 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid (1-piperidin-4-ylmethylpiperidin-4-ylmethyl)amide (0.22 g, 0.55 mmol) and *N,N*-diisopropylethylamine (0.19 mL) in dichloromethane (5.0 mL) was added phenyl chloroformate (70 µL). The mixture was stirred at room temperature for 10 min, then concentrated in vacuo and purified via preparative reverse phase HPLC to afford the bis trifluoroacetate salt of the title compound as a white solid (98.4 mg, 0.13 mmol, 24 %). (*m/z*): [M+H]⁺ calcd for C₃₀H₃₉N₅O₃ 518.32; found 518.6. ¹H NMR (300MHz, MeOD-*d*₃): δ (ppm) 1.14-1.28 (m, 2H), 1.39-1.53 (m, 6H), 1.52-1.62 (m, 2H), 1.70-1.78 (m, 2H), 1.92-2.06 (m, 4H), 2.82-2.97 (m, 6H), 3.32-3.38 (m, 2H), 3.43-3.50 (m, 1H), 3.52-3.69 (m, 2H), 4.04-4.12 (m, 1H), 4.18-4.26 (m, 1H), 6.91-6.98 (m, 1H), 7.08-7.13 (m, 1H), 7.21-7.28 (m, 1H), 7.45-7.50 (m, 1H), 7.73-7.77 (m, 1H), 7.81-7.87 (m, 1H), 9.02-9.32 (brs, 1H).

Example 3: Synthesis of 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(2-chlorobenzoyl) piperidin-4-ylmethyl] piperidin-4-ylmethyl}amide

To a suspension of 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid (1-piperidin-4-ylmethylpiperidin-4-ylmethyl)amide (2.1 g, 5.29 mmol) in tetrahydrofuran (26 mL) at room temperature was added *N,N*-diisopropylethylamine (2.05 g, 15.87 mmol), dichloromethane (12 mL) and *N,N*-dimethylformamide (5 mL). To the resulting suspension was slowly added *o*-chlorobenzoyl chloride (1.02 g, 5.82 mmol), and the reaction mixture was stirred for 0.5 h at room temperature. The solution was concentrated in vacuo, the resulting residue diluted with acetic acid (7.5 mL) and water (0.5 mL), and the product purified by reverse phase preparative HPLC. The purified salt was partitioned between dichloromethane and 1M aqueous sodium hydroxide, the organic layer removed and the aqueous layer re-extracted with dichloromethane, and the combined organic layers washed with brine, dried over sodium sulfate and concentrated in vacuo to give the title compound as a white foam (1.75 g, 3.26 mmol, 62 %).

(*m/z*): [M+H]⁺ calcd for C₃₀H₃₈ClN₅O₂) 536.28; found 536.3. ¹H NMR (300 MHz, DMSO- *d*₆): 0.90 (br m, 2H), 1.24 (d, 6H), 1.45 (br m, 2H), 1.68 (br m, 8H), 1.96 (m, 1H), 2.72 (br m, 5H), 3.08 (m, 2H), (3.20, m, 3H), 4.40 (br m, 1H), 7.14 (t, 1H), 7.28 (m, 2H), 7.39 (m, 1H), 7.49 (dd, 1H), 7.66 (dd, 1H).

5 **Examples 4-6**

Using processes similar to that of Example 3, except replacing the *o*-chlorobenzoyl chloride with the appropriate chloride reagent, the compounds of Examples 4-6 were prepared.

10 **Example 4** 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(2,4-difluorobenzoyl)piperidin-4-ylmethyl]piperidin-4-ylmethyl}amide; (*m/z*): [M+H]⁺ calcd for C₃₀H₃₇F₂N₅O₂, 538.30; found 538.2. Retention time (anal. HPLC: 2-60% MeCN/H₂O over 4 min) = 2.12 min. ¹H NMR (300 MHz, DMSO- *d*₆): 0.92 (m, 2H), 1.30 (m, 2H), 1.38 (d, 6H), 1.53 (m, 2H), 1.60-1.90 (m, 6H), 2.07 (d, 2H), 2.73-2.85 (br m, 3H), 3.05 (t, 1H), 3.22 (septet, 1H), 3.38 (br m, 3H), 4.44 (br d, 1H), 7.10-7.50 (m, 4H), 7.62 (d, 1H), 7.77 (d, 1H), 10.10 (br s, 1H).

15 **Example 5** 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(furan-2-carbonyl)piperidin-4-ylmethyl]piperidin-4-ylmethyl}amide; (*m/z*): [M+H]⁺ calcd for C₂₈H₃₇N₅O₃ 492.30; found 492.2. Retention time (anal. HPLC: 2-65% MeCN/H₂O over 4 min) = 1.68 min.

20 **Example 6** 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(thiophene-2-carbonyl)piperidin-4-ylmethyl]piperidin-4-ylmethyl}amide; (*m/z*): [M+H]⁺ calcd for C₂₈H₃₇N₅O₂S 508.28; found 508.2. Retention time (anal. HPLC: 2-65% MeCN/H₂O over 4 min) = 1.94 min.

25 **Example 7: Synthesis of 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(2-fluoro-5-trifluoromethylbenzoyl)piperidin-4-ylmethyl]piperidin-4-ylmethyl}amide**

To a solution of 2-fluoro-5-trifluoromethyl benzoic acid (100 mg, 0.48 mmol) in dimethylformamide (4 mL) at room temperature was added O-(7-azabenzotriazol-1-yl)-
30 N,N,N',N'-tetramethyluronium hexafluorophosphate (200 mg, 0.48mmol). The mixture was stirred at room temperature for 0.25 h, then 2-isopropyl-1*H*-benzoimidazole-4-

5 carboxylic acid (1-piperidin-4-ylmethylpiperidin-4-ylmethyl)amide (210 mg, 0.48 mmol) and *N,N*-diisopropylethylamine (0.184 mL, 0.96 mmol) were added and stirring continued for a further 0.5 h. The solution was evaporated in vacuo and the crude product purified by reverse phase HPLC [gradient of 5-10-25% (5-10% over 10 min; 10-25% over 50 min); flow rate 15 mL/min; detection at 280 nm] to afford the bis trifluoroacetate salt of the title compound as a white solid (70 mg, 0.09 mmol, 18 %). (*m/z*): [M+H]⁺ calcd for C₃₁H₃₇F₄N₅O₂ 588.30; found 588.2. Retention time (anal. HPLC: 2-60% MeCN/H₂O over 4 min) = 2.39 min.

10 **Example 8: Synthesis of 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(2-fluoro-phenylcarbamoyl)piperidin-4-ylmethyl]piperidin-4-ylmethyl}-amide**

2-Isopropyl-1*H*-benzoimidazole-4-carboxylic acid (1-piperidin-4-ylmethylpiperidin-4-ylmethyl)amide (220 mg, 0.55 mmol) was dissolved in *N,N*-dimethylformamide (2.0 mL) at room temperature. To this solution was added *N,N*-diisopropylethylamine (143.2 mg, 1.1 mmol) followed by *o*-fluorophenylisocyanate (75.4 mg, 0.55 mmol). The resulting mixture was stirred at room temperature overnight, concentrated in vacuo and the residue purified by preparative reverse-phase HPLC to afford the bis trifluoroacetate salt of the title compound as a white solid (92.6 mg, 0.12 mmol, 22 %). (*m/z*): [M+H]⁺ calcd for C₃₀H₃₉FN₆O₂ 535.32; found 535.2. Retention time (anal. HPLC: 2-65% MeCN/H₂O over 4 min) = 2.09 min.

Example 9: Synthesis of 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid [1-(1-methanesulfonylpiperidin-4-ylmethyl)piperidin-4-ylmethyl]amide

2-Isopropyl-1*H*-benzoimidazole-4-carboxylic acid (1-piperidin-4-ylmethylpiperidin-4-ylmethyl)amide (40 mg, 0.1 mmol) was dissolved in *N,N*-dimethylformamide (1.0 mL) at room temperature. To this solution was added *N,N*-diisopropylethylamine (0.175 mL, 1 mmol) followed by methanesulfonyl chloride (11.5 mg, 0.1 mmol). The mixture was stirred at room temperature for 16 h, then concentrated in vacuo and the residue purified by preparative reverse-phase HPLC to afford the bis trifluoroacetate salt of the title compound as a white solid (27.2 mg, 0.04 mmol, 40 %). (*m/z*): [M+H]⁺ calcd for C₂₄H₃₇N₅O₃S 476.27; found 476.2. Retention time (anal. HPLC: 2-65% MeCN/H₂O over 4 min) = 1.66 min.

Example 10: Synthesis of 4-(4-[[2-*tert*-butyl-1*H*-benzimidazole-4-carbonyl]amino]-methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester

To the crude product of Preparation 3 (2.4 g, 5.8 mmol) in dichloromethane (29 mL) at room temperature was added *N,N*-diisopropylethylamine (1.5 g, 11.6 mmol). The resulting mixture was cooled to 0 °C and methyl chloroformate (660 mg, 6.98 mmol) was added dropwise. The reaction was allowed to warm to room temperature and stirred for a further 10 min. The solution was concentrated, re-dissolved in 50 % acetic acid in water, filtered and purified by reversed phase preparative HPLC. The resulting solid was dissolved in dichloromethane and washed with 1M aqueous sodium hydroxide. The aqueous layer was extracted twice with dichloromethane, and the combined organic layers washed with brine, dried over sodium sulfate, filtered and concentrated to give the title compound as a white foam (1.3 g, 2.8 mmol, 48 %). (*m/z*): [M+H]⁺ calcd for C₂₆H₃₉N₅O₃ 470.32, found 470.6. ¹H NMR (300 MHz, MeOD-*d*₃): δ (ppm) 1.02-1.16 (m, 2H), 1.49 (s, 9H), 1.47-1.7 (m, 4H), 1.82-2.03 (m, 4H), 2.74-2.94 (m, 6H), 3.31-3.40 (m, 2H), 3.54-3.58 (m, 2H), 3.56 (s, 3H), 3.98-4.03 (m, 2H), 7.41-7.46 (m, 1H), 7.71-7.74 (m, 1H), 7.79-7.82 (m, 1H), 9.35 (brs, 1H).

Examples 11-13

Using processes similar to that of Example 10, except replacing the methyl chloroformate with the appropriate chloride reagent, the compounds of Examples 11-13 were prepared.

Example 11 2-*tert*-butyl-1*H*-benzimidazole-4-carboxylic acid {1-[1-(2-fluoro-benzoyl)-piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide; (*m/z*): [M+H]⁺ calcd for C₃₁H₄₀FN₅O₂ 534.33; found 534.4. Retention time (anal. HPLC: 2-65% MeCN/H₂O over 4 min) = 2.09 min.

Example 12 2-*tert*-butyl-1*H*-benzimidazole-4-carboxylic acid {1-[1-(3-methyl-benzoyl)-piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide; (*m/z*): [M+H]⁺ calcd for C₃₂H₄₃N₅O₂ 530.35; found 530.42. Retention time (anal. HPLC: 2-65% MeCN/H₂O over 4 min) = 2.22.

Example 13 2-*tert*-butyl-1*H*-benzimidazole-4-carboxylic acid {1-[1-(4-fluorobenzoyl)-piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide; (*m/z*): [M+H]⁺ calcd for

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 $C_{31}H_{40}FN_5O_2$ 534.33; found 534.4. Retention time (anal. HPLC: 2-65% MeCN/H₂O over 4 min) = 2.17.

Example 14. Synthesis of 4-[4-([2-(1-hydroxy-1-methylethyl)-1H-benzoimidazole-4-carbonyl]amino)methyl]piperidin-1-ylmethyl]piperidine-1-carboxylic acid methyl ester

5 **a. Preparation of 2-(1-hydroxy-1-methylethyl)-1H-benzoimidazole-4-carboxylic acid**

To a solution of 2,3-diaminobenzoic acid methyl ester (1.5 g, 9.2 mmol) in 4 M HCl (50 mL) was added 2-hydroxyisobutyric acid (2.87 g, 27.6 mmol). The mixture was stirred at ~90°C for 24 h. It was neutralized by use of aqueous sodium hydroxide solution to pH~3, and concentrated to dryness. The residue was suspended in methanol, and filtered through a filter paper. The filtrate was concentrated and the residue was rinsed with ether. The remaining solid residue was dissolved in ethyl acetate, and washed with brine solution. After drying over MgSO₄, the organic solution was evaporated in vacuo, yielding the title intermediate as a pale yellow oil (~800 mg). The crude product was used in the next step without further purification. (*m/z*): [M+H]⁺ calcd for C₁₁H₁₂N₂O₃ 221.09; found 221.1. ¹H-NMR (CD₃OD) δ (ppm) 7.8 (dd, 1H), 7.7 (dd, 1H), 7.2 (m, 1H), 1.3 (s, 6H).

b. Synthesis of 4-[4-([2-(1-hydroxy-1-methylethyl)-1H-benzoimidazole-4-carbonyl]amino)methyl]piperidin-1-ylmethyl]piperidine-1-carboxylic acid methyl ester

20 To a solution of the benzoimidazole carboxylic acid product of the previous step (0.7 g, 3.18 mmol), the aminomethylpiperidine product of Preparation 4 as the bis-TFA salt (1.2 g, 3.13 mmol), and hydroxybenzotriazole (HOBt) (0.43 g, 3.18 mmol) in dimethylformamide (50 mL) was added triethylamine (1.3 mL, 9.3 mmol) and *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (0.67 g, 3.5 mmol). The mixture was stirred at room temperature for 12 h, and concentrated to dryness in vacuo. The residue was partitioned between dichloromethane (150 mL) and saturated sodium bicarbonate. The organic layer was dried over MgSO₄, and evaporated to dryness, yielding a pale yellow oily residue. It was purified by preparative HPLC to provide the bis-trifluoroacetate salt of the title compound. (*m/z*): [M+H]⁺ calcd for C₂₅H₃₇N₅O₄ 472.29; found 472.5. Retention time (anal. HPLC: 5-30% MeCN/H₂O over 6 min) = 3.67 min. ¹H-NMR (CD₃OD) δ (ppm) 7.9-7.8 (m, 2H), 7.6-7.5 (t, 1H), 4.0 (br d, 2H), 3.6 (s, 5H), 2.9-2.75 (br m, 5H), 2.05-1.9 (br d, 3H), 1.68 (m, 6H), 1.15 (m, 4H).

Example 15. Synthesis of 4-[4-({[2-(2-hydroxy-1-methylethyl)-1H-benzimidazole-4-carbonyl]amino}methyl)piperidin-1-ylmethyl]piperidine-1-carboxylic acid methyl ester

a. Preparation of 2-(2-hydroxy-1-methylethyl)-1H-benzimidazole-4-carboxylic acid

5 To a solution of 2,3-diaminobenzoic acid methyl ester (2.1 g, 14.1 mmol) in 4 M HCl (90 mL) was added 2-methyl-3-hydroxypropionic acid methyl ester (5 g, 42.3 mmol). The mixture was stirred at ~90°C for 24 h. It was neutralized by use of aqueous sodium hydroxide solution to pH~3, and concentrated to dryness. The residue was suspended in methanol, and filtered through a filter paper. After the filtrate was
10 concentrated, the remaining solid residue was dissolved in water and washed with ethyl acetate. The aqueous solution was evaporated in vacuo yielding the title intermediate as a pale yellow oil (~800 mg). The crude product was used in the next step without further purification. (*m/z*): [M+H]⁺ calcd for C₁₁H₁₂N₂O₃ 221.09; found 221.3. ¹H-NMR (CD₃OD) δ (ppm) 8.1 (d, 1H), 7.9 (m, 1H), 7.6 (t, 1H), 3.8 (m, 2H), 3.6 (m, 1H), 1.4 (d,
15 3H).

b. Synthesis of 4-[4-({[2-(2-hydroxy-1-methylethyl)-1H-benzimidazole-4-carbonyl]amino}methyl)piperidin-1-ylmethyl]piperidine-1-carboxylic acid methyl ester

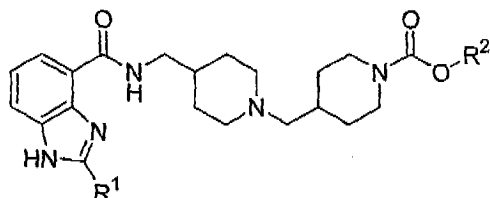
To a solution of the benzimidazole carboxylic acid product of the previous step (0.45 g, 1.75 mmol), the aminomethylpiperidine product of Preparation 4 as the bis-trifluoroacetate salt (0.8 g, 1.6 mmol), and HOBt (0.237 g, 1.75 mmol) in
20 dimethylformamide (50 mL) was added triethylamine (0.98 mL, 7.0 mmol) and EDC (0.353 g, 1.84 mmol). The mixture was stirred at room temperature for 12 h, and concentrated to dryness under reduced pressure. The residue was partitioned between dichloromethane (150 mL) and saturated sodium bicarbonate. The organic layer was dried
25 over MgSO₄, and evaporated to dryness, yielding pale yellow oily residue. It was purified by preparative HPLC, yielding the bis-trifluoroacetate salt of the title compound (0.2 g). (*m/z*): [M+H]⁺ calcd for C₂₅H₃₇N₅O₄ 472.29; found 472.5. Retention time (anal. HPLC: 10-40% MeCN/H₂O over 6 min) = 3.31 min. ¹H-NMR (CD₃OD) δ (ppm) 7.9-7.8 (m, 2H), 7.6-7.5 (m, 1H), 4.0 (br d, 2H), 3.85-3.7 (m, 2H), 3.6 (br s, 6H), 3.3 (br, 2H), 2.9-2.6
30 (br m, 6H), 2.0-1.8 (br, 4H), 1.7-1.5 (m, 6H), 1.4 (m, 3H), 1.1-1.0 (m, 4H).

Additional Compounds of the Invention

Using the procedures of Examples 1-13 and variations thereof, the compounds of Tables I to IX were prepared and characterized by mass spectrometry. In the following tables, blank entries denote hydrogen.

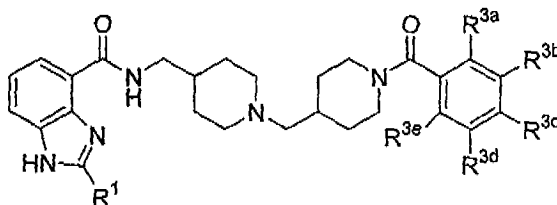
5

Table I



Example No.	R ¹	R ²	Molecular Formula	[M+H] ⁺ calcd	[M+H] ⁺ found
16	iPr	-CH ₂ -phenyl	C ₃₁ H ₄₁ N ₅ O ₃	532.32	532.2
17	iPr	iPr	C ₂₇ H ₄₁ N ₅ O ₃	484.32	484.2
18	tBu	phenyl	C ₃₁ H ₄₁ N ₅ O ₃	532.32	532.2
19	tBu	-CH ₂ -phenyl	C ₃₂ H ₄₃ N ₅ O ₃	546.34	546.4
20	tBu	iPr	C ₂₈ H ₄₃ N ₅ O ₃	498.34	498.4

Table II

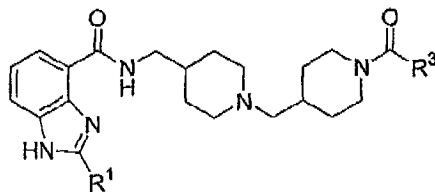


Example No.	R ¹	R ^{3a}	R ^{3b}	R ^{3c}	R ^{3d}	R ^{3e}	Molecular Formula	[M+H] ⁺ calcd	[M+H] ⁺ found
21	tBu	Cl					C ₃₁ H ₄₀ ClN ₅ O ₂	550.29	550.6
22	iPr						C ₃₀ H ₃₉ N ₅ O ₂	502.31	502.4
23	iPr	F					C ₃₀ H ₃₈ FN ₅ O ₂	520.30	520.2
24	iPr	CH ₃					C ₃₁ H ₄₁ N ₅ O ₂	516.33	516.4
25	iPr	CF ₃					C ₃₁ H ₃₈ F ₃ N ₅ O ₂	570.30	570.2
26	iPr		F				C ₃₀ H ₃₈ FN ₅ O ₂	520.30	520.2
27	iPr		CH ₃				C ₃₁ H ₄₁ N ₅ O ₂	516.33	516.4
28	iPr		CF ₃				C ₃₁ H ₃₈ F ₃ N ₅ O ₂	570.30	570.2

Example No.	R ¹	R ^{3a}	R ^{3b}	R ^{3c}	R ^{3d}	R ^{3e}	Molecular Formula	[M+H] ⁺ calcd	[M+H] ⁺ found
29	iPr		Cl				C ₃₀ H ₃₈ ClN ₅ O ₂	536.27	536.2
30	iPr			CF ₃			C ₃₁ H ₃₈ F ₃ N ₅ O ₂	570.30	570.2
31	iPr			CH ₃			C ₃₁ H ₄₁ N ₅ O ₂	516.33	516.4
32	iPr			Cl			C ₃₀ H ₃₈ ClN ₅ O ₂	536.27	536.2
33	iPr			OCH ₃			C ₃₁ H ₄₁ N ₅ O ₃	532.32	532.2
34	iPr			F			C ₃₀ H ₃₈ FN ₅ O ₂	520.30	520.2
35	iPr	F				F	C ₃₀ H ₃₇ F ₂ N ₅ O ₂	538.29	538.2
36	iPr	F			F		C ₃₀ H ₃₇ F ₂ N ₅ O ₂	538.29	538.2
37	iPr		F	F			C ₃₀ H ₃₇ F ₂ N ₅ O ₂	538.29	538.2
38	iPr	F	F				C ₃₀ H ₃₇ F ₂ N ₅ O ₂	538.29	538.2
39	iPr		F		F		C ₃₀ H ₃₇ F ₂ N ₅ O ₂	538.29	538.2
40	iPr	F		Cl			C ₃₀ H ₃₇ ClFN ₅ O ₂	554.26	554.4
41	iPr		CF ₃		CF ₃		C ₃₂ H ₃₇ F ₆ N ₅ O ₂	638.29	638.2
42	iPr	Cl		F			C ₃₀ H ₃₇ ClFN ₅ O ₂	554.26	554.2
43	iPr		Cl		Cl		C ₃₀ H ₃₇ Cl ₂ N ₅ O ₂	570.23	570.2
44	iPr			OCF ₃			C ₃₁ H ₃₈ F ₃ N ₅ O ₃	586.29	586.2
45	iPr		CF ₃	F			C ₃₁ H ₃₇ F ₄ N ₅ O ₂	588.29	588.2
46	iPr		Cl	F			C ₃₀ H ₃₇ ClFN ₅ O ₂	554.26	554.2
47	iPr	OCH ₃		Cl			C ₃₁ H ₄₀ ClN ₅ O ₃	566.28	566.2
48	iPr	CN					C ₃₁ H ₃₈ N ₆ O ₂	527.31	527.2
49	iPr	Cl		Cl			C ₃₀ H ₃₇ Cl ₂ N ₅ O ₂	570.23	570.2
50	iPr		F	CF ₃			C ₃₁ H ₃₇ F ₄ N ₅ O ₂	588.29	588.2
51	iPr			CN			C ₃₁ H ₃₈ N ₆ O ₂	527.31	527.2
52	iPr			OCHF ₂			C ₃₁ H ₃₉ F ₂ N ₅ O ₃	568.30	568.8
53	tBu	Cl		F			C ₃₁ H ₃₉ ClFN ₅ O ₂	568.28	568.2
54	tBu		Cl		Cl		C ₃₁ H ₃₉ Cl ₂ N ₅ O ₂	584.25	584.2
55	tBu		CN				C ₃₂ H ₄₀ N ₆ O ₂	541.32	541.4
56	tBu			OCF ₃			C ₃₂ H ₄₀ F ₃ N ₅ O ₃	600.31	600.2
57	tBu		CF ₃	F			C ₃₂ H ₃₉ F ₄ N ₅ O ₂	602.30	602.2
58	tBu		Cl	F			C ₃₁ H ₃₉ ClFN ₅ O ₂	568.28	568.2
59	tBu	OCH ₃		Cl			C ₃₂ H ₄₂ ClN ₅ O ₃	580.30	580.2
60	tBu	CN					C ₃₂ H ₄₀ N ₆ O ₂	541.32	541.4
61	tBu	Cl		Cl			C ₃₁ H ₃₉ Cl ₂ N ₅ O ₂	584.25	584.2

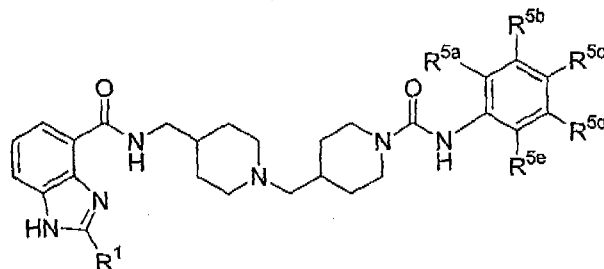
Example No.	R ¹	R ^{3a}	R ^{3b}	R ^{3c}	R ^{3d}	R ^{3e}	Molecular Formula	[M+H] ⁺ calcd	[M+H] ⁺ found
62	tBu		F	CF ₃			C ₃₂ H ₃₉ F ₄ N ₅ O ₂	602.30	602.2
63	tBu	F			CF ₃		C ₃₂ H ₃₉ F ₄ N ₅ O ₂	602.30	602.4
64	tBu			CN			C ₃₂ H ₄₀ N ₆ O ₂	541.32	541.2
65	tBu			OCHF ₂			C ₃₂ H ₄₁ F ₂ N ₅ O ₃	582.32	582.4
66	tBu						C ₃₁ H ₄₁ N ₅ O ₂	516.33	516.2
67	tBu	CH ₃					C ₃₂ H ₄₃ N ₅ O ₂	530.34	530.4
68	tBu	CF ₃					C ₃₂ H ₄₀ F ₃ N ₅ O ₂	584.31	584.4
69	tBu		F				C ₃₁ H ₄₀ FN ₅ O ₂	534.32	534.2
70	tBu		CF ₃				C ₃₂ H ₄₀ F ₃ N ₅ O ₂	584.31	584.4
71	tBu		Cl				C ₃₁ H ₄₀ ClN ₅ O ₂	550.29	550.2
72	tBu			CF ₃			C ₃₂ H ₄₀ F ₃ N ₅ O ₂	584.31	584.4
73	tBu			CH ₃			C ₃₂ H ₄₃ N ₅ O ₂	530.34	530.4
74	tBu			Cl			C ₃₁ H ₄₀ ClN ₅ O ₂	550.29	550.2
75	tBu			OCH ₃			C ₃₂ H ₄₃ N ₅ O ₃	546.34	546.4
76	tBu	F				F	C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552.31	552.4
77	tBu	F			F		C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552.31	552.2
78	tBu		F	F			C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552.31	552.2
79	tBu	F	F				C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552.31	552.4
80	tBu		F		F		C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552.31	552.4
81	tBu	F		F			C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552.31	552.2
82	tBu	F			Cl		C ₃₁ H ₃₉ ClFN ₅ O ₂	568.28	568.2
83	tBu		CF ₃		CF ₃		C ₃₃ H ₃₉ F ₆ N ₅ O ₂	652.30	652.2
84	iPr	OCF ₃					C ₃₁ H ₃₈ F ₃ N ₅ O ₃	586.29	586.2
85	iPr		OCF ₃				C ₃₁ H ₃₈ F ₃ N ₅ O ₃	586.29	586.2
86	tBu	OCF ₃					C ₃₂ H ₄₀ F ₃ N ₅ O ₃	600.31	600.2
87	tBu		OCF ₃				C ₃₂ H ₄₀ F ₃ N ₅ O ₃	600.31	600.2

Table III



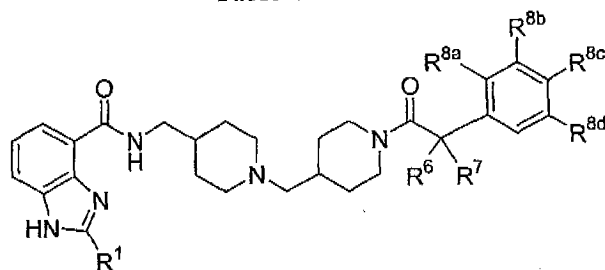
Example No.	R ¹	R ³	Molecular Formula	[M+H] ⁺ calcd	[M+H] ⁺ found
88	iPr	N(CH ₃) ₂	C ₂₆ H ₄₀ N ₆ O ₂	469.32	469.4
89	iPr	morpholin-1-yl	C ₂₈ H ₄₂ N ₆ O ₃	511.33	511.4
90	iPr	CH ₃	C ₂₅ H ₃₇ N ₅ O ₂	440.30	440.2
91	iPr	tetrahydrofuran-2-yl	C ₂₈ H ₄₁ N ₅ O ₃	496.32	496.4
92	iPr	-CH ₂ -thiophen-3-yl	C ₂₉ H ₃₉ N ₅ O ₂ S	522.28	522.2
93	iPr	2,2-dimethylpropyl	C ₂₉ H ₄₅ N ₅ O ₂	496.36	496.4
94	iPr	-CH ₂ -thiophen-2-yl	C ₂₉ H ₃₉ N ₅ O ₂ S	522.28	522.2
95	iPr	cyclohexyl	C ₃₀ H ₄₅ N ₅ O ₂	508.36	508.4
96	iPr	(S)-1-methylpropyl	C ₂₈ H ₄₃ N ₅ O ₂	482.34	482.4
97	iPr	-CH ₂ -naphth-1-yl	C ₃₅ H ₄₃ N ₅ O ₂	566.34	566.4
98	iPr	cyclopentyl	C ₃₀ H ₄₅ N ₅ O ₂	508.36	508.4
99	iPr	(R)-tetrahydrofuran-2-yl	C ₂₈ H ₄₁ N ₅ O ₃	496.32	496.4
100	tBu	furan-2-yl	C ₂₉ H ₃₉ N ₅ O ₃	506.31	506.2
101	tBu	-CH ₂ -thiophen-3-yl	C ₃₀ H ₄₁ N ₅ O ₂ S	536.30	536.2
102	tBu	2,2-dimethylpropyl	C ₃₀ H ₄₇ N ₅ O ₂	510.37	510.4
103	tBu	-CH ₂ -thiophen-2-yl	C ₃₀ H ₄₁ N ₅ O ₂ S	536.30	536.2
104	tBu	(S)-1-methylpropyl	C ₂₉ H ₄₅ N ₅ O ₂	496.36	496.4
105	tBu	-CH ₂ -naphth-1-yl	C ₃₆ H ₄₅ N ₅ O ₂	580.36	580.4
106	tBu	(R)-tetrahydrofuran-2-yl	C ₂₉ H ₄₃ N ₅ O ₃	510.34	510.4
107	tBu	(S)-4-oxo-azetidin-2-yl	C ₂₈ H ₄₀ N ₆ O ₃	509.32	510.4
108	tBu	pyridin-2-yl	C ₃₁ H ₄₂ N ₆ O ₂	531.34	531.2

Table IV



Example No.	R ¹	R ^{5a}	R ^{5b}	R ^{5c}	R ^{5d}	R ^{5e}	Molecular Formula	[M+H] ⁺ calcd	[M+H] ⁺ found
109	iPr	CH ₃					C ₃₁ H ₄₂ N ₆ O ₂	531.34	531.4
110	iPr		F				C ₃₀ H ₃₉ FN ₆ O ₂	535.31	535.2
111	iPr	CF ₃					C ₃₁ H ₃₉ F ₃ N ₆ O ₂	585.31	585.2
112	iPr	OCF ₃					C ₃₁ H ₃₉ F ₃ N ₆ O ₃	601.30	601.2
113	iPr			OCHF ₂			C ₃₁ H ₄₀ F ₂ N ₆ O ₃	583.31	583.2
114	iPr						C ₃₀ H ₄₀ N ₆ O ₂	517.32	517.4
115	iPr	CH ₃				CH ₃	C ₃₂ H ₄₄ N ₆ O ₂	545.35	545.4
116	iPr			OCF ₃			C ₃₁ H ₃₉ F ₃ N ₆ O ₃	601.30	601.2
117	iPr	tBu					C ₃₄ H ₄₈ N ₆ O ₂	573.38	573.4
118	iPr	Cl					C ₃₀ H ₃₉ ClN ₆ O ₂	551.28	551.2
119	tBu	Cl					C ₃₁ H ₄₁ ClN ₆ O ₂	565.30	565.2
120	tBu	CH ₃					C ₃₂ H ₄₄ N ₆ O ₂	545.35	545.5
121	tBu	F					C ₃₁ H ₄₁ FN ₆ O ₂	549.33	549.2
122	tBu	CF ₃					C ₃₂ H ₄₁ F ₃ N ₆ O ₂	599.32	599.2
123	tBu	OCF ₃					C ₃₂ H ₄₁ F ₃ N ₆ O ₃	615.32	615.2
124	tBu			OCHF ₂			C ₃₂ H ₄₂ F ₂ N ₆ O ₃	597.33	597.4
125	tBu	F					C ₃₁ H ₄₁ FN ₆ O ₂	549.33	549.2
126	tBu						C ₃₁ H ₄₂ N ₆ O ₂	531.34	531.4
127	tBu	CH ₃				CH ₃	C ₃₃ H ₄₆ N ₆ O ₂	559.37	559.4
128	tBu			OCF ₃			C ₃₂ H ₄₁ F ₃ N ₆ O ₃	615.32	615.2
129	tBu	tBu					C ₃₅ H ₅₀ N ₆ O ₂	587.40	587.4

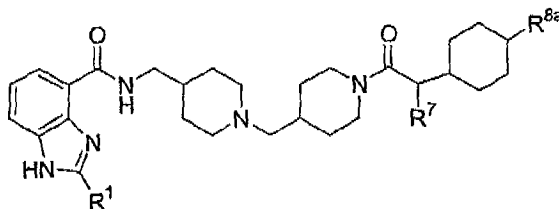
Table V



Example No.	R ¹	R ⁶	R ⁷	R ^{8a}	R ^{8b}	R ^{8c}	R ^{8d}	Molecular Formula	[M+H] ⁺ calcd	[M+H] ⁺ found
130	iPr		(S)-OH					C ₃₁ H ₄₁ N ₅ O ₃	532.32	532.2
131	iPr		oxo					C ₃₁ H ₃₉ N ₅ O ₃	530.31	530.2
132	iPr					Cl		C ₃₁ H ₄₀ ClN ₅ O ₂	550.29	550.2
133	iPr		(S)-CH ₃					C ₃₂ H ₄₃ N ₅ O ₂	530.34	530.4
134	iPr		-(CH ₂) ₂ -					C ₃₃ H ₄₃ N ₅ O ₂	542.34	542.4
135	iPr			F		F		C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552.31	552.2
136	iPr				F			C ₃₁ H ₄₀ FN ₅ O ₂	534.32	534.2
137	iPr				Cl			C ₃₁ H ₄₀ ClN ₅ O ₂	550.29	551.2
138	iPr				F	F		C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552.31	552.2
139	iPr				F		F	C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552.31	553.2
140	iPr							C ₃₁ H ₄₁ N ₅ O ₂	516.33	516.4
141	tBu		(S)-OH					C ₃₂ H ₄₃ N ₅ O ₃	546.34	546.4
142	tBu					F		C ₃₂ H ₄₂ FN ₅ O ₂	548.33	548.2
143	tBu		oxo					C ₃₂ H ₄₁ N ₅ O ₃	544.32	544.4
144	tBu					Cl		C ₃₂ H ₄₂ ClN ₅ O ₂	564.30	565.2
145	tBu		CH ₃			2-methylpropyl		C ₃₇ H ₅₃ N ₅ O ₂	600.42	600.4
146	tBu		(S)-CH ₃					C ₃₃ H ₄₅ N ₅ O ₂	544.36	544.4
147	tBu		-(CH ₂) ₂ -					C ₃₄ H ₄₅ N ₅ O ₂	556.36	556.4
148	tBu			Cl		Cl		C ₃₂ H ₄₁ Cl ₂ N ₅ O ₂	598.26	599.2
149	tBu			F		F		C ₃₂ H ₄₁ F ₂ N ₅ O ₂	566.32	566.2
150	tBu				F			C ₃₂ H ₄₂ FN ₅ O ₂	548.33	548.4
151	tBu					CF ₃		C ₃₃ H ₄₂ F ₃ N ₅ O ₂	598.33	598.4
152	tBu				CF ₃			C ₃₃ H ₄₂ F ₃ N ₅ O ₂	598.33	598.2
153	tBu				Cl			C ₃₂ H ₄₂ ClN ₅ O ₂	564.30	564.2
154	tBu				F	F		C ₃₂ H ₄₁ F ₂ N ₅ O ₂	566.32	566.2

Example No.	R ¹	R ⁶	R ⁷	R ^{8a}	R ^{8b}	R ^{8c}	R ^{8d}	Molecular Formula	[M+H] ⁺ calcd	[M+H] ⁺ found
155	tBu				F		F	C ₃₂ H ₄₁ F ₂ N ₅ O ₂	566.32	566.4
156	tBu							C ₃₂ H ₄₃ N ₅ O ₂	530.34	530.4

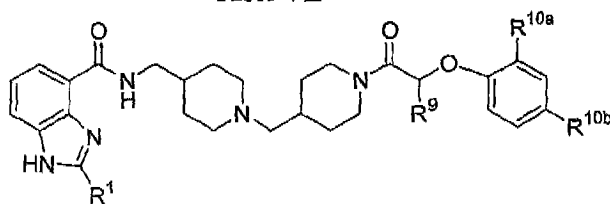
Table VI



Example No.	R ¹	R ⁷	R ^{8a}	Molecular Formula	[M+H] ⁺ calcd	[M+H] ⁺ found
157	iPr	(R)-OH		C ₃₁ H ₄₇ N ₅ O ₃	538.37	538.4
158	iPr			C ₃₁ H ₄₇ N ₅ O ₂	522.37	522.4
159	tBu	(R)-OH		C ₃₂ H ₄₉ N ₅ O ₃	552.38	552.4
160	tBu			C ₃₂ H ₄₉ N ₅ O ₂	536.39	536.4

5

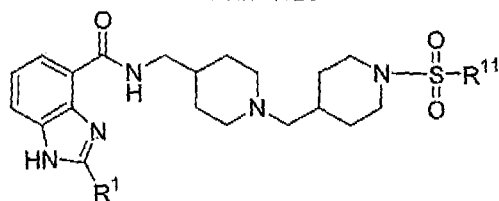
Table VII



Example No.	R ¹	R ⁹	R ^{10a}	R ^{10b}	Molecular Formula	[M+H] ⁺ calcd	[M+H] ⁺ found
161	iPr			CH ₃	C ₃₂ H ₄₃ N ₅ O ₃	546.34	546.4
162	iPr		Cl	Cl	C ₃₁ H ₃₉ Cl ₂ N ₅ O ₃	600.24	601.2
163	iPr	CH ₃		Cl	C ₃₂ H ₄₂ ClN ₅ O ₃	580.30	581.2
164	iPr	CH ₃			C ₃₂ H ₄₃ N ₅ O ₃	546.34	546.4
165	tBu			CH ₃	C ₃₃ H ₄₅ N ₅ O ₃	560.35	560.4
166	tBu		Cl	Cl	C ₃₂ H ₄₁ Cl ₂ N ₅ O ₃	614.26	615.2
167	tBu	CH ₃		Cl	C ₃₃ H ₄₄ ClN ₅ O ₃	594.31	595.2
168	tBu	CH ₃			C ₃₃ H ₄₅ N ₅ O ₃	560.35	560.4

10

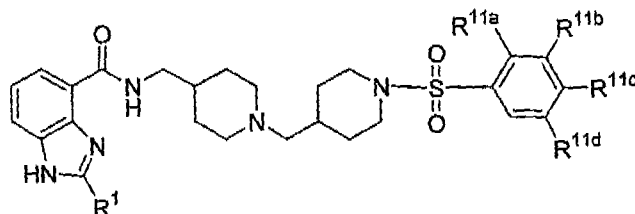
Table VIII



Example No.	R¹	R¹¹	Molecular Formula	[M+H] ⁺ calcd	[M+H] ⁺ found
169	iPr	CH ₃	C ₂₄ H ₃₇ N ₅ O ₃ S	476.26	476.2
170	iPr	2,4-dimethylisoxazol-2-yl	C ₂₈ H ₄₀ N ₆ O ₄ S	557.28	557.2
171	iPr	-CH ₂ -phenyl	C ₃₀ H ₄₁ N ₅ O ₃ S	552.29	552.2
172	tBu	2,4-dimethylisoxazol-2-yl	C ₂₉ H ₄₂ N ₆ O ₄ S	571.30	571.2
173	tBu	-CH ₂ -phenyl	C ₃₁ H ₄₃ N ₅ O ₃ S	566.31	566.2

5

Table IX



Example No.	R¹	R¹¹ᵃ	R¹¹ᵇ	R¹¹ᶜ	R¹¹ᵈ	Molecular Formula	[M+H] ⁺ calcd	[M+H] ⁺ found
174	iPr	CF ₃				C ₃₀ H ₃₈ F ₃ N ₅ O ₃ S	606.27	606.2
175	iPr	CN				C ₃₀ H ₃₈ N ₆ O ₃ S	563.27	563.2
176	iPr			OCH ₃		C ₃₀ H ₄₁ N ₅ O ₄ S	568.29	568.2
177	iPr		Cl			C ₂₉ H ₃₈ ClN ₅ O ₃ S	572.24	572.2
178	iPr	F				C ₂₉ H ₃₈ FN ₅ O ₃ S	556.27	556.2
179	iPr			CF ₃		C ₃₀ H ₃₈ F ₃ N ₅ O ₃ S	606.27	606.2
180	iPr			iPr		C ₃₂ H ₄₅ N ₅ O ₃ S	580.32	580.4
181	iPr	Cl				C ₂₉ H ₃₈ ClN ₅ O ₃ S	572.24	572.2
182	iPr	CH ₃			F	C ₃₀ H ₄₀ FN ₅ O ₃ S	570.28	570.2
183	iPr	Cl		F		C ₂₉ H ₃₇ ClFN ₅ O ₃ S	590.23	590.2
184	iPr	CH ₃	Cl			C ₃₀ H ₄₀ ClN ₅ O ₃ S	586.25	586.2
185	iPr			tBu		C ₃₃ H ₄₇ N ₅ O ₃ S	594.34	594.4
186	iPr	OCH ₃			Cl	C ₃₀ H ₄₀ ClN ₅ O ₄ S	602.25	602.2
187	iPr			Cl		C ₂₉ H ₃₈ ClN ₅ O ₃ S	572.24	572.2

Example No.	R ^I	R ^{IIa}	R ^{IIb}	R ^{IIc}	R ^{IIId}	Molecular Formula	[M+H] ⁺ calcd	[M+H] ⁺ found
188	iPr					C ₂₉ H ₃₉ N ₅ O ₃ S	538.28	538.2
189	iPr			F		C ₂₉ H ₃₈ FN ₅ O ₃ S	556.27	556.2
190	iPr		CH ₃			C ₃₀ H ₄₁ N ₅ O ₃ S	552.29	552.2
191	iPr			CH ₃		C ₃₀ H ₄₁ N ₅ O ₃ S	552.29	552.2
192	iPr		CF ₃			C ₃₀ H ₃₈ F ₃ N ₅ O ₃ S	606.27	606.2
193	iPr	CH ₃				C ₃₀ H ₄₁ N ₅ O ₃ S	552.29	552.2
194	tBu	CN				C ₃₁ H ₄₀ N ₆ O ₃ S	577.29	577.2
195	tBu			CF ₃		C ₃₁ H ₄₀ F ₃ N ₅ O ₃ S	620.28	620.2
196	tBu			iPr		C ₃₃ H ₄₇ N ₅ O ₃ S	594.34	594.4
197	tBu	Cl				C ₃₀ H ₄₀ ClN ₅ O ₃ S	586.25	586.2
198	tBu	CH ₃			F	C ₃₁ H ₄₂ FN ₅ O ₃ S	584.30	584.2
199	tBu	Cl		F		C ₃₀ H ₃₉ ClFN ₅ O ₃ S	604.24	604.2
200	tBu	CH ₃	Cl			C ₃₁ H ₄₂ ClN ₅ O ₃ S	600.27	600.2
201	tBu			tBu		C ₃₄ H ₄₉ N ₅ O ₃ S	608.36	608.4
202	tBu	OCH ₃			Cl	C ₃₁ H ₄₂ ClN ₅ O ₄ S	616.26	616.2
203	tBu		CH ₃			C ₃₁ H ₄₃ N ₅ O ₃ S	566.31	566.4
204	tBu			CH ₃		C ₃₁ H ₄₃ N ₅ O ₃ S	566.31	566.2
205	tBu		CF ₃			C ₃₁ H ₄₀ F ₃ N ₅ O ₃ S	620.28	620.2
206	tBu	CH ₃				C ₃₁ H ₄₃ N ₅ O ₃ S	566.31	566.2
207	tBu	CF ₃				C ₃₁ H ₄₀ F ₃ N ₅ O ₃ S	620.28	620.2
208	tBu			OCH ₃		C ₃₁ H ₄₃ N ₅ O ₄ S	582.30	582.2
209	tBu		Cl			C ₃₀ H ₄₀ ClN ₅ O ₃ S	586.25	586.2
210	tBu	F				C ₃₀ H ₄₀ FN ₅ O ₃ S	570.28	570.2
211	tBu			Cl		C ₃₀ H ₄₀ ClN ₅ O ₃ S	586.25	586.2
212	tBu					C ₃₀ H ₄₁ N ₅ O ₃ S	552.29	552.2
213	tBu			F		C ₃₀ H ₄₀ FN ₅ O ₃ S	570.28	570.2

Example 214. Alternate synthesis of 4-(4-[(2-isopropyl-1*H*-benzimidazole-4-carbonyl)-amino]methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester

a. Preparation of 4-hydroxymethyl-piperidine-1-carboxylic acid methyl ester

5 4-Hydroxymethylpiperidine (1.0 g, 8.6 mmol) was dissolved in water (15 mL) and cooled to 0 °C. To this solution was added dropwise a solution of potassium carbonate (4.8 g, 34.7 mmol) in water (10 mL), followed by methyl chloroformate (2.68 mL, 34.7 mmol). The mixture was stirred vigorously and allowed to warm to room temperature over 2 h. After stirring overnight (16 h), the reaction mixture was acidified
10 with 6M aqueous hydrochloric acid and extracted with dichloromethane (3 x 60 mL). The extracts were combined, dried over sodium sulfate and filtered. The filtrate was evaporated to yield the title intermediate (1.4 g, 8.1 mmol, 93%) as a colorless oil. (*m/z*): C₈H₁₅NO₃ calcd. 173.11; found 156.2 [M-H₂O+H]⁺. ¹H NMR (300MHz, DMSO-*d*₆): δ (ppm) 0.98 (m, 2H), 1.52 (m, 1H), 1.63 (br d, 2H), 2.72 (br m, 2H), 3.23 (d, 2H), 3.56 (s, 3H), 3.95 (br d, 2H), 4.48 (br s, 1H).
15

b. Preparation of 4-formylpiperidine-1-carboxylic acid methyl ester

To a solution of oxalyl chloride (4.1 mL, 8.2 mmol) in dichloromethane (4 mL) at -78 °C was added dropwise a solution of dimethylsulfoxide (1.2 mL, 16.4 mmol) in dichloromethane (4 mL). After stirring for 5 min, a solution of 4-hydroxymethyl-
20 piperidine-1-carboxylic acid methyl ester (1.3 g, 7.5 mmol) in dichloromethane (5 mL) was added. The resulting solution was stirred for another 5 min, then triethylamine (5.2 mL, 37.3 mmol) was added and the mixture allowed to warm to -10 °C. After stirring for 1 h, dichloromethane (100 mL) was added, and the organic layer was washed with 1M aqueous phosphoric acid, 1M aqueous sodium hydroxide, and brine. The
25 solution was dried over sodium sulfate then evaporated to afford the title intermediate as a wheat colored oil (1.0 g, 5.8 mmol, 78%). ¹H NMR (300MHz, DMSO-*d*₆): δ (ppm) 1.36 (m, 2H), 1.83 (m, 2H), 2.48 (br m, 1H), 2.93 (br t, 2H), 3.56 (s, 3H), 3.80 (br d, 2H), 9.56 (s, 1H).

c. Synthesis of 4-(4-[(2-isopropyl-1*H*-benzimidazole-4-carbonyl)-amino]methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester
30

2-Isopropyl-1*H*-benzimidazole-4-carboxylic acid (piperidin-4-ylmethyl)amide, (bis TFA salt; 1.1 g, 2.0 mmol) was suspended in dichloromethane (20 mL) and *N,N*-diisopropylethylamine (0.72 mL, 4.0 mmol) was added. When the suspension became a

clear solution, acetic acid (0.13 mL, 2.0 mmol) was added, followed by a solution of 4-formylpiperidine-1-carboxylic acid methyl ester (0.54 g, 3.1 mmol) in dichloromethane (20 mL). After stirring for 5 minutes at room temperature, sodium triacetoxyborohydride (0.628 g, 3.1 mmol) was added, and the reaction stirred for an additional 1 h. The aqueous layer was then made alkaline with 1M aqueous sodium hydroxide (35 mL) and extracted with dichloromethane (2 x 20 mL). The combined organic layers were washed with brine, dried over sodium sulfate and evaporated to yield crude product as a brown solid (1.41 g).

The crude product was purified via preparative HPLC (reverse phase) [gradient of 5-10-25%: 5% MeCN/water (0.1% TFA) to 10% MeCN linear over 10 min; 10% MeCN to 25% MeCN linear over 50 min; flow rate = 15 mL/min; detection at 280 nm] to provide the title compound as the bis trifluoroacetate salt, which was then lyophilized. A mixture of 1M sodium hydroxide and dichloromethane (1:1, 100 mL) was added to the lyophilized bis trifluoroacetate salt. The organic layer was dried over sodium sulfate, filtered, and evaporated, and the resulting solid was lyophilized to provide the title compound as a white solid (0.93 g, 2 mmol, 98 % yield, purity 97.5 %). (m/z): $[M+H]^+$ calcd for $C_{25}H_{37}N_5O_3$ 456.30; found 456.3. Retention time (anal. HPLC: 2-50% MeCN/H₂O over 6 min) = 3.06 min. ¹H NMR (300 MHz, DMSO- d_6): 0.92 (m, 2H), 1.30 (m, 2H), 1.38 (d, 6H), 1.53 (m, 1H), 1.60-1.90 (m, 7H), 2.07 (d, 2H), 2.73 (br m, 2H), 2.83 (br d, 2H), 3.22 (septet, 1H), 3.33 (t, 2H), 3.56 (s, 3H), 3.93 (br d, 2H), 7.23 (t, 1H), 7.62 (d, 1H), 7.77 (d, 1H), 10.10 (br s, 1H).

Example 215. Synthesis of crystalline 4-(4-[(2-isopropyl-1H-benzimidazole-4-carbonyl)-amino]methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester (Form I)

4-(4-[(2-isopropyl-1H-benzimidazole-4-carbonyl)-amino]methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester in amorphous solid form, prepared according to the process of Example 214 (300 mg) was dissolved in acetonitrile (15 mL), mixed until complete dissolution, and exposed to the atmosphere resulting in partial evaporation. Crystals were observed to have nucleated within 2 h. Chemical composition of the crystals was confirmed by ¹H NMR, liquid chromatography/mass spectrometry (LC/MS), and x-ray structure analysis. Crystalline nature of the solid product was confirmed by powder x-ray diffraction, differential scanning calorimetry, and x-ray structure analysis.

Example 216. Synthesis of crystalline 4-(4-[(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)-amino]methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester (Form I)

a. Preparation of 4-hydroxymethyl-piperidine-1-carboxylic acid methyl ester

5 4-Hydroxymethylpiperidine (47.6 g, 1.0 eq) and water (300 mL) were charged to a flask. The resulting mixture was cooled to 0-10°C. Potassium carbonate (85.7 g, 1.5 eq) dissolved in water (150 mL) and methyl chloroformate (38.4 mL, 1.1 eq) were added while maintaining the temperature at below 10°C. When the addition was complete, the reaction mixture was warmed up to 20-30°C for 1 hour. After the reaction
10 was complete, dichloromethane (500 mL) was added to the reaction mixture. The organic layer was collected and washed with 1 M phosphoric acid solution (200 mL), saturated sodium bicarbonate solution (200 mL) and saturated sodium chloride solution (200 mL). The organic layer was dried over sodium sulfate (50 g, 1 w/w eq) and then distilled under vacuum to produce the title intermediate. (67.0 g, 90% yield)

b. Preparation of 4-formylpiperidine-1-carboxylic acid methyl ester

 4-Hydroxymethylpiperidine-1-carboxylic acid methyl ester (34.7 g, 1.0 eq) was dissolved in dichloromethane and cooled to 0-10°C. A solution of sodium bicarbonate (2.35 g, 0.14 eq) and sodium bromide (2.40 g, 0.10 eq) in water (100 mL) was added over
15 15 min while maintaining the temperature at 0-10 °C. 2,2,6,6-Tetramethyl-1-
20 piperidinyloxy free radical (TEMPO) (0.32 g, 0.01 eq) was added to the mixture, followed by 10-13% w/v sodium hypochlorite solution (135 mL, 1.1 eq) over 1 h with good agitation while maintaining the temperature at 0-10°C. After the reaction was complete, the layers were separated and the organic layer washed with water (150 mL) and dried over sodium sulfate (30 g, 1 w/w eq). The solvent was removed by distillation
25 to provide the title intermediate. (31.0 g, 90% yield)

c. Preparation of 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid (piperidin-4-ylmethyl)amide

 Trifluoroacetic acid (56.0 mL, 10 eq) was added to a flask containing a ~5°C solution of 4-[(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)amino]methyl-piperidine-
30 1-carboxylic acid tert-butyl ester (30.0 g, 1.0 eq) in dichloromethane (300 mL) while maintaining the temperature below 10°C. The resulting mixture was stirred at 20-30 °C for 2 h. When the reaction was complete, triethylamine (73.2 mL, 7.0 eq) and acetic acid

(4.3 mL, 1.0 eq) were added to provide a solution of the title intermediate with an apparent pH of approximately 4 that was used directly in the next step.

d. Synthesis of 4-(4-[(2-isopropyl-1H-benzoimidazole-4-carbonyl)-amino]methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester

5 4-Formylpiperidine-1-carboxylic acid methyl ester (25.7 g, 2.0 eq) was added to the solution prepared in the previous step while maintaining the temperature at 20-30 °C. After stirring for 30 min, sodium triacetoxyborohydride (24.3 g, 1.5 eq) was added while maintaining the temperature at 20-30°C. The reaction mixture was stirred at 20-30°C for 30 min. After the reaction was complete, 1 M hydrochloric acid (300 mL) was added to
10 quench the reaction. The product-containing aqueous layer was collected and washed with dichloromethane (150 mL). The aqueous layer was treated with activated carbon (Darco G60, 6 g, 20% w/w) to remove color. The suspension was stirred for 1 hr, and then filtered through a bed of Celite. Dichloromethane (300 mL) was added to the aqueous solution and the product free-based using 4 N sodium hydroxide by adjusting the pH of
15 the aqueous layer to 12-13. The organic layer was collected and washed with water (300 mL). The organic layer was distilled at 80 °C and solvent exchanged with acetonitrile (2 x 300 mL), to remove dichloromethane and residual triethylamine. The solids were suspended in acetonitrile (600 mL), and the mixture heated until the solids were dissolved (~75°C). The solution was cooled until nucleation occurred (~55 - 65°C) and held for 1 h.
20 The slurry was cooled to 20°C over 2 h, and then to 0-5°C over 30 min, followed by stirring at 0-5°C for 30 min. The solids were filtered and washed with cold acetonitrile (60 mL). The wet cake was dried under vacuum at 60 °C for 6 h to provide the title compound. (28.3 g, 85% yield).

25 **Example 217: Synthesis of crystalline 4-(4-[(2-isopropyl-1H-benzoimidazole-4-carbonyl)-amino]methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester (Form I)**

 4-(4-[(2-isopropyl-1H-benzoimidazole-4-carbonyl)-amino]methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester in amorphous solid form, prepared according to the process of Example 214 was dispersed in the inert diluents listed in
30 Table X below. The mixtures were exposed to the atmosphere and allowed to completely evaporate. The resulting solids were characterized by powder x-ray diffraction. All solids were demonstrated to be crystalline with a powder x-ray diffraction pattern consistent

with that reported below in Example 220, which was obtained from the sample of Example 215.

Table X: Crystalline Form Synthesis

Diluent	Compound of Formula (I) (mg)	Volume of Diluent (mL)
Ether	4.60	0.230
Cyclohexane	4.87	0.486
Ethyl acetate	5.67	0.284

Example 218: Synthesis of crystalline 4-(4-((2-isopropyl-1*H*-benzimidazole-4-carbonyl)-amino)methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester (Form II)

4-(4-((2-Isopropyl-1*H*-benzimidazole-4-carbonyl)-amino)methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester in amorphous solid form (42.2 mg), was dispersed in hexane (4.22 mL) at ambient temperature to a final concentration of 10 mg/mL. The solution was sonicated to disperse larger solids. After 24 h at ambient temperature (approximately 22 °C) crystallization had occurred. The crystalline solids were isolated via vacuum filtration, prior to analysis.

Example 219: Synthesis of crystalline 4-(4-((2-isopropyl-1*H*-benzimidazole-4-carbonyl)-amino)methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester (Form III)

4-(4-((2-Isopropyl-1*H*-benzimidazole-4-carbonyl)-amino)methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester in amorphous solid form (38 mg) was dissolved in a 1:1 ethanol:water solvent mixture (1.9 mL) at ambient temperature to a final concentration of 20 mg/mL. The solution was sonicated for 30 seconds to ensure complete dissolution. The solution was then left to slowly evaporate in an uncapped vial. After 24 h at ambient temperature crystallization had occurred. The crystalline solids were isolated via vacuum filtration. The filter cake was washed once with a 1:1 ethanol:water solvent mixture, prior to analysis.

Example 220: Powder X-Ray Diffraction

Powder x-ray diffraction patterns were obtained with a Thermo ARL X-Ray Diffractometer Model X'TRA (Thermo ARL SA, Switzerland) using Cu K α radiation at 1.542 Å (45 kV, 40 mA) with a Si(Li) solid-state detector. The analysis was typically

performed at a scan rate of $2^\circ/\text{min}$ with a step size of 0.03° per point over a range of 2° to 35° in two-theta angle. Samples, either as received or ground to a fine powder, were gently packed into a quartz insert 7.8 mm in diameter and 0.5 mm in depth designed to fit into the instrument top-loading sample cup for analysis. The instrument calibration to within $\pm 0.02^\circ$ two-theta angle was verified weekly by comparison with a silicon metal standard. A representative PXRD pattern for the crystalline compound of Example 215 (Form I), which was hand ground to a powder, is shown in Figure 1. A representative PXRD pattern for a sample of crystalline Form III obtained with a Rigaku diffractometer using Cu K α (30 kV, 15 mA) radiation is shown in Figure 5.

Example 221: X-ray Structure Analysis

a. Form I

A chunk crystal produced in Example 215 having dimensions of $0.33 \times 0.17 \times 0.11$ mm was mounted on a glass fiber. X-ray structure data was obtained using a Bruker SMART 6K CCD θ -ray area detector with window diameter of 13.5 cm, controlled by SMART version 5.630 software (Bruker, 2003) using Cu K α radiation. The sample to detector distance was 5.039 cm. Data was collected at a temperature of $-153 \pm 1^\circ\text{C}$ and was analyzed using SHELXS version 6.14 (Bruker, 2003) software. The following lattice parameters were derived; unit cell is orthorhombic with dimensions $a = 16.9053 \text{ \AA}$, $b = 9.5172 \text{ \AA}$, $c = 15.4659 \text{ \AA}$; space group is $\text{Pna}2_1$; calculated density is 1.22 g/cm^3 . Powder x-ray diffraction peaks predicted from the derived atomic positions are in excellent agreement with the observed results obtained as described in Example 220, as shown in Table XI.

Table XI: PXRD Peak Positions

Observed 2θ (degrees)	Predicted 2θ (degrees)
15.08 ± 0.20	15.1 ± 0.2
15.41 ± 0.20	15.6 ± 0.2
19.00 ± 0.20	19.2 ± 0.2
19.70 ± 0.20	19.5 ± 0.2
23.68 ± 0.20	23.7 ± 0.2

b. Form III

A chunk crystal produced by the process of Example 219 having dimensions of 0.35 x 0.12 x 0.09 mm was analyzed by the method described above. The following lattice parameters were derived: unit cell is monoclinic with dimensions $a = 14.8101 \text{ \AA}$, $b = 9.9985 \text{ \AA}$, $c = 17.9222 \text{ \AA}$; $\beta = 106.3020^\circ$, space group is $P2_1/n$; calculated density is 1.23 g/cm^3 .

Example 222: Thermal Analysis

Differential scanning calorimetry (DSC) was performed using a TA Instruments Model Q-100 module. Data were collected and analyzed using TA Instruments Thermal Advantage for Q Series™ software. A sample of about 7 mg was accurately weighed into an aluminum pan with lid. The sample was evaluated using a linear heating ramp of 10°C/min from 5°C to about 200°C . The DSC cell was purged with dry nitrogen during use.

Thermogravimetric analysis (TGA) was performed using a TA Instruments Model Q-500 module. Data were collected and analyzed using TA Instruments Thermal Advantage for Q Series™ software. A sample weighing about 2 mg was placed in an aluminum pan on a platinum cradle and scanned from ambient temperature to about 300°C with a linear heating rate of 10°C/min . The balance and furnace chambers were purged with nitrogen during use.

Representative DSC and TGA traces for crystalline Form I (prepared according to the process of Example 216), Form II, and Form III material are shown in Figures 2, 4, and 6, respectively.

Example 223: Dynamic Moisture Sorption Assessment

Dynamic moisture sorption (DMS) assessment was performed at 25°C using a VTI atmospheric microbalance, SGA-100 system (VTI Corp., Hialeah, FL 33016). A sample size of approximately 5-10 mg was used and the humidity was set at the ambient value at the start of the analysis. A typical DMS analysis consisted of three scans: ambient to 2% relative humidity (RH), 2% RH to 90% RH, 90% RH to 5% RH at a scan rate of 5 % RH/step. The mass was measured every two minutes and the RH was changed to the next value ($\pm 5\% \text{ RH}$) when the mass of the sample was stable to within 0.02 % for 5 consecutive points. A representative DMS isotherm for the crystalline compound of Example 215 (Form I) is shown in Figure 3.

The crystalline compound of the invention exhibits a reversible sorption/desorption profile with a weight change of less than 0.25 % over the entire range of 2 % to 90 % RH and a weight change of less than 0.1 % over the critical humidity range of 40 % to 75 % RH.

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Example 224: Infrared Analysis

The infrared (IR) absorption spectrum of the crystalline compound of Example 215 (Form I) was determined over the frequency range 4000 to 675 cm^{-1} using an Avatar 360 FT-IR spectrometer equipped with a Nicolet attenuated total reflection (ATR) sample holder. A representative IR absorption spectrum for a sample of the crystalline compound of the invention had significant absorption bands at 766 ± 1 , 1097 ± 1 , 1251 ± 1 , 1413 ± 1 , 1449 ± 1 , 1579 ± 1 , 1609 ± 1 , 1640 ± 1 , and 1696 ± 1 cm^{-1} .

10

Example 225: Solid State Stability Assessment

Samples of the crystalline compound of Form I, prepared according to the process of Example 216, were stored in multiple open glass vials at 40 °C and 75 % RH. At specific intervals, the contents of a representative vial was removed and analyzed by DSC, TGA, PXRD, and by HPLC for chemical purity. After three months of storage, there was no detectable change in the DSC or TGA thermograms nor in the PXRD pattern. The chemical purity of the stored sample was 99.5 %.

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Assay 1: Radioligand Binding Assay on 5-HT_{4(c)} Human Receptors

a. Membrane Preparation 5-HT_{4(c)}

HEK-293 (human embryonic kidney) cells stably-transfected with human 5-HT_{4(c)} receptor cDNA ($B_{\text{max}} = \sim 6.0$ pmol/mg protein, as determined using [³H]-GR113808 membrane radioligand binding assay) were grown in T-225 flasks in Dulbecco's Modified Eagles Medium (DMEM) containing 4,500 mg/L D-glucose and pyridoxine hydrochloride (GIBCO-Invitrogen Corp., Carlsbad CA: Cat #11965) supplemented with 10% fetal bovine serum (FBS) (GIBCO-Invitrogen Corp.: Cat #10437), 2 mM L-glutamine and (100 units) penicillin-(100 µg) streptomycin/ml (GIBCO-Invitrogen Corp.: Cat #15140) in a 5% CO₂, humidified incubator at 37 °C. Cells were grown under continuous selection

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pressure by the addition of 800 µg/mL geneticin (GIBCO-Invitrogen Corp.: Cat #10131) to the medium.

Cells were grown to roughly 60-80% confluency (< 35 subculture passages). At 20-22 hours prior to harvesting, cells were washed twice and fed with serum-free DMEM.

- 5 All steps of the membrane preparation were performed on ice. The cell monolayer was lifted by gentle mechanical agitation and trituration with a 25 mL pipette. Cells were collected by centrifugation at 1000 rpm (5 min).

- For the membrane preparation, cell pellets were resuspended in ice-cold 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), pH 7.4 (membrane
10 preparation buffer) (40 mL/total cell yield from 30-40 T225 flasks) and homogenized using a polytron disrupter (setting 19, 2 x 10 s) on ice. The resultant homogenates were centrifuged at 1200 g for 5 min at 4°C. The pellet was discarded and the supernatant centrifuged at 40,000 g (20 min). The pellet was washed once by resuspension with membrane preparation buffer and centrifugation at 40,000 g (20 min). The final pellet
15 was resuspended in 50 mM HEPES, pH 7.4 (assay buffer) (equivalent 1 T225 flask/1 mL). Protein concentration of the membrane suspension was determined by the method of Bradford (Bradford, 1976). Membranes were stored frozen in aliquots at -80 °C.

b. Radioligand Binding Assays

- 20 Radioligand binding assays were performed in 1.1 mL 96- deep well polypropylene assay plates (Axygen) in a total assay volume of 400 µL containing 2 µg membrane protein in 50 mM HEPES pH 7.4, containing 0.025% bovine serum albumin (BSA). Saturation binding studies for determination of K_d values of the radioligand were performed using [3 H]-GR113808 (Amersham Inc., Bucks, UK: Cat #TRK944; specific
25 activity ~82 Ci/mmol) at 8-12 different concentrations ranging from 0.001 nM – 5.0 nM. Displacement assays for determination of pK_i values of compounds were performed with [3 H]-GR113808 at 0.15 nM and eleven different concentrations of compound ranging from 10 pM - 100 µM.

- Test compounds were received as 10 mM stock solutions in DMSO and diluted to
30 400 µM into 50 mM HEPES pH 7.4 at 25°C, containing 0.1% BSA, and serial dilutions (1:5) then made in the same buffer. Non-specific binding was determined in the presence of 1 µM unlabeled GR113808. Assays were incubated for 60 min at room temperature,

and then the binding reactions were terminated by rapid filtration over 96-well GF/B glass fiber filter plates (Packard BioScience Co., Meriden, CT) presoaked in 0.3% polyethyleneimine. Filter plates were washed three times with filtration buffer (ice-cold 50mM HEPES, pH7.4) to remove unbound radioactivity. Plates were dried, 35 μ L 5 Microscint-20 liquid scintillation fluid (Packard BioScience Co., Meriden, CT) was added to each well and plates were counted in a Packard Topcount liquid scintillation counter (Packard BioScience Co., Meriden, CT).

Binding data were analyzed by nonlinear regression analysis with the GraphPad Prism Software package (GraphPad Software, Inc., San Diego, CA) using the 3-parameter 10 model for one-site competition. The BOTTOM (curve minimum) was fixed to the value for nonspecific binding, as determined in the presence of 1 μ M GR113808. K_i values for test compounds were calculated, in Prism, from the best-fit IC_{50} values, and the K_d value of the radioligand, using the Cheng-Prusoff equation (Cheng and Prusoff, *Biochemical Pharmacology*, 1973, 22, 3099-108): $K_i = IC_{50} / (1 + [L]/K_d)$ where $[L]$ = concentration 15 $[^3H]$ -GR113808. Results are expressed as the negative decadic logarithm of the K_i values, pK_i .

Test compounds having a higher pK_i value in this assay have a higher binding affinity for the 5-HT₄ receptor. The compounds of the invention which were tested in this assay had a pK_i value ranging from about 7.0 to about 10.0.

20 **Assay 2: Radioligand Binding Assay on 5-HT_{3A} Human Receptors: Determination of Receptor Subtype Selectivity**

a. Membrane Preparation 5-HT_{3A}

HEK-293 (human embryonic kidney) cells stably-transfected with human 5-HT_{3A} receptor cDNA were obtained from Dr. Michael Bruess (University of Bonn, GDR) 25 (Bmax $\approx$ ~ 9.0 pmol/mg protein, as determined using $[^3H]$ -GR65630 membrane radioligand binding assay). Cells were grown in T-225 flasks or cell factories in 50% Dulbecco's Modified Eagles Medium (DMEM) (GIBCO-Invitrogen Corp., Carlsbad, CA: Cat #11965) and 50% Ham's F12 (GIBCO-Invitrogen Corp.: Cat #11765) supplemented with 10% heat inactivated fetal bovine serum (FBS) (Hyclone, Logan, UT: Cat 30 #SH30070.03) and (50 units) penicillin-(50 μ g) streptomycin/ml (GIBCO-Invitrogen Corp.: Cat #15140) in a 5% CO₂, humidified incubator at 37 °C.

Cells were grown to roughly 70-80% confluency (< 35 subculture passages). All steps of the membrane preparation were performed on ice. To harvest the cells, the media was aspirated and cells were rinsed with Ca^{2+} , Mg^{2+} -free Dulbecco's phosphate buffered saline (dPBS). The cell monolayer was lifted by gentle mechanical agitation. Cells were
5 collected by centrifugation at 1000 rpm (5 min). Subsequent steps of the membrane preparation followed the protocol described above for the membranes expressing 5-HT_{4(c)} receptors.

b. Radioligand Binding Assays

Radioligand binding assays were performed in 96-well polypropylene assay plates
10 in a total assay volume of 200 μL containing 1.5-2 μg membrane protein in 50 mM HEPES pH 7.4, containing 0.025% BSA assay buffer. Saturation binding studies for determination of K_d values of the radioligand were performed using [³H]-GR65630 (PerkinElmer Life Sciences Inc., Boston, MA: Cat #NET1011, specific activity ~85 Ci/mmol) at twelve different concentrations ranging from 0.005 nM to 20 nM.
15 Displacement assays for determination of pK_i values of compounds were performed with [³H]-GR65630 at 0.50 nM and eleven different concentrations of compound ranging from 10 pM to 100 μM . Compounds were received as 10 mM stock solutions in DMSO (see section 3.1), diluted to 400 μM into 50 mM HEPES pH 7.4 at 25°C, containing 0.1% BSA, and serial (1:5) dilutions then made in the same buffer. Non-specific binding
20 was determined in the presence of 10 μM unlabeled MDL72222. Assays were incubated for 60 min at room temperature, then the binding reactions were terminated by rapid filtration over 96-well GF/B glass fiber filter plates (Packard BioScience Co., Meriden, CT) presoaked in 0.3% polyethyleneimine. Filter plates were washed three times with filtration buffer (ice-cold 50mM HEPES, pH7.4) to remove unbound
25 radioactivity. Plates were dried, 35 μL Microscint-20 liquid scintillation fluid (Packard BioScience Co., Meriden, CT) was added to each well and plates were counted in a Packard Topcount liquid scintillation counter (Packard BioScience Co., Meriden, CT).

Binding data were analyzed using the non-linear regression procedure described above to determine K_i values. The BOTTOM (curve minimum) was fixed to the value for
30 nonspecific binding, as determined in the presence of 10 μM MDL72222. The quantity [L] in the Cheng-Prusoff equation was defined as the concentration [³H]-GR65630.

Selectivity for the 5-HT₄ receptor subtype with respect to the 5-HT₃ receptor subtype was calculated as the ratio $K_i(5\text{-HT}_{3A})/K_i(5\text{-HT}_{4(c)})$. The compounds of the invention which were tested in this assay had a 5-HT₄/5-HT₃ receptor subtype selectivity ranging from about 4000 to upwards of 400,000.

5 **Assay 3: Whole-cell cAMP Accumulation Flashplate Assay with HEK-293 cells expressing human 5-HT_{4(c)} Receptors**

In this assay, the functional potency of a test compound was determined by measuring the amount of cyclic AMP produced when HEK-293 cells expressing 5-HT₄ receptors were contacted with different concentrations of test compound.

10 a. Cell Culture

HEK-293 (human embryonic kidney) cells stably-transfected with cloned human 5-HT_{4(c)} receptor cDNA were prepared expressing the receptor at two different densities: (1) at a density of about 0.5-0.6 pmol/mg protein, as determined using a [³H]-GR113808 membrane radioligand binding assay, and (2) at a density of about 6.0 pmol/mg protein.

- 15 The cells were grown in T-225 flasks in Dulbecco's Modified Eagles Medium (DMEM) containing 4,500 mg/L D-glucose (GIBCO-Invitrogen Corp.: Cat #11965) supplemented with 10% fetal bovine serum (FBS) (GIBCO-Invitrogen Corp.: Cat #10437) and (100 units) penicillin-(100 µg) streptomycin/ml (GIBCO-Invitrogen Corp.: Cat #15140) in a 5% CO₂, humidified incubator at 37°C. Cells were grown under continuous selection
20 pressure by the addition of geneticin (800 µg/mL: GIBCO-Invitrogen Corp.: Cat #10131) to the medium.

b. Cell Preparation

- Cells were grown to roughly 60-80% confluency. Twenty to twenty-two hours prior to assay, cells were washed twice, and fed, with serum-free DMEM containing
25 4,500 mg/L D-glucose (GIBCO-Invitrogen Corp.: Cat #11965). To harvest the cells, the media was aspirated and 10 mL Versene (GIBCO-Invitrogen Corp.: Cat #15040) was added to each T-225 flask. Cells were incubated for 5 min at RT and then dislodged from the flask by mechanical agitation. The cell suspension was transferred to a centrifuge tube containing an equal volume of pre-warmed (37°C) dPBS and centrifuged for 5 min at
30 1000 rpm. The supernatant was discarded and the pellet was re-suspended in pre-warmed (37°C) stimulation buffer (10mL equivalent per 2-3 T-225 flasks). This time was noted and marked as time zero. The cells were counted with a Coulter counter (count above 8

75
μm, flask yield was $1-2 \times 10^7$ cells/flask). Cells were resuspended at a concentration of 5×10^5 cells/ml in pre-warmed (37°C) stimulation buffer (as provided in the flashplate kit) and preincubated at 37°C for 10 min.

cAMP assays were performed in a radioimmunoassay format using the Flashplate
5 Adenylyl Cyclase Activation Assay System with ^{125}I -cAMP (SMP004B, PerkinElmer Life Sciences Inc., Boston, MA), according to the manufacturer's instructions.

Cells were grown and prepared as described above. Final cell concentrations in the assay were 25×10^3 cells/well and the final assay volume was 100 μL. Test compounds were received as 10 mM stock solutions in DMSO, diluted to 400 μM into
10 50 mM HEPES pH 7.4 at 25°C, containing 0.1% BSA, and serial (1:5) dilutions then made in the same buffer. Cyclic AMP accumulation assays were performed with 11 different concentrations of compound ranging from 10 pM to 100 μM (final assay concentrations). A 5-HT concentration-response curve (10 pM to 100 μM) was included on every plate. The cells were incubated, with shaking, at 37°C for 15 min and the
15 reaction terminated by addition of 100 μl of ice-cold detection buffer (as provided in the flashplate kit) to each well. The plates were sealed and incubated at 4°C overnight. Bound radioactivity was quantified by scintillation proximity spectroscopy using the Topcount (Packard BioScience Co., Meriden, CT).

The amount of cAMP produced per mL of reaction was extrapolated from the
20 cAMP standard curve, according to the instructions provided in the manufacturer's user manual. Data were analyzed by nonlinear regression analysis with the GraphPad Prism Software package using the 3-parameter sigmoidal dose-response model (slope constrained to unity). Potency data are reported as pEC_{50} values, the negative decadic logarithm of the EC_{50} value, where EC_{50} is the effective concentration for a 50 %
25 maximal response.

Test compounds exhibiting a higher pEC_{50} value in this assay have a higher potency for agonizing the 5-HT₄ receptor. The compounds of the invention which were tested in this assay, for example, in the cell line (1) having a density of about 0.5-0.6 pmol/mg protein, had a pEC_{50} value ranging from about 7.5 to about 9.5.

Assay 4: *In vitro* Voltage Clamp Assay of Inhibition of Potassium Ion Current in Whole Cells Expressing the hERG Cardiac Potassium Channel

CHO-K1 cells stably transfected with hERG cDNA were obtained from Gail Robertson at the University of Wisconsin. Cells were held in cryogenic storage until
5 needed. Cells were expanded and passaged in Dulbecco's Modified Eagles Medium/F12 supplemented with 10 % fetal bovine serum and 200 µg/mL geneticin. Cells were seeded onto poly-D-lysine (100 µg/mL) coated glass coverslips, in 35 mm² dishes (containing 2 mL medium) at a density that enabled isolated cells to be selected for whole cell voltage-clamp studies. The dishes were maintained in a humidified, 5% CO₂ environment at 37°C.

10 Extracellular solution was prepared at least every 7 days and stored at 4°C when not in use. The extracellular solution contained (mM): NaCl (137), KCl (4), CaCl₂ (1.8), MgCl₂ (1), Glucose (10), 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) (10), pH 7.4 with NaOH. The extracellular solution, in the absence or presence of test compound, was contained in reservoirs, from which it flowed into the recording chamber
15 at approximately 0.5 mL/min. The intracellular solution was prepared, aliquoted and stored at -20°C until the day of use. The intracellular solution contained (mM): KCl (130), MgCl₂ (1), ethylene glycol-bis(beta-aminoethyl ether) *N,N,N',N'*-tetra acetic acid salt (EGTA) (5), MgATP (5), 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) (10), pH 7.2 with KOH. All experiments were performed at room temperature (20-22°C).

20 The coverslips on which the cells were seeded were transferred to a recording chamber and perfused continuously. Gigaohm seals were formed between the cell and the patch electrode. Once a stable patch was achieved, recording commenced in the voltage clamp mode, with the initial holding potential at -80 mV. After a stable whole-cell current was achieved, the cells were exposed to test compound. The standard voltage
25 protocol was: step from the holding potential of -80 mV to +20 mV for 4.8 sec, repolarize to -50 mV for 5 sec and then return to the original holding potential (-80 mV). This voltage protocol was run once every 15 sec (0.067 Hz). Peak current amplitudes during the repolarization phase were determined using pClamp software. Test compounds at a concentration of 3 µM were perfused over the cells for 5 minutes, followed by a 5-minute
30 washout period in the absence of compound. Finally a positive control (cisapride, 20 nM) was added to the perfusate to test the function of the cell. The step from -80 mV to +20 mV activates the hERG channel, resulting in an outward current. The step back to -50 mV

results in an outward tail current, as the channel recovers from inactivation and deactivates.

Peak current amplitudes during the repolarization phase were determined using pCLAMP software. The control and test article data were exported to Origin® (OriginLab Corp., Northampton MA) where the individual current amplitudes were normalized to the initial current amplitude in the absence of compound. The normalized current means and standard errors for each condition were calculated and plotted versus the time course of the experiment.

Comparisons were made between the observed K^+ current inhibitions after the five-minute exposure to either the test article or vehicle control (usually 0.3 % DMSO). Statistical comparisons between experimental groups were performed using a two-population, independent t-test (Microcal Origin v. 6.0). Differences were considered significant at $p < 0.05$.

The smaller the percentage inhibition of the potassium ion current in this assay, the smaller the potential for test compounds to change the pattern of cardiac repolarization when used as therapeutic agents. For example, the compounds of Examples 1-14 which were tested in this assay at a concentration of 3 μ M exhibited an inhibition of the potassium ion current of less than about 30 %, including, less than about 20 %.

Assay 5: *In vitro* Model of Oral Bioavailability: Caco-2 Permeation Assay

The Caco-2 permeation assay was performed to model the ability of test compounds to pass through the intestine and get into the blood stream after oral administration. The rate at which test compounds in solution permeate a cell monolayer designed to mimic the tight junction of human small intestinal monolayers was determined.

Caco-2 (colon, adenocarcinoma; human) cells were obtained from ATCC (American Type Culture Collection; Rockville, MD). For the permeation study, cells were seeded at a density of 63,000 cells/cm² on pre-wetted transwells polycarbonate filters (Costar; Cambridge, MA). A cell monolayer was formed after 21 days in culture. Following cell culture in the transwell plate, the membrane containing the cell monolayer was detached from the transwell plate and inserted into the diffusion chamber (Costar; Cambridge, MA). The diffusion chamber was inserted into the heating block which was equipped with circulating external, thermostatically regulated 37 °C water for temperature

control. The air manifold delivered 95% O₂/5% CO₂ to each half of a diffusion chamber, and created a laminar flow pattern across the cell monolayer, which was effective in reducing the unstirred boundary layer.

The permeation study was performed with test compound concentrations at 5 100 µM and with ¹⁴C-mannitol to monitor the integrity of the monolayer. All experiments were conducted at 37 °C for 60 min. Samples were taken at 0, 30 and 60 min from both the donor and receiver sides of the chamber. Samples were analyzed by HPLC or liquid scintillation counting for test compound and mannitol concentrations. The permeation coefficient (K_p) in cm/sec was calculated.

10 In this assay, a K_p value greater than about 10 x 10⁻⁶ cm/sec is considered indicative of favorable bioavailability. Those compounds of the invention which were tested in this assay typically exhibited K_p values of between about 10 x 10⁻⁶ cm/sec and about 50 x 10⁻⁶ cm/sec.

Assay 6: Pharmacokinetic Study in the Rat

15 Aqueous solution formulations of test compounds were prepared in 0.1 % lactic acid at a pH of between about 5 and about 6. Male Sprague-Dawley rats (CD strain, Charles River Laboratories, Wilmington, MA) were dosed with test compounds via intravenous administration (IV) at a dose of 2.5 mg/kg or by oral gavage (PO) at a dose of 5 mg/kg. The dosing volume was 1 mL/kg for IV and 2 mL/kg for PO administration. 20 Serial blood samples were collected from animals pre-dose, and at 2 (IV only), 5, 15, and 30 min, and at 1, 2, 4, 8, and 24 hours post-dose. Concentrations of test compounds in blood plasma were determined by liquid chromatography-mass spectrometry analysis (LC-MS/MS) (MDS SCIEX, API 4000, Applied Biosystems, Foster City, CA) with a lower limit of quantitation of 1 ng/mL.

25 Standard pharmacokinetic parameters were assessed by non-compartmental analysis (Model 201 for IV and Model 200 for PO) using WinNonlin (Version 4.0.1, Pharsight, Mountain View, CA). The maximum in the curve of test compound concentration in blood plasma vs. time is denoted C_{max}. The area under the concentration vs. time curve from the time of dosing to the last measurable concentration (AUC(0-t)) 30 was calculated by the linear trapezoidal rule. Oral bioavailability (F(%)), i.e. the dose-normalized ratio of AUC(0-t) for PO administration to AUC(0-t) for IV administration, was calculated as:

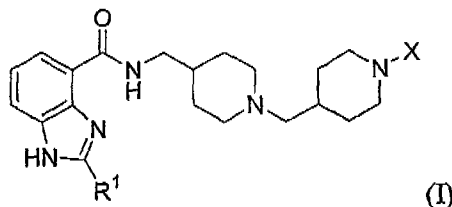
$$F(\%) = AUC_{PO}/AUC_{IV} \times Dose_{IV}/Dose_{PO} \times 100\%$$

Test compounds which exhibit larger values of the parameters C_{max} , $AUC(0-t)$, and $F(\%)$ in this assay are expected to have greater bioavailability when administered orally. Preferred compounds of the invention had C_{max} values typically ranging from
5 about 0.06 to about 0.8 $\mu\text{g/mL}$ and $AUC(0-t)$ values typically ranging from about 0.14 to about 1.2 $\mu\text{g}\cdot\text{hr/mL}$. By way of example, the compound of Example 1 had a C_{max} value of 0.8 $\mu\text{g/mL}$, an $AUC(0-t)$ value of 1.2 $\mu\text{g}\cdot\text{hr/mL}$ and oral bioavailability ($F(\%)$) in the rat model of about 75 %.

While the present invention has been described with reference to the specific
10 embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true
7 spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended
15 to be within the scope of the claims appended hereto. Additionally, all publications, patents, and patent documents cited hereinabove are incorporated by reference herein in full, as though individually incorporated by reference.

WHAT IS CLAIMED IS:

1. A compound of formula (I):



5 wherein:

R^1 is C_{3-5} alkyl, optionally substituted with -OH; and

X is selected from

(a) $-C(O)OR^2$ wherein R^2 is C_{1-4} alkyl or $-(CH_2)_n$ -phenyl wherein n is 0 or 1;

(b) $-C(O)R^3$ wherein R^3 is selected from:

10 phenyl, optionally substituted with 1, 2, or 3 substituents selected from

C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, $-OCHF_2$, and $-CN$,

C_{1-5} alkyl,

C_{4-5} cycloalkyl, and

$-(CH_2)_m$ -A wherein m is 0 or 1 and A is selected from amino, furanyl,

15 thiophenyl, morpholinyl, tetrahydrofuranyl, pyridinyl, naphthalenyl,

pyrrolyl, thiomorpholinyl, pyrrolidinyl, piperidinyl, oxoazetidyl,

thiazolidinyl, 1,1-dioxo isothiazolidinyl, and 2,4-

dimethylisoxazolyl;

(c) $-C(O)NR^4R^5$ wherein R^4 is hydrogen or C_{1-3} alkyl, and R^5 is phenyl

20 optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl,

halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, and $-OCHF_2$;

(d) $-C(O)C(R^6R^7)R^8$ wherein R^6 is hydrogen or C_{1-3} alkyl and R^7 is hydrogen,

$-OH$, or C_{1-3} alkyl; or R^6 and R^7 taken together form oxo or $-(CH_2)_2$; and

R^8 is phenyl or cyclohexyl, wherein phenyl or cyclohexyl are optionally

25 substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl, halo,

C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, $-OCHF_2$, and $-CN$;

(e) $-C(O)C(HR^9)OR^{10}$ wherein R^9 is hydrogen or C_{1-3} alkyl, and R^{10} is phenyl

optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl,

halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, and $-OCHF_2$; and

(f) $-S(O)_2R^{11}$ wherein R^{11} is selected from C_{1-3} alkyl, $-CH_2$ -phenyl, furanyl, thiophenyl, morpholinyl, tetrahydrofuranyl, pyridinyl, naphthalenyl, pyrrolyl, thiomorpholinyl, pyrrolidinyl, piperidinyl, oxoazetidiny, thiazolidinyl, 1,1-dioxo isothiazolidinyl, 2,4-dimethylisoxazolyl, and phenyl optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, $-OCHF_2$, and $-CN$;
or a pharmaceutically-acceptable salt or solvate or stereoisomer thereof.

2. The compound of Claim 1 wherein R^1 is C_{3-5} alkyl.
3. The compound of Claim 2 wherein R^1 is isopropyl or *tert*-butyl.
4. The compound of Claim 2 wherein X is $-C(O)OR^2$.
5. The compound of Claim 4 wherein R^2 is C_{1-3} alkyl or phenyl.
6. The compound of Claim 2 wherein X is $-C(O)R^3$.
7. The compound of Claim 6 wherein R^3 is phenyl, optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, and $-OCHF_2$.
8. The compound of Claim 2 wherein X is $-C(O)NR^4R^5$.
9. The compound of Claim 2 wherein:
 R^1 is C_{3-4} alkyl; and
X is selected from:
 - (a) $-C(O)OR^2$ wherein R^2 is C_{1-3} alkyl or phenyl;
 - (b) $-C(O)R^3$ wherein R^3 is phenyl, optionally substituted with 1 or 2 substituents selected from C_{1-4} alkyl, halo, and $-CF_3$; furanyl; or thiophenyl;
 - (c) $-C(O)NR^4R^5$ wherein R^4 is hydrogen and R^5 is phenyl optionally substituted with 1 or 2 substituents selected from C_{1-4} alkyl and halo;

(d) $-C(O)C(R^6R^7)R^8$ wherein R^6 is hydrogen and R^7 is hydrogen, $-OH$, or methyl; or R^6 and R^7 taken together form oxo or $-(CH_2)_2$; and R^8 is phenyl or cyclohexyl, wherein phenyl or cyclohexyl are optionally substituted with 1 or 2 substituents selected from C_{1-4} alkyl and halo;

(e) $-C(O)C(HR^9)OR^{10}$ wherein R^9 is hydrogen or methyl and R^{10} is phenyl optionally substituted with 1 or 2 substituents selected from C_{1-4} alkyl and halo; and

(f) $-S(O)_2R^{11}$ wherein R^{11} is methyl or phenyl, optionally substituted with 1 or 2 substituents selected from C_{1-4} alkyl and halo.

10. The compound of Claim 9 wherein:

R^1 is isopropyl or *tert*-butyl; and

X is selected from:

(a) $-C(O)OR^2$ wherein R^2 is methyl or phenyl;

(b) $-C(O)R^3$ wherein R^3 is phenyl, optionally substituted with 1 or 2 substituents selected from methyl, chloro, fluoro, and $-CF_3$; furan-2-yl; and thiophen-2-yl; and

(c) $-C(O)NR^4R^5$ wherein R^4 is hydrogen and R^5 is phenyl optionally substituted with 1 fluoro or chloro.

11. The compound of Claim 2 wherein the compound is selected from:

4-(4-[(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)-amino]methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester;

4-(4-[(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)-amino]-methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid phenyl ester;

2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(2-chlorobenzoyl)piperidin-4-ylmethyl] piperidin-4-ylmethyl} amide;

2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(2,4-difluorobenzoyl)piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide;

2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(furan-2-carbonyl)piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide;

2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(thiophene-2-carbonyl)piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide;

2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(2-fluoro-5-trifluoromethylbenzoyl)piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide;

2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(2-fluoro-phenylcarbamoyl)piperidin-4-ylmethyl]piperidin-4-ylmethyl}-amide;

5 4-(4-[[2-*tert*-butyl-1*H*-benzoimidazole-4-carbonyl]-amino]-methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester;

2-*tert*-butyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(2-fluoro-benzoyl)-piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide;

10 2-*tert*-butyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(3-methyl-benzoyl)-piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide;

2-*tert*-butyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(4-fluorobenzoyl)-piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide; and

pharmaceutically-acceptable salts and solvates and stereoisomers thereof.

15 12. The compound of Claim 2 wherein the compound is selected from:

4-(4-[[2-isopropyl-1*H*-benzoimidazole-4-carbonyl]-amino]methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester;

4-(4-[[2-*tert*-butyl-1*H*-benzoimidazole-4-carbonyl]-amino]-methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester;

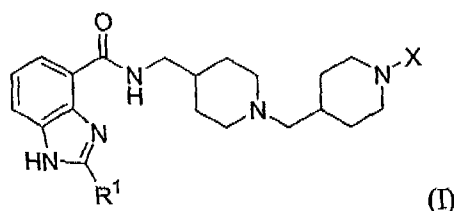
20 2-*tert*-butyl-1*H*-benzoimidazole-4-carboxylic acid {1-[1-(2-fluoro-benzoyl)-piperidin-4-ylmethyl]piperidin-4-ylmethyl} amide; and

pharmaceutically-acceptable salts and solvates and stereoisomers thereof.

13. Crystalline 4-(4-[[2-isopropyl-1*H*-benzoimidazole-4-carbonyl]-amino]methyl)piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester or a solvate thereof.

14. The crystalline compound of Claim 13, wherein the crystalline compound is characterized by a powder x-ray diffraction pattern having two or more diffraction
30 peaks at 2θ values selected from 15.08 ± 0.20 , 15.41 ± 0.20 , 19.00 ± 0.20 , 19.70 ± 0.20 , and 23.68 ± 0.20 .

15. The crystalline compound of Claim 14, wherein the crystalline compound is characterized by a differential scanning calorimetry profile which shows a maximum in endothermic heat flow at a temperature in the range of about 146 °C to about 148 °C.
- 5 16. The crystalline compound of Claim 13, wherein the crystalline compound is characterized by a differential scanning calorimetry profile which shows a maximum in endothermic heat flow at a temperature in the range of about 143 °C to about 145 °C.
- 10 17. The crystalline compound of Claim 13, wherein the crystalline compound is a monohydrate.
18. The crystalline compound of Claim 17 wherein the crystalline compound is characterized by a powder x-ray diffraction pattern having two or more diffraction peaks at 2θ values selected from 9.14 ± 0.20 , 12.41 ± 0.20 , 12.74 ± 0.20 , 17.75 ± 0.20 ,
15 18.47 ± 0.20 , 20.63 ± 0.20 , 21.13 ± 0.20 , and 27.05 ± 0.20 .
19. A pharmaceutical composition comprising a therapeutically effective amount of the compound of any one of Claims 1 to 18 and a pharmaceutically acceptable carrier.
- 20 20. A compound as claimed in any one of Claims 1 to 18 for use in therapy.
21. Use of a compound of any one of Claims 1 to 18 for manufacture of a medicament.
- 25 22. The use of Claim 21 wherein the medicament is for the treatment of a medical condition in a mammal associated with 5-HT₄ receptor activity.
23. The use of Claim 22 wherein the disease or condition is a disorder of
30 reduced motility of the gastrointestinal tract.
24. A process for preparing a compound of formula (I),



wherein:

R^1 is C_{3-5} alkyl, optionally substituted with -OH; and

X is selected from

(a) $-C(O)OR^2$ wherein R^2 is C_{1-4} alkyl or $-(CH_2)_n$ -phenyl wherein n is 0 or 1;

(b) $-C(O)R^3$ wherein R^3 is selected from:

phenyl, optionally substituted with 1, 2, or 3 substituents selected from

C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, and $-OCHF_2$,

C_{1-5} alkyl,

C_{4-5} cycloalkyl, and

$-(CH_2)_m$ -A wherein m is 0 or 1 and A is selected from furanyl,

thiophenyl, morpholinyl, tetrahydrofuranyl, pyridinyl, naphthalenyl,

pyrrolyl, thiomorpholinyl, pyrrolidinyl, piperidinyl, oxoazetidiny,

thiazolidinyl, 1,1-dioxo isothiazolidinyl, and 2,4-

dimethylisoxazoly;

(c) $-C(O)NR^4R^5$ wherein R^4 is hydrogen or C_{1-3} alkyl, and R^5 is phenyl

optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl,

halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, and $-OCHF_2$;

(d) $-C(O)C(R^6R^7)R^8$ wherein R^6 is hydrogen or C_{1-3} alkyl and R^7 is hydrogen,

-OH, C_{1-3} alkyl, or oxo; or R^6 and R^7 form $-(CH_2)_2$; and R^8 is phenyl or

cyclohexyl, wherein phenyl or cyclohexyl are optionally substituted with

1, 2, or 3 substituents selected from C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$,

$-OCF_3$, $-OCHF_2$, and $-CN$;

(e) $-C(O)C(HR^9)OR^{10}$ wherein R^9 is hydrogen or C_{1-3} alkyl, and R^{10} is phenyl

optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl,

halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, and $-OCHF_2$; and

(f) $-S(O)_2R^{11}$ wherein R^{11} is selected from C_{1-3} alkyl, $-CH_2$ -phenyl, furanyl,

thiophenyl, morpholinyl, tetrahydrofuranyl, pyridinyl, naphthalenyl,

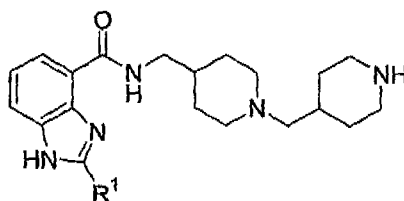
pyrrolyl, thiomorpholinyl, pyrrolidinyl, piperidinyl, oxoazetidiny,

thiazolidinyl, 1,1-dioxo isothiazolidinyl, 2,4-dimethylisoxazolyl, and phenyl optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, $-OCHF_2$, and $-CN$;

or a pharmaceutically-acceptable salt or solvate or stereoisomer thereof, the process

5 comprising:

(i) reacting a compound of formula (II):



(II)

with a compound of formula (III):

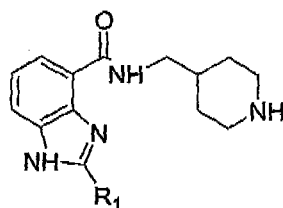
10



(III)

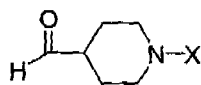
wherein L is leaving group;

(ii) reacting a compound of formula (VIII):



(VIII)

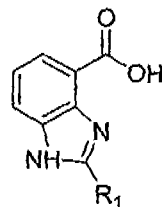
15 with a compound of formula (XIII):



(XIII)

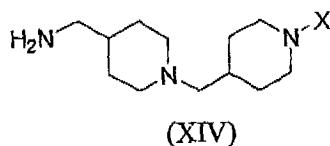
; or

(iii) reacting a compound of formula (VI):



(VI)

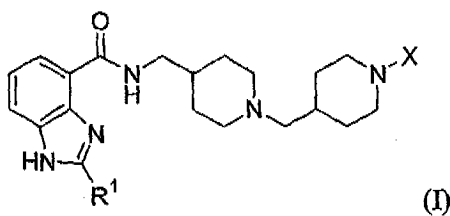
with a compound of formula (XIV):



to provide a compound of formula (I), or a pharmaceutically-acceptable salt or solvate or stereoisomer thereof.

5

25. A process for preparing a compound of formula (I),



wherein:

R^1 is C_{3-5} alkyl; and

10

X is selected from

(b) $-C(O)R^3$ wherein R^3 is selected from:

phenyl, optionally substituted with 1, 2, or 3 substituents selected from

C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, and $-OCHF_2$,

C_{1-5} alkyl,

15

C_{4-5} cycloalkyl, and

$-(CH_2)_m-A$ wherein m is 0 or 1 and A is selected from furanyl,

thiophenyl, morpholinyl, tetrahydrofuranyl, pyridinyl, naphthalenyl,

pyrrolyl, thiomorpholinyl, pyrrolidinyl, piperidinyl, oxoazetidiny,

thiazolidinyl, 1,1-dioxo isothiazolidinyl, and 2,4-

20

dimethylisoxazolyl;

(d) $-C(O)C(R^6R^7)R^8$ wherein R^6 is hydrogen or C_{1-3} alkyl and R^7 is hydrogen,

$-OH$, C_{1-3} alkyl, or oxo; or R^6 and R^7 form $-(CH_2)_2-$; and R^8 is phenyl or

cyclohexyl, wherein phenyl or cyclohexyl are optionally substituted with

1, 2, or 3 substituents selected from C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$,

25

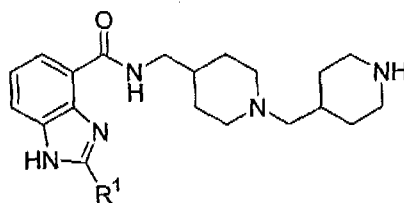
$-OCF_3$, $-OCHF_2$, and $-CN$; and

(e) $-C(O)C(HR^9)OR^{10}$ wherein R^9 is hydrogen or C_{1-3} alkyl, and R^{10} is phenyl optionally substituted with 1, 2, or 3 substituents selected from C_{1-4} alkyl, halo, C_{1-4} alkoxy, $-CF_3$, $-OCF_3$, and $-OCHF_2$;

or a pharmaceutically-acceptable salt or solvate or stereoisomer thereof, the process

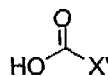
5 comprising:

reacting a compound of formula (II):



(II)

with a compound of formula (IV):

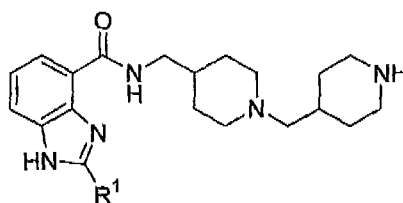


(IV)

wherein X' is selected from R^3 , $C(R^6R^7)R^8$, and $C(HR^9)OR^{10}$ to provide a compound of formula (I) or a pharmaceutically-acceptable salt or solvate or stereoisomer thereof.

15 26. The product prepared by the process of Claim 24 or Claim 25.

27. A compound of formula (II):



(II)

20 wherein R^1 is C_{3-5} alkyl,

or a salt or stereoisomer or protected derivative thereof.

28. A process for preparing crystalline 4-(4- {[(2-isopropyl-1H-benzimidazole-4-carbonyl)-amino]methyl}piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester, the process comprising:

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(a) dispersing 4-(4-{{(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)-amino}methyl}piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester in an inert diluent selected from acetonitrile, ether, cyclohexane, and ethyl acetate in a proportion of between about 15 mg and 25 mg of 4-(4-{{(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)-amino}methyl}piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester per milliliter of diluent to form a mixture; and

(b) allowing the mixture to evaporate to provide crystalline 4-(4-{{(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)-amino}methyl}piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester.

10

29. A process for preparing crystalline 4-(4-{{(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)-amino}methyl}piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester, the process comprising:

(a) reacting 2-isopropyl-1*H*-benzoimidazole-4-carboxylic acid (piperidin-4-ylmethyl)amide with 4-formylpiperidine-1-carboxylic acid methyl ester in a polar aprotic diluent;

(b) adding acetonitrile while distilling the product of step (a) to remove the polar aprotic diluent from the product of step (a);

(c) preparing a mixture of the residue from the distillation of step (b) in acetonitrile at a concentration of between about 50 and about 125 mg of 4-(4-{{(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)-amino}methyl}piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester per milliliter of acetonitrile at a temperature sufficient to dissolve the residue; and

(d) cooling the mixture of step (c) to a temperature of no more than about 20 °C to provide crystalline 4-(4-{{(2-isopropyl-1*H*-benzoimidazole-4-carbonyl)-amino}methyl}piperidin-1-ylmethyl)piperidine-1-carboxylic acid methyl ester.

30. The product prepared by the process of Claim 29 or Claim 30.

31. A method of treating a mammal having a medical condition associated with 5-HT₄ receptor activity, the method comprising administering to the mammal, a therapeutically effective amount of a pharmaceutical composition comprising a pharmaceutically-acceptable carrier and a compound of any one of Claims 1 to 18.

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32. The method of Claim 31 wherein the medical condition is selected from irritable bowel syndrome, chronic constipation, functional dyspepsia, delayed gastric emptying, gastroesophageal reflux disease, gastroparesis, post-operative ileus, intestinal
5 pseudo-obstruction, and drug-induced delayed transit.

33. A method of treating a disorder of reduced motility of the gastrointestinal tract in a mammal, the method comprising administering to the mammal, a therapeutically effective amount of a pharmaceutical composition comprising a pharmaceutically-
10 acceptable carrier and a compound of any one of Claims 1 to 18.

34. The method of Claim 33, wherein the disorder of reduced motility is selected from chronic constipation, constipation-predominant irritable bowel syndrome, diabetic and idiopathic gastroparesis, and functional dyspepsia.
15

35. A method of studying a biological system or sample comprising a 5-HT₄ receptor, the method comprising:

(a) contacting the biological system or sample with a compound of any one of Claims 1 to 18; and

20 (b) determining the effect caused by the compound on the biological system or sample.

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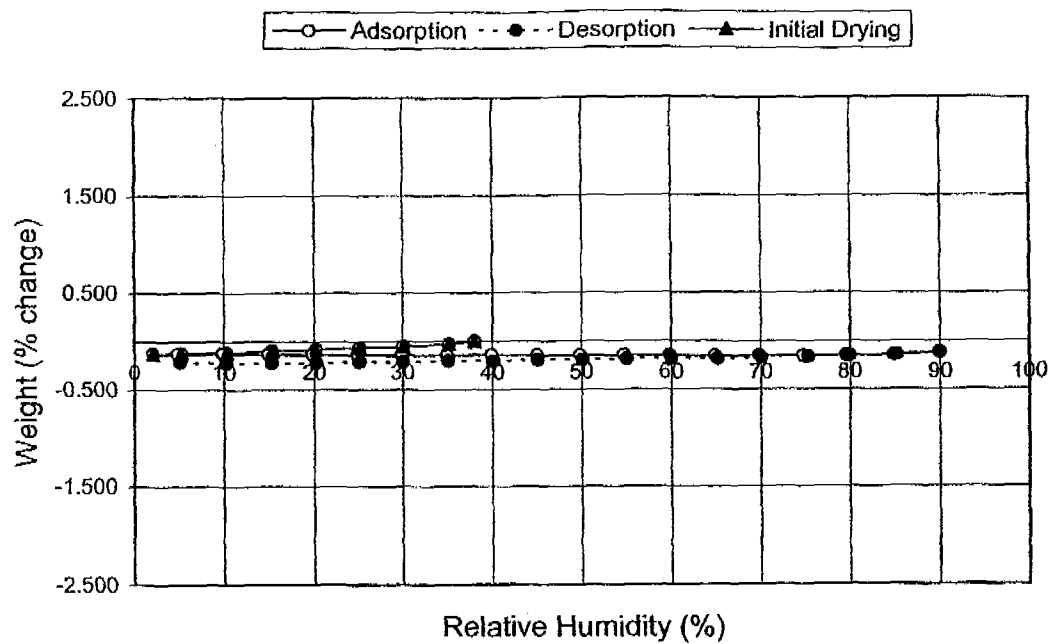


Figure 3

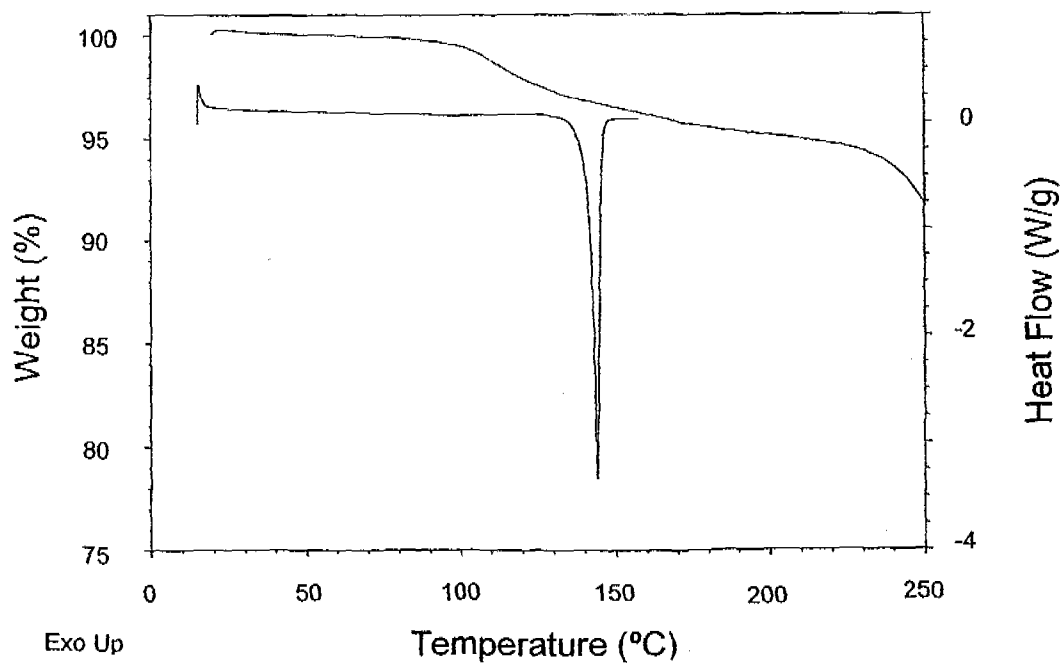


Figure 4

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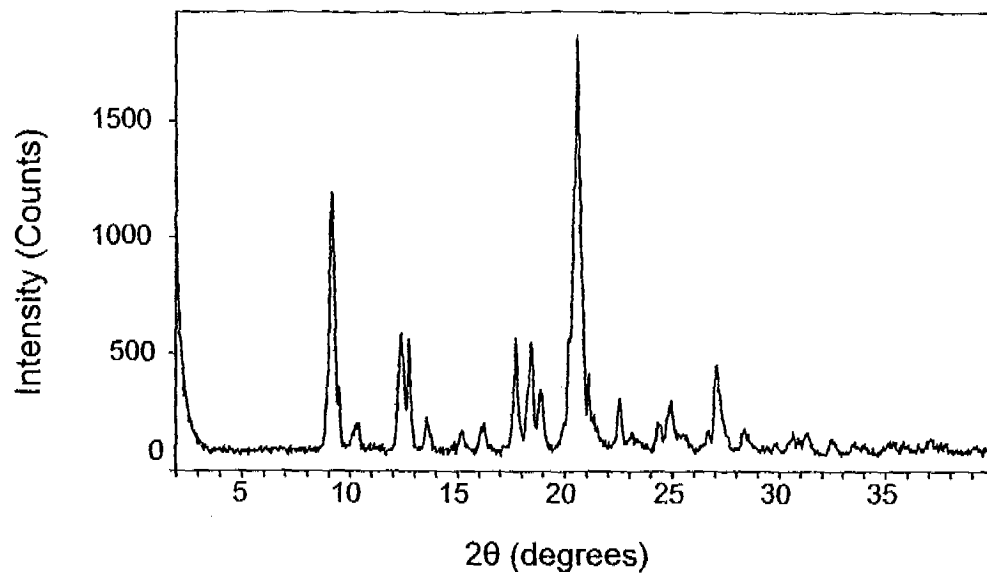


Figure 5

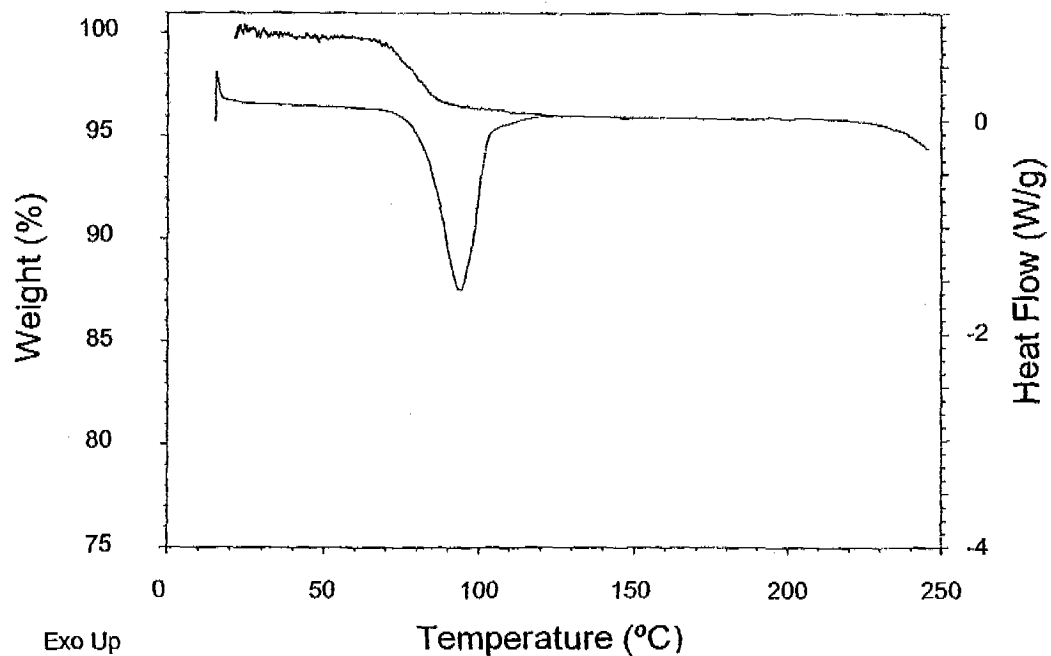


Figure 6

94

(19) World Intellectual Property Organization
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KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,
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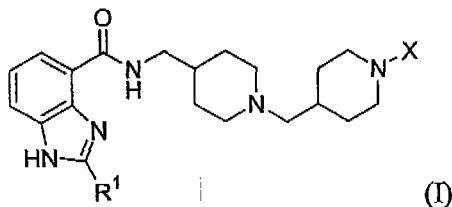
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For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: BENZIMIDAZOLE-CARBOXAMIDE COMPOUNDS AS 5-HT₄ RECEPTOR AGONISTS



(I)

(57) Abstract: The invention relates to benzimidazole-carboxamide
5-HT₄ receptor agonist compounds of formula (I), wherein R¹ and X
are as defined in the specification, or a pharmaceutically acceptable
salt or solvate or stereoisomer thereof. The invention also relates to
pharmaceutical compositions comprising such compounds, methods
of using such compounds to treat diseases associated with 5-HT₄ re-
ceptor activity, and processes and intermediates useful for preparing
such compounds. The invention further relates to crystalline forms of
a compound of formula (I).

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/020085

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D401/14 C07D405/14 C07D409/14 A61K31/4545 A61P1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LOPEZ-RODRIGUEZ MARIA L ET AL: "3-D-QSAR/CoMFA and recognition models of benzimidazole derivatives at the 5-HT4 receptor" BIOORGANIC AND MEDICINAL CHEMISTRY LETTERS, vol. 11, no. 21, 5 November 2001 (2001-11-05), pages 2807-2811, XP002434296 ISSN: 0960-894X the whole document in particular compounds 5 and 6	1-35
Y	JP 2004 277318 A (DAINIPPON PHARMACEUTICAL CO) 7 October 2004 (2004-10-07) abstract; tables 3-8 ----- -/-	1-35



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

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Authorized officer

Papathoma, Sofia

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2006/020085

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005/021539 A (PFIZER JAPAN INC [JP]; PFIZER [US]; IGUCHI SATORU [JP]; KATSU YASUHIRO) 10 March 2005 (2005-03-10) abstract; claims 1-19; examples 1-16 -----	1-35
A	WO 2004/026868 A (PFIZER PHARMA [JP]; KATSU YASUHIRO [JP]; KON-I KANA [JP]; MORITA MIKIO) 1 April 2004 (2004-04-01) abstract; claims 1-16; examples 7-9,19,20,22 -----	1-35

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 31-34 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.

Although claim 35 is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2006/020085

Box 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.: 31-35
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 III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

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COMPUESTOS BENZIMIDAZOL-CARBOXAMIDA COMO AGONISTAS DEL RECEPTOR 5-HT₄

ANTECEDENTES DE LA INVENCION

CAMPO DE LA INVENCION

La invención se relaciona con compuestos benzimidazol-carboxamida que son útiles como agonistas del receptor 5-HT₄. La invención también tiene como objetos las composiciones farmacéuticas que comprenden dichos compuestos, métodos para usar dichos compuestos para tratar o prevenir condiciones médicas mediadas por la actividad del receptor 5-HT₄, y procesos e intermediarios útiles para preparar dichos compuestos.

ESTADO DE LA TÉCNICA

La serotonina (5-hidroxitriptamina, 5-HT) es un neurotransmisor ampliamente distribuido a través de todo el cuerpo, tanto en el sistema nervioso central como en los sistemas periféricos. Se han identificado por lo menos siete subtipos de receptores de serotonina y la interacción de la serotonina con dichos diferentes receptores se relaciona con una amplia variedad de funciones fisiológicas. Por lo tanto, ha habido un interés sustancial en el desarrollo de agentes terapéuticos que se dirigen a subtipos específicos del receptor 5-HT.

En particular, la caracterización de los receptores 5-HT₄ y la identificación de agentes farmacéuticos que interactúan con ellos ha sido el foco reciente de una gran actividad. (Véase, por ejemplo, la revisión de Langlois y Fischmeister, *J. Med. Chem.* **2003**, 46, 319-344). Los agonistas del receptor 5-HT₄ son útiles para el tratamiento de trastornos de motilidad reducida del tracto gastrointestinal. Dichos trastornos incluyen síndrome del intestino irritable (IBS), constipación crónica, dispepsia funcional, retardo del

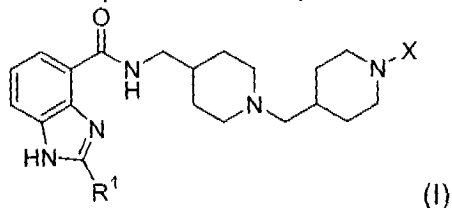
vaciado gástrico, enfermedad de reflujo gastroesofágico (GERD), gastroparesis, íleo postoperatorio, pseudoobstrucción intestinal, y tránsito con retardo inducido por drogas. Además, se ha sugerido que algunos compuestos agonistas del receptor 5-HT₄ se pueden utilizar en el tratamiento de trastornos del sistema nervioso central incluyendo trastornos cognitivos, trastornos del comportamiento, trastornos del estado de ánimo, y trastornos de control de la función autónoma.

A pesar de la amplia utilidad de los agentes farmacéuticos que modulan la actividad del receptor 5-HT₄, pocos compuestos agonistas del receptor 5-HT₄ están en uso clínico en el presente. Por lo tanto, existe la necesidad de nuevos agonistas del receptor 5-HT₄ que consigan sus efectos deseados con mínimos efectos secundarios. Los agentes preferidos pueden poseer, entre otras propiedades, selectividad, potencia, propiedades farmacocinéticas, y/o duración de la acción mejoradas. — P. Ventaja.

RESUMEN DE LA INVENCION

La invención provee nuevos compuestos que poseen actividad agonista del receptor 5-HT₄. Entre otras propiedades, los compuestos de la invención resultaron ser agonistas del receptor 5-HT₄ potentes y selectivos. Además, se ha descubierto que los compuestos preferidos de la invención presentan propiedades farmacocinéticas favorables en un modelo animal, que permiten predecir una buena biodisponibilidad al realizar una administración oral.

En forma acorde, la invención provee un compuesto de fórmula (I):



(I)

en donde:

R^1 es C_{3-5} alquilo, opcionalmente sustituido con -OH; y

X se selecciona entre

(a) $-C(O)OR^2$ en donde R^2 es C_{1-4} alquilo o $-(CH_2)_n$ -fenilo en donde n es 0 o 1;

(b) $-C(O)R^3$ en donde R^3 se selecciona entre :

fenilo, opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre

~~C_{1-4} alquilo~~, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, $-OCHF_2$, y $-CN$,

~~C_{1-5} alquilo,~~

C_{4-5} cicloalquilo, y

$-(CH_2)_m$ -A en donde m es 0 o 1 y A se selecciona entre amino, furanilo, tiofenilo, morfolinilo, tetrahidrofuranilo, piridinilo, naftalenilo, pirrolilo, tiomorfolinilo, pirrolidinilo, piperidinilo, oxoazetidinilo, tiazolidinilo, 1,1-dioxo isotiazolidinilo, y 2,4-dimetilisoxazolilo;

(c) $-C(O)NR^4R^5$ en donde R^4 es hidrógeno o C_{1-3} alquilo, y R^5 es fenilo opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, y $-OCHF_2$;

(d) $-C(O)C(R^6R^7)R^8$ en donde R^6 es hidrógeno o C_{1-3} alquilo y R^7 es hidrógeno, -OH, o C_{1-3} alquilo; o R^6 y R^7 juntos forman oxo o $-(CH_2)_2$ -; y R^8 es fenilo o ciclohexilo, en donde fenilo o ciclohexilo se sustituyen opcionalmente con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, $-OCHF_2$, y $-CN$;

(e) $-C(O)C(HR^9)OR^{10}$ en donde R^9 es hidrógeno o C_{1-3} alquilo, y R^{10} es fenilo opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, y $-OCHF_2$; y

(f) $-S(O)_2R^{11}$ en donde R^{11} se selecciona entre C_{1-3} alquilo, $-CH_2$ -fenilo, furanilo, tiofenilo, morfolinilo, tetrahidrofuranilo, piridinilo, naftalenilo, pirrolilo, tiomorfolinilo, pirrolidinilo, piperidinilo, oxoazetidínilo, tiazolidínilo, 1,1-dioxo isotiazolidínilo, 2,4-dimetilisoxazolilo, y fenilo opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, $-OCHF_2$, y $-CN$;
o una sal aceptable para uso farmacéutico o solvato o estereoisómero del mismo.

La invención también provee una composición farmacéutica que comprende un compuesto de la invención y un vehículo aceptable para el uso farmacéutico.

En otro aspecto, en la invención se provee un compuesto de fórmula (I) particular en forma de una base cristalina libre. Se ha descubierto que el metil éster de ácido 4-(4-(((2-isopropil-1H-benzoimidazol-4-carbonil)amino)metil)-piperidin-1-ilmetil)piperidin-1-carboxílico cristalino tiene una temperatura de fusión en el rango de entre aproximadamente 145°C y aproximadamente 155°C, típicamente entre aproximadamente 146 y aproximadamente 148°C, una temperatura de degradación superior a aproximadamente 240°C, y presenta cambios de peso menores que aproximadamente 0,25% cuando se expone a un rango de humedad relativa de entre aproximadamente 2% y aproximadamente 90% a temperatura ambiente. En otros aspectos de la invención se proporcionan formas cristalinas adicionales del metil éster de ácido 4-(4-(((2-isopropil-1H-benzoimidazol-4-carbonil)amino)metil)-piperidin-1-ilmetil)piperidin-1-carboxílico.

no Higroscópica

En un aspecto del método, en la invención se provee un método para tratar una enfermedad o una condición asociada con la actividad del receptor 5-HT₄, por ejemplo, un trastorno de motilidad reducida del tracto gastrointestinal, donde el método comprende administrarle al mamífero una cantidad eficiente para el uso terapéutico de un compuesto de la invención.

Además, la invención provee un método para tratar una enfermedad o condición asociada con la actividad del receptor 5-HT₄ en un mamífero, y el método comprende administrar al mamífero, una cantidad eficaz en términos terapéuticos de una composición farmacéutica de la invención.

actividad
Los compuestos de la invención también pueden utilizarse como herramientas de investigación, es decir para estudiar sistemas o muestras biológicas, o para estudiar la actividad de otros compuestos químicos. En consecuencia, en uno de sus aspectos de método, la invención provee un método para utilizar un compuesto de fórmula (I), o una sal o un solvato o un estereoisómero aceptable para el uso farmacéutico del mismo, como una herramienta de investigación para estudiar un sistema o una muestra biológica, o para descubrir nuevos agonistas del receptor 5-HT₄, y el método comprende poner en contacto un sistema o muestra biológica con un compuesto de la invención y determinar los efectos causados por el compuesto en el sistema o muestra biológica.

En aspectos independientes y diferenciados, la invención también provee procesos sintéticos e intermediarios descritos en la presente, que son útiles para preparar compuestos de la invención.

La invención también provee un compuesto de la invención como se ha descrito en la presente para su uso en terapia médica, así como el uso de un

compuesto de la invención en la fabricación de una formulación o medicamento para tratar una enfermedad o una afección asociada a la actividad del receptor 5-HT₄, por ejemplo un trastorno de motilidad reducida del tracto gastrointestinal, en un mamífero.

BREVE DESCRIPCIÓN DE LAS FIGURAS

Varios aspectos de la presente invención se ilustran con referencia a los dibujos adjuntos.

En la Figura 1 se ilustra un patrón de difracción de rayos X en polvo (PXRD) del metil éster de ácido 4-(4-[[[2-isopropil-1H-benzoimidazol-4-carbonil)amino]metil]-piperidin-1-ilmetil]piperidin-1-carboxílico cristalino (Forma I).

En la Figura 2 se ilustra una representación de calorimetría diferencial de barrido (DSC) (representación superior, eje vertical derecho) y una representación de un análisis gravimétrico térmico (TGA) (representación inferior, eje vertical izquierdo) del metil éster de ácido 4-(4-[[[2-isopropil-1H-benzoimidazol-4-carbonil)amino]metil]-piperidin-1-ilmetil]piperidin-1-carboxílico cristalino (Forma I).

En la Figura 3 se ilustra una isoterma de absorción dinámica de humedad (DMS) del metil éster de ácido 4-(4-[[[2-isopropil-1H-benzoimidazol-4-carbonil)amino]metil]-piperidin-1-ilmetil]piperidin-1-carboxílico cristalino (Forma I).

En la Figura 4 se ilustra una representación de calorimetría diferencial de barrido (DSC) (representación superior, eje vertical derecho) y una representación de un análisis gravimétrico térmico (TGA) (representación inferior, eje vertical izquierdo) del metil éster de ácido 4-(4-[[[2-isopropil-1H-

benzoimidazol-4-carbonil)amino]metil}-piperidin-1-ilmetil)piperidin-1-carboxílico cristalino (Forma II).

En la Figura 5 se ilustra un patrón de difracción de rayos X en polvo (PXRD) del metil éster de ácido 4-(4-[[[2-isopropil-1H-benzoimidazol-4-carbonil)amino]metil]-piperidin-1-ilmetil)piperidin-1-carboxílico cristalino (Forma III).

Figura 6 se ilustra una representación de calorimetría diferencial de barrido (DSC) (representación superior, eje vertical derecho) y una representación de un análisis gravimétrico térmico (TGA) (representación inferior, eje vertical izquierdo) del metil éster de ácido 4-(4-[[[2-isopropil-1H-benzoimidazol-4-carbonil)amino]metil]-piperidin-1-ilmetil)piperidin-1-carboxílico cristalino (Forma III).

DESCRIPCIÓN DETALLADA DE LA INVENCION

La invención provee nuevos agonistas del receptor 5-HT₄ de benzimidazol-carboxamida de la Fórmula (I), sus sales aceptables para el uso farmacéutico o solvatos o estereoisómeros de los mismos. Los siguientes sustituyentes y valores se incluyen con el fin de proveer ejemplos representativos de diversos aspectos de esta invención. Estos valores representativos tienen como finalidad definir tales aspectos sin intención de excluir otros valores ni limitar el alcance de la invención.

En un aspecto específico de la invención, R¹ es C₃₋₅alquilo, opcionalmente sustituido con -OH.

En otro aspecto específico, R¹ es C₃₋₅alquilo.

En otros aspectos específicos, R¹ es C₃₋₄alquilo; o R¹ es isopropilo o *ter*-butilo.

En otro aspecto específico, R¹ es isopropilo.

En otros aspectos específicos, R^1 es 1-hidroxi-1-metiletilo, o 2-hidroxi-1-metiletilo.

En un aspecto específico, X es $-C(O)OR^2$ en donde R^2 es C_{1-4} alquilo o $-(CH_2)_n$ -fenilo en donde n es 0 o 1.

En otro aspecto específico, X es $-C(O)OR^2$ en donde R^2 es C_{1-3} alquilo o fenilo.

En otros aspectos específicos, X es $-C(O)OR^2$ en donde R^2 es metilo o fenilo, o en donde R^2 es metilo.

En un aspecto específico, X es $-C(O)R^3$ en donde R^3 se selecciona entre fenilo, opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, $-OCHF_2$, y $-CN$; C_{1-5} alquilo; C_{4-5} cicloalquilo; y $-(CH_2)_m-A$ en donde m es 0 o 1 y A se selecciona entre amino, furanilo, tiofenilo, morfolinilo, tetrahidrofuranilo, piridinilo, naftalenilo, pirrolilo, tiomorfolinilo, pirrolidinilo, piperidinilo, oxoazetidinilo, tiazolidinilo, 1,1-dioxo isotiazolidinilo, y 2,4-dimetilisoxazolilo.

En otro aspecto específico, X es $-C(O)R^3$ en donde R^3 es fenilo, opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, $-OCHF_2$, y $-CN$.

En otro aspecto específico, X es $-C(O)R^3$ en donde R^3 es C_{1-5} alquilo o C_{4-5} cicloalquilo.

En otro aspecto específico, X es $-C(O)R^3$ en donde R^3 es $-(CH_2)_m-A$ en donde m es 0 y A se selecciona entre amino, furanilo, tiofenilo, morfolinilo, tetrahidrofuranilo, piridinilo, naftalenilo, pirrolilo, tiomorfolinilo, pirrolidinilo, piperidinilo, oxoazetidinilo, tiazolidinilo, 1,1-dioxo isotiazolidinilo, y 2,4-dimetilisoxazolilo.

En otro aspecto específico, X es $-C(O)R^3$ en donde R^3 es fenilo, opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre C_{1-4} alquilo, halo, y $-CF_3$; furanilo; o tiofenilo.

En otros aspectos específicos, X es $-C(O)R^3$ en donde R^3 es fenilo opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre metilo, cloro, fluoro, y $-CF_3$; o R^3 es furan-2-ilo o tiofen-2-ilo.

En un aspecto específico, X es $-C(O)NR^4R^5$ en donde R^4 es hidrógeno o C_{1-3} alquilo, y R^5 es fenilo opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, y $-OCHF_2$.

En otro aspecto específico, X es $-C(O)NR^4R^5$ en donde R^4 es hidrógeno.

En otro aspecto específico, X es $-C(O)NR^4R^5$ en donde R^4 es hidrógeno y R^5 es fenilo opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre C_{1-4} alquilo y halo.

En otros aspectos específicos, X es $-C(O)NR^4R^5$ en donde R^4 es hidrógeno y R^5 es fenilo opcionalmente sustituido con 1 halo, o con un fluoro o cloro.

En un aspecto específico, X es $-C(O)C(R^6R^7)R^8$ en donde R^6 es hidrógeno o C_{1-3} alquilo y R^7 es hidrógeno, $-OH$, o C_{1-3} alquilo; o R^6 y R^7 juntos forman oxo o $-(CH_2)_2$; y R^8 es fenilo o ciclohexilo, en donde fenilo o ciclohexilo se sustituyen opcionalmente con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, $-OCHF_2$, y $-CN$.

En otro aspecto específico, X es $-C(O)C(R^6R^7)R^8$ en donde R^6 es hidrógeno.

En otro aspecto específico, X es $-C(O)C(R^6R^7)R^8$ en donde R^8 es fenilo opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, $-OCHF_2$, y $-CN$.

En otro aspecto específico, X es $-\text{C}(\text{O})\text{C}(\text{R}^6\text{R}^7)\text{R}^8$ en donde R^8 es ciclohexilo opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-\text{CF}_3$, $-\text{OCF}_3$, $-\text{OCHF}_2$, y $-\text{CN}$.

En otros aspectos específicos, X es $-\text{C}(\text{O})\text{C}(\text{R}^6\text{R}^7)\text{R}^8$ en donde R^6 es hidrógeno y R^7 es hidrógeno, $-\text{OH}$, o metilo; o R^6 y R^7 juntos forman oxo o $-(\text{CH}_2)_2-$; y R^8 es fenilo o ciclohexilo, en donde fenilo o ciclohexilo se sustituyen opcionalmente con 1 o 2 sustituyentes seleccionados entre C_{1-4} alquilo y halo; o R^8 es fenilo o ciclohexilo, en donde fenilo o ciclohexilo se sustituyen opcionalmente con 1 o 2 sustituyentes seleccionados entre metilo, fluoro, y cloro.

En un aspecto específico, X es $-\text{C}(\text{O})\text{C}(\text{HR}^9)\text{OR}^{10}$ en donde R^9 es hidrógeno o C_{1-3} alquilo, y R^{10} es fenilo opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-\text{CF}_3$, $-\text{OCF}_3$, y $-\text{OCHF}_2$.

En otro aspecto específico, X es $-\text{C}(\text{O})\text{C}(\text{HR}^9)\text{OR}^{10}$ en donde R^9 es hidrógeno o metilo.

En otros aspectos específicos, X es $-\text{C}(\text{O})\text{C}(\text{HR}^9)\text{OR}^{10}$ en donde R^9 es hidrógeno o metilo y R^{10} es fenilo opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre C_{1-4} alquilo y halo, o fenilo opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre metilo, fluoro, y cloro.

En un aspecto específico, X es $-\text{S}(\text{O})_2\text{R}^{11}$ en donde R^{11} se selecciona entre C_{1-3} alquilo, $-\text{CH}_2$ -fenilo, furanilo, tiofenilo, morfolinilo, tetrahidrofuranilo, piridinilo, naftalenilo, pirrolilo, tiomorfolinilo, pirrolidinilo, piperidinilo, oxoazetidinilo, tiazolidinilo, 1,1-dioxo isotiazolidinilo, 2,4-dimetilisoxazolilo, y fenilo opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-\text{CF}_3$, $-\text{OCF}_3$, $-\text{OCHF}_2$, y $-\text{CN}$.

En otro aspecto específico, X es $-\text{S}(\text{O})_2\text{R}^{11}$ en donde R^{11} es C_{1-3} alquilo, 2,4-dimetilisoxazolilo, o fenilo, opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-\text{CF}_3$, $-\text{OCF}_3$, $-\text{OCHF}_2$, y $-\text{CN}$. En otros aspectos específicos, X es $-\text{S}(\text{O})_2\text{R}^{11}$ en donde R^{11} es metilo o fenilo opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre C_{1-4} alquilo y halo; o con 1 o 2 sustituyentes seleccionados entre metilo, fluoro, y cloro.

En un aspecto, la invención provee un compuesto de fórmula (I) en donde R^1 es C_{3-4} alquilo; y

X se selecciona entre :

(a) $-\text{C}(\text{O})\text{OR}^2$ en donde R^2 es C_{1-3} alquilo o fenilo;

(b) $-\text{C}(\text{O})\text{R}^3$ en donde R^3 es fenilo, opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre C_{1-4} alquilo, halo, y $-\text{CF}_3$; furanilo; o tiofenilo;

(c) $-\text{C}(\text{O})\text{NR}^4\text{R}^5$ en donde R^4 es hidrógeno y R^5 es fenilo opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre C_{1-4} alquilo y halo;

Definición (d) $-\text{C}(\text{O})\text{C}(\text{R}^6\text{R}^7)\text{R}^8$ en donde R^6 es hidrógeno y R^7 es hidrógeno, $-\text{OH}$, o metilo; o R^6 y R^7 juntos forman oxo o $-(\text{CH}_2)_2-$; y R^8 es fenilo o ciclohexilo, en donde fenilo o ciclohexilo se sustituyen opcionalmente con 1 o 2 sustituyentes seleccionados entre C_{1-4} alquilo y halo;

(e) $-\text{C}(\text{O})\text{C}(\text{HR}^9)\text{OR}^{10}$ en donde R^9 es hidrógeno o metilo y R^{10} es fenilo opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre C_{1-4} alquilo y halo; y

(f) $-\text{S}(\text{O})_2\text{R}^{11}$ en donde R^{11} es metilo o fenilo, opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre C_{1-4} alquilo y halo.

La invención además provee un compuesto de fórmula (I) en donde:

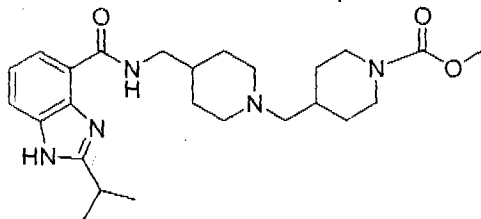
R¹ es isopropilo o *ter*-butilo; y

X se selecciona entre :

- (a) -C(O)OR² en donde R² es metilo o fenilo;
- (b) -C(O)R³ en donde R³ es fenilo, opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre metilo, cloro, fluoro, y -CF₃; furan-2-ilo; o tiofen-2-ilo; y
- (c) -C(O)NR⁴R⁵ en donde R⁴ es hidrógeno y R⁵ es fenilo opcionalmente sustituido con 1 fluoro o cloro.

En otros aspectos específicos, la invención provee los compuestos listados en los Ejemplos y en las Tablas I a IX a continuación.

La convención de nomenclatura química usada en la presente documentación se ilustra para el compuesto del Ejemplo 1:



que se designa metil éster de ácido 4-(4-[[[2-isopropil-1H-benzimidazol-4-carbonil)amino]metil]-piperidin-1-ilmetil)piperidin-1-carboxílico de acuerdo con el software AutoNom, proporcionado por MDL Information Systems, GmbH (Frankfurt, Alemania). Como alternativa, la estructura de anillos fusionados "benzimidazol" se denomina "benzimidazol". Tal como se los usa en la presente documentación, los dos términos son equivalentes.

Como se presenta a modo de ejemplo con los compuestos particulares indicados en las tablas más adelante, los compuestos de la invención pueden contener un centro quiral. Por consiguiente, la invención incluye mezclas

racémicas, estereoisómeros puros y mezclas enriquecidas en estereoisómeros de dichos isómeros, a menos que se indique de otra manera. Cuando se muestra un estereoisómero particular, los especialistas en la técnica comprenderán que puede haber cantidades menores de otros estereoisómeros en las composiciones de la invención a menos que se indique de otra manera, siempre que no se elimine ninguna utilidad de la composición como un todo por la presencia de dichos isómeros adicionales.

En un aspecto, la invención provee un compuesto seleccionado entre:

metiléster de ácido 4-(4-[(2-isopropil-1*H*-benzoimidazol-4-carbonil)-amino]metil)piperidin-1-ilmetil)piperidin-1-carboxílico;

feniléster de ácido 4-(4-[(2-isopropil-1*H*-benzoimidazol-4-carbonil)-amino]metil)piperidin-1-ilmetil)piperidin-1-carboxílico;

{1-[1-(2-clorobenzoil) piperidin-4-ilmetil] piperidin-4-ilmetil}amida de ácido 2-isopropil-1*H*-benzoimidazol-4-carboxílico;

{1-[1-(2,4-difluoro-benzoil)piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-isopropil-1*H*-benzoimidazol-4-carboxílico;

{1-[1-(furan-2-carbonil)-piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-isopropil-1*H*-benzoimidazol-4-carboxílico;

{1-[1-(tiofeno-2-carbonil)piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-isopropil-1*H*-benzoimidazol-4-carboxílico;

{1-[1-(2-fluoro-5-trifluorometilbenzoil)piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-isopropil-1*H*-benzoimidazol-4-carboxílico;

{1-[1-(2-fluoro-fenilcarbamoil)piperidin-4-ilmetil]piperidin-4-ilmetil}-amida de ácido 2-isopropil-1*H*-benzoimidazol-4-carboxílico;

metiléster de ácido 4-(4-[[[(2-*ter*-butil-1*H*-benzoimidazol-4-carbonil)-amino]-metil]piperidin-1-ilmetil]piperidin-1-carboxílico;

{1-[1-(2-fluoro-benzoil)-piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-*ter*-butil-1*H*-benzoimidazol-4-carboxílico;

{1-[1-(3-metil-benzoil)-piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-*ter*-butil-1*H*-benzoimidazol-4-carboxílico; y

{1-[1-(4-fluorobenzoil)-piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-*ter*-butil-1*H*-benzoimidazol-4-carboxílico.

Definiciones

Al describir los compuestos, composiciones y métodos de la invención, los siguientes términos tienen los siguientes significados, salvo que se indique lo contrario.

El término "alquilo" significa un grupo hidrocarbonado saturado monovalente que puede ser lineal o ramificado o combinaciones de los mismos. Salvo que se defina lo contrario, dichos grupos alquilo contienen típicamente entre 1 y 10 átomos de carbono. Algunos grupos alquilo representativos con, a modo de ejemplo, metilo (Me), etilo, *n*-propilo (*n*-Pr), isopropilo (*i*-Pr), *n*-butilo (*n*-Bu), *sec*-butilo, isobutilo, *ter*-butilo (*t*Bu), *n*-pentilo, *n*-hexilo, *n*-heptilo, *n*-octilo, *n*-nonilo, *n*-decilo y similares.

El término "alcoxilo" significa un grupo monovalente -o-alquilo, donde alquilo es como se ha definido. Algunos grupos alcoxilo representativos son, a modo de ejemplo, metoxilo, etoxilo, propoxilo, butoxilo y similares.

El término "cicloalquilo" hace referencia a un grupo carbocíclico saturado monovalente que puede ser monocíclico o multicíclico. A menos que se especifique de otro modo, dichos grupos cicloalquilo típicamente contienen

entre 3 y 10 átomos de carbono. Los grupos cicloalquilo representativos incluyen, a modo de ejemplo, ciclopropilo, ciclobutilo, ciclopentilo, ciclohexilo, cicloheptilo, ciclooctilo, y similares.

El término "halo" significa flúor, cloro, bromo o iodo.

El término "oxo" hace referencia a un átomo de oxígeno con un enlace doble ($=O$).

El término "compuesto" se refiere a un compuesto que fue preparado mediante síntesis o producido de cualquier otra manera, tal como por una vía metabólica.

El término "cantidad eficaz para terapia" significa una cantidad suficiente para efectuar un tratamiento cuando se administra a un paciente que necesite tratamiento.

El término "tratamiento" como se lo utiliza en la presente significa el tratamiento de una enfermedad, trastorno o afección clínica en un paciente, como por ejemplo un mamífero (particularmente un humano) que incluye:

- (a) prevenir que ocurra la enfermedad, trastorno o afección clínica, es decir, tratamiento profiláctico de un paciente;
- (b) mejorar la enfermedad, trastorno o afección clínica, es decir, eliminar o causar una regresión de la enfermedad, trastorno o afección clínica en un paciente;
- (c) suprimir la enfermedad, trastorno o afección clínica, es decir, demorar o detener el desarrollo de la enfermedad, trastorno o afección clínica en un paciente; o
- (d) aliviar los síntomas de la enfermedad, trastorno o afección clínica en un paciente.

El término "sal aceptable para el uso farmacéutico" hace referencia a una sal preparada a partir de un ácido o base que es aceptable para su administración a un paciente, tal como un mamífero. Tales sales se pueden derivar a partir de ácidos inorgánicos u orgánicos aceptables para el uso farmacéutico y a partir de bases aceptables para el uso farmacéutico. En general, las sales aceptables para el uso farmacéutico de los compuestos de la presente invención se preparan a partir de ácidos.

Las sales derivadas de ácidos aceptables para el uso farmacéutico incluyen, pero no taxativamente, ácido acético, adípico, bencensulfónico, benzoico, alcanforsulfónico, cítrico, etansulfónico, fumárico, glucónico, glutámico, bromhídrico, clorhídrico, láctico, maleico, málico, mandélico, metansulfónico, mícico, nítrico, pantoténico, fosfórico, succínico, sulfúrico, tartárico, p-toluensulfónico, xinafoico (ácido 1-hidroxi-2-naftoico), ácido naftalen-1,5-disulfónico y similares.

El término "solvato" significa un complejo o agregado formado por una o más moléculas de un soluto, es decir un compuesto de la invención o una sal aceptable para el uso farmacéutico del mismo, y una o más moléculas de un solvente. Dichos solvatos son típicamente sólidos cristalinos con una relación molar de soluto y solvente sustancialmente fija. Algunos solventes representativos son a modo de ejemplo, agua, metanol, etanol, isopropanol, ácido acético, y similares. Cuando el solvente es agua, el solvato formado es un hidrato.

Se apreciará que el término "o una sal o solvato de estereoisómero aceptable para el uso farmacéutico del mismo" pretende incluir todas las permutaciones de sales, solvatos y estereoisómeros, como por ejemplo un

solvato de una sal aceptable para el uso farmacéutico de un estereoisómero de un compuesto de fórmula (I).

El término "grupo protector de amino" significa un grupo protector adecuado para prevenir reacciones indeseadas en un nitrógeno de amino. Algunos grupos protectores de amino son, sin que esto constituya una limitación, formilo; grupos acilo, por ejemplo grupos alcanilo, como por ejemplo acetilo; grupos alcoxycarbonilo, como por ejemplo *ter*-butoxycarbonilo (Boc); grupos arilmetoxycarbonilo, como por ejemplo benciloxycarbonilo (Cbz) y 9-fluorenilmetoxycarbonilo (Fmoc); grupos arilmetilo, como por ejemplo bencilo (Bn), tritilo (Tr) y 1,1-di-(4'-metoxifenil)metilo; grupos sililo, como por ejemplo trimetilsililo (TMS) y *ter*-butildimetilsililo (TBDMS); y similares.

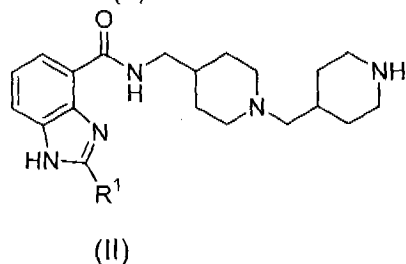
Procedimientos Generales de Síntesis

Los compuestos de la invención pueden prepararse a partir de materiales de partida disponibles utilizando los siguientes métodos y procesos generales. A pesar de que se ilustra un aspecto específico de la presente invención en los esquemas a continuación, los especialistas en la materia reconocerán que todos los aspectos de la presente invención pueden prepararse utilizando los métodos descritos en la presente o utilizando otros métodos, reactivos y materiales de partida conocidos por los especialistas en la materia. También se apreciará que cuando se dan condiciones de proceso preferidas o típicas (es decir, temperaturas de reacción, tiempos, relaciones molares de reactivos, solventes, presiones, etc.), también pueden utilizarse otras condiciones de proceso salvo que se especifique lo contrario. Las condiciones de reacción óptimas pueden variar con los reactivos o los solventes particulares usados, pero estas condiciones pueden ser

determinadas por aquél entrenado en la técnica utilizando procedimientos de optimización convencionales.

Adicionalmente, como será evidente para aquellos entrenados en la técnica, pueden ser necesarios grupos protectores convencionales para evitar que determinados grupos funcionales se vean sometidos a reacciones indeseadas. La elección de un grupo protector adecuado para un grupo funcional específico, así como condiciones adecuadas para la protección y desprotección, son bien conocidos en la especialidad. Por ejemplo, diversos grupos protectores, y su introducción y eliminación, se describen en T. W. Greene y G. M. Wuts, *Protecting Groups in Organic Synthesis*, Tercera Edición, Wiley, Nueva York, 1999, y las referencias allí citadas.

En un método de síntesis, los compuestos de fórmula (I) se preparan haciendo reaccionar un intermediario de piperidinilmetil-piperidinilmetilo de fórmula (II):



con un reactivo de fórmula (III):

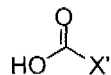


(III),

donde L es un grupo saliente, por ejemplo, un halógeno, tal como cloro, o un aciloxilo, un éster sulfónico, u oxisuccinimida, y R¹ y X son como se los define en la fórmula (I).

La reacción típicamente se realiza poniendo en contacto el intermediario (II) con entre aproximadamente 1 y aproximadamente 1,5 equivalentes de intermediario (III) en un diluyente aprótico polar, tal como diclorometano, en presencia de al menos un equivalente de una base de amina, tal como *N,N*-diisopropiletilamina. Los diluyentes inertes apropiados para este proceso y los que se describirán más adelante también incluyen *N,N*-dimetilformamida, triclorometano, 1,1,2,2-tetracloroetano, tetrahidrofurano, y semejantes. Las bases de amina apropiadas para los procesos de la presente invención también incluyen trietilamina, piridina, y semejantes. La reacción se lleva a cabo típicamente a una temperatura en un rango que comprende entre aproximadamente 0°C y aproximadamente 30°C durante aproximadamente un cuarto de hora a aproximadamente 2 horas, o hasta que se haya completado sustancialmente la reacción. Los ejemplos de reactivos L—X, donde L es cloro, incluyen cloroformato de metilo, cloroformato de fenilo, cloruro de clorobenzoilo y cloruro de metanosulfonilo.

En un método de síntesis alternativo, los compuestos de fórmula (I) donde X se selecciona entre $-C(O)R^3$, $-C(O)C(R^6R^7)R^8$ y $-C(O)C(HR^9)OR^{10}$ pueden prepararse por medio de la reacción de unión de amida de un intermediario de fórmula (II) con un ácido carboxílico de fórmula (IV):



(IV).

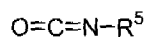
En la fórmula (IV), X' representa R^3 , $C(R^6R^7)R^8$ o $C(HR^9)OR^{10}$, de modo que $-C(O)X'$ corresponde a X, como se detalló en la fórmula (IV) anterior. En la reacción de unión de amida del intermediario (II), primero se ponen en contacto entre aproximadamente 1 y aproximadamente 1,5 equivalentes del ácido

10

carboxílico (IV) con entre 1 y aproximadamente 1,5 equivalentes de un agente de unión, tal como hexafluorofosfato de O-(7-axabenzotrizol-1-il)-*N,N,N',N'*-tetrametiluronio (HATU), en un solvente polar aprótico, tal como dimetilformamida o aquellos descritos con anterioridad. Después, la mezcla ácida se pone en contacto con el intermediario (II) en presencia de entre aproximadamente 2 y aproximadamente 4 equivalentes de una base de amina, por ejemplo, *N,N*-diisopropiletilamina. La reacción se lleva a cabo típicamente a una temperatura en un rango que comprende entre aproximadamente 0°C y aproximadamente 30°C durante aproximadamente un cuarto de hora a aproximadamente 2 horas, o hasta que se haya completado sustancialmente la reacción.

Los agentes de unión alternativos apropiados incluyen clorhidrato de *N*-etil-*N'*-(3-dimetilaminopropil)carbodiimida (EDC), 1,1'-carbonildiimidazol (CDI), 1,3 diciohexilcarbodiimida (DCC) y hexafluorofosfato de benzotriazol-1-iloxitripirrolidino-fosfonio (PyBop). Los agentes de unión pueden combinarse con agentes promotores, por ejemplo, 1-hidroxi-7-azabenzotriazol (HOAt), hidroxibenzotriazol (HOBt) o 1,4-diazabicyclo[2,2,2]octano (DABCO).

En aún otro proceso alternativo, los compuestos de fórmula (I) donde X es -C(O)NHR⁵ pueden prepararse haciendo reaccionar un intermediario de fórmula (II) con un isocianato de la siguiente forma:



(V).

La reacción típicamente se realiza poniendo en contacto el intermediario (II) con entre aproximadamente 1 y aproximadamente 1,5 equivalentes del intermediario (V) en un diluyente aprótico polar, en presencia de entre

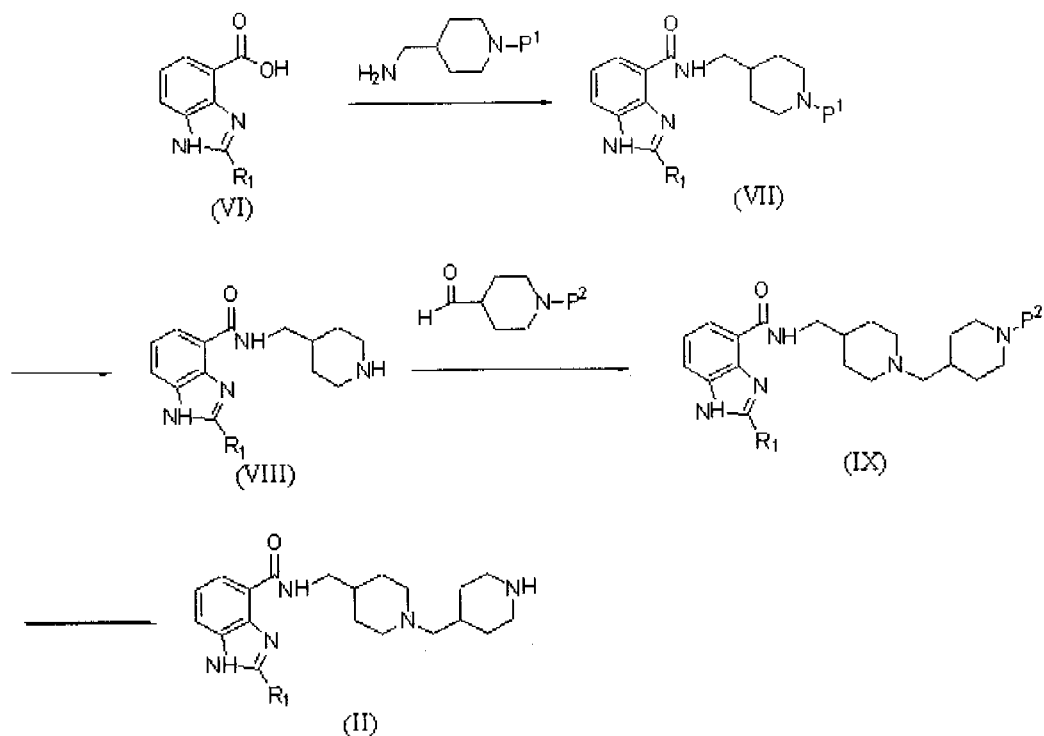
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aproximadamente 2 y aproximadamente 4 equivalentes de una base de amina. La reacción se lleva a cabo típicamente a una temperatura en un rango que comprende entre aproximadamente 0°C y aproximadamente 30°C durante aproximadamente un cuarto de hora a aproximadamente 24 horas, o hasta que se haya completado sustancialmente la reacción.

El producto de la fórmula (I) se aísla y purifica utilizando los procedimientos convencionales. Por ejemplo, el producto puede concentrarse hasta secarlo a presión reducida, y el residuo puede purificarse por cromatografía HPLC.

Los intermediarios de piperidinilmetil-piperidinilmetilo de fórmula (II) se preparan a partir de materiales iniciales disponibles comercialmente, de acuerdo con el procedimiento ilustrado en el Esquema A.

Esquema A



donde P^1 y P^2 independientemente representan un grupo protector de amino, tal como *ter*-butoxicarbonilo (Boc).

Primero se hace reaccionar un ácido carboxílico de fórmula (VI) con una piperidina protegida con aminometilo para formar un intermediario protegido de fórmula (VII). Esta reacción típicamente se realiza poniendo en contacto (VI) con entre aproximadamente 1 y aproximadamente 2 equivalentes de aminometilpiperidina protegida en un diluyente aprótico polar, en presencia de un agente de unión de amida descrito con anterioridad, por ejemplo, clorhidrato de *N*-etil-*N'*-(3-dimetilaminopropil)carbodiimida (EDC), en combinación con hidroxibenzotriazol (HOBt) o 1,1'-carbonyldiimidazol (CDI), en combinación con 1,4-diazabicyclo[2,2,2]octano (DABCO). La reacción típicamente se realiza a una temperatura en el rango de entre

aproximadamente 0°C y aproximadamente 60°C, por entre aproximadamente 1 y aproximadamente 24 horas, o hasta que la reacción está sustancialmente completa.

El grupo protector P¹ se elimina del intermediario (VII) por medios convencionales para proporcionar el intermediario (VIII). Por ejemplo, cuando se usa Boc como grupo protector, puede eliminárselo con un tratamiento con un ácido, tal como ácido trifluoroacético o ácido clorhídrico.

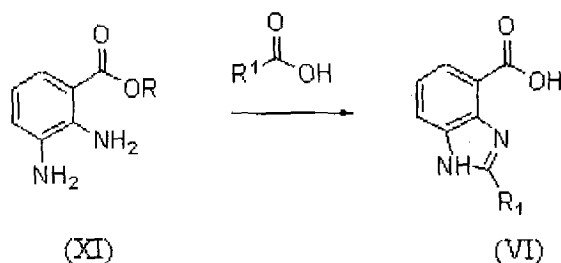
Después se forma un intermediario de fórmula (IX) mediante la aminación reductiva del intermediario (VIII) con un carboxaldehído protegido con piperidina. Esta reacción típicamente se realiza poniendo en contacto (VIII) con entre aproximadamente 1 y aproximadamente 2 equivalentes del carboxaldehído protegido con piperidina en un diluyente inerte, en presencia de entre aproximadamente 1 y aproximadamente 2 equivalentes de un agente reductor. Opcionalmente, puede incluirse aproximadamente un equivalente de un ácido débil, tal como ácido acético, para acelerar la reacción. La reacción puede efectuarse a una temperatura de entre aproximadamente 0°C y aproximadamente 30°C, típicamente entre aproximadamente 20°C y aproximadamente 30°C, por entre aproximadamente 0,25 y aproximadamente 2 horas, o hasta que la reacción está sustancialmente completa.

Los diluyentes inertes adecuados incluyen diclorometano, triclorometano, 1,1,2,2-tetracloroetano y semejantes. Los agentes reductores típicos incluyen triacetoxiborohidruro de sodio, borohidruro de sodio y cianoborohidruro de sodio. El producto (IX) se aísla con procedimientos convencionales. Cuando la amina (VIII) se proporciona como una sal ácida, en la reacción típicamente se incluyen entre aproximadamente 1 y

aproximadamente 3 equivalentes de una base de amina, tal como *N,N*-diisopropiletilamina. Finalmente, el grupo protector P^2 se elimina del intermediario (IX) de acuerdo con procedimientos convencionales, para proporcionar el intermediario de piperidinilmetil-piperidinilmetilo (II).

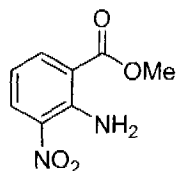
Un ácido carboxílico A de fórmula (VI) puede prepararse a partir de un ácido diaminobenzoico o un éster de acuerdo con el proceso ilustrado en el Esquema B:

Esquema B



donde R representa metilo o hidrógeno. El intermediario (XI) se hace reaccionar con un ácido carboxílico $R^1C(O)OH$ para formar el intermediario ácido (VI). Esta reacción típicamente se realiza poniendo en contacto el ácido o el éster (XI) con entre aproximadamente 2 y aproximadamente 4 equivalentes del ácido carboxílico $R^1C(O)OH$ en una solución ácida acuosa. La reacción típicamente se realiza a una temperatura en el rango de entre aproximadamente 80°C y aproximadamente 100°C, por entre aproximadamente 12 y aproximadamente 72 horas. Luego se incrementa el pH de la solución agregando una base, tal como hidróxido de sodio, y el producto se aísla por medios convencionales.

En un proceso conveniente para proporcionar el intermediario (XI) como éster de metilo se usa metil éster de ácido 2-amino-3-nitrobenzoico (X):

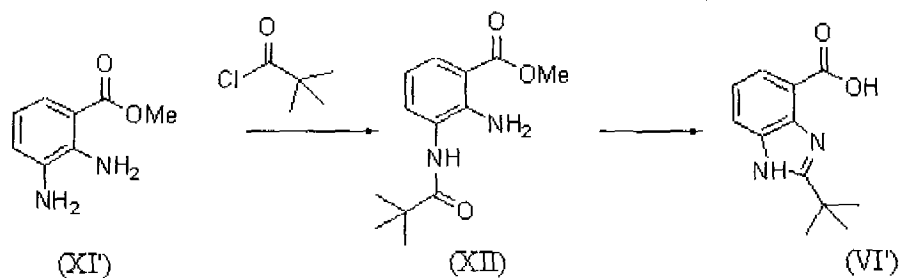


(X)

como material inicial. Típicamente se disuelve metil éster de ácido 2-amino-3-nitrobenzoico (X) en un diluyente polar, y se lo reduce exponiéndolo a una atmósfera de hidrógeno en presencia de un catalizador de metal de transición, para proporcionar el metil éster de ácido diaminobenzoico (XI). La reacción tiene lugar típicamente a temperatura ambiente durante aproximadamente 12 a aproximadamente 72 horas.

Cuando el sustituyente R^1 presenta impedimentos estéricos, tal como por ejemplo, cuando R^1 es *ter*-butilo, el ácido *ter*-butilbenzimidazol carboxílico (VI') puede prepararse convirtiendo el éster de metilo (XI') en un proceso de dos pasos, como se ilustra, por ejemplo, en el Esquema C:

Esquema C

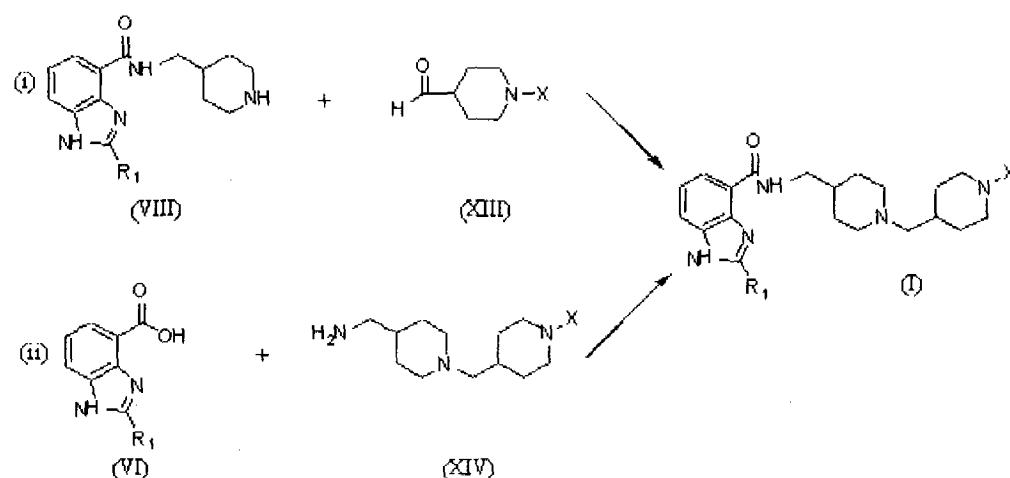


Como se describe en detalle en la preparación 3 más adelante, el éster de metilo (XI') se hace reaccionar primero con cloruro de 2,2-dimetilpropionilo para proporcionar el intermediario (XII), que se calienta a reflujo en una solución ácida fuerte, típicamente por entre aproximadamente 12 y

aproximadamente 72 horas, para proporcionar el ácido *ter*-butilbenzimidazol carboxílico (VI').

En métodos de síntesis alternativos, los compuestos de fórmula (I) pueden prepararse de acuerdo con las rutas de procesamiento ilustradas en el Esquema D, usando la aminación reductiva y otras reacciones descriptas con anterioridad, y/o usando reacciones alternativas bien conocidas por aquellos entrenados en la técnica.

Esquema D

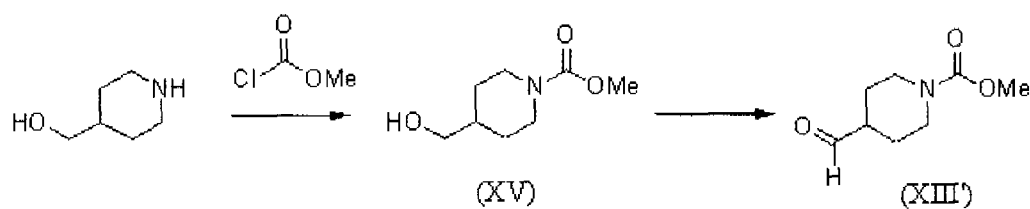


Como se ilustra en la ruta de procesamiento (i), un intermediario de fórmula (VIII) se hace reaccionar con un intermediario de fórmula (XIII) para proporcionar un compuesto de fórmula (I). La reacción típicamente se efectúa bajo las condiciones descriptas con anterioridad para la reacción de la amina (VIII) con el carboxaldehído protegido con piperidina en el Esquema A.

El intermediario (XIII) puede prepararse mediante la reacción de 4-hidroxi metilpiperidina con un reactivo L—X de fórmula (III), a lo que sigue la oxidación del intermediario resultante. Por ejemplo, para el caso particular

donde X es $-\text{C}(\text{O})\text{OCH}_3$, el intermediario (XIII) puede prepararse como se indica en el Esquema E.

Esquema E

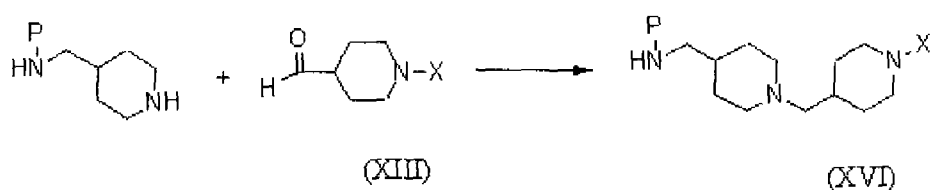


Primero se hace reaccionar 4-hidroximetilpiperidina con metilcloroformato para formar el intermediario de hidroximetilpiperidina (XV). La reacción típicamente se realiza poniendo en contacto 4-hidroximetilpiperidina en una solución acuosa con entre aproximadamente 3 y aproximadamente 5 equivalentes de metilcloroformato, en presencia de entre aproximadamente 3 y aproximadamente 5 equivalentes de base. La reacción típicamente se realiza a una temperatura en el rango de entre aproximadamente 0°C y aproximadamente 30°C , por entre aproximadamente 12 y aproximadamente 72 horas, o hasta que la reacción está sustancialmente completa. Después, el intermediario (XV) se oxida para formar el intermediario de formilpiperidinilo (XIII'). En la reacción de oxidación típicamente se usa un reactivo de oxidación, tal como una combinación de cloruro de oxalio y dimetilsulfóxido (oxidación de Swern), un reactivo de cromato, tal como clorocromato de piridinio, o un agente oxidante, tal como hipoclorito de sodio, junto con un catalizador, tal como el radical libre 2,2,6,6-tetrametil-1-piperidiniloxilo (TEMPO).

La preparación de un compuesto de fórmula (I) de acuerdo con la ruta de procesamiento (i) del Esquema D usando el intermediario (XIII') se describe en los Ejemplos 214 y 216 más adelante.

Un compuesto de fórmula (I) también puede prepararse haciendo reaccionar un ácido carboxílico de fórmula (VI) con un intermediario de fórmula (XIV), como se ilustra en la ruta de procesamiento (ii). Como se ilustra en el Esquema F, el intermediario (XIV) pueden prepararse haciendo reaccionar una aminometilpiperidina protegida con el intermediario (XIII):

Esquema F



donde P es un grupo protector de amino, para proporcionar un intermediario protegido de fórmula (XVI), a lo que sigue un paso de desprotección. La preparación de los compuestos de acuerdo con la ruta de procesamiento (ii) se describe en la Preparación 4 y los Ejemplos 14 y 15 más adelante.

Los reactivos $L-X$ (III), $X'C(O)OH$ (IV), $O=C=N=R^5$ (V) y $R^1C(O)OH$ están disponibles comercialmente o se preparan fácilmente a partir de materiales iniciales comunes, de acuerdo con procedimientos convencionales.

Otros detalles con respecto a las condiciones de reacción específicas y otros procedimientos para preparar compuestos representativos de la invención, o intermediarios de los mismos, se describirán más adelante en los ejemplos.

Por consiguiente, en un aspecto del método, en la invención se provee un proceso para preparar un compuesto de fórmula (I), o una sal o un estereoisómero de éste, donde el proceso comprende (a) hacer reaccionar un compuesto de fórmula (II) con un compuesto de fórmula (III); (b) hacer

reaccionar un compuesto formula (VIII) con un compuesto de fórmula (XIII); o
(c) hacer reaccionar un compuesto de fórmula (VI) con un compuesto de
fórmula (XIV), para proporcionar un compuesto de fórmula (I), o una sal o un
estereoisómero de éste.

En un aspecto adicional del método, en la invención se provee un
proceso para preparar un compuesto de fórmula (I) donde X se selecciona
entre $-C(O)R^3$, $-C(O)C(R^6R^7)R^8$ y $-C(O)C(HR^9)OR^{10}$, o una sal o un
estereoisómero de éste, donde el proceso comprende hacer reaccionar un
compuesto de fórmula (II) con un compuesto de fórmula (IV), donde X'
representa R^3 , $C(R^6R^7)R^8$ o $C(HR^9)OR^{10}$, para proporcionar un compuesto de
fórmula (I), o una sal o un estereoisómero de éste.

Además, en la invención se provee un compuesto de fórmula (II), o una
sal o un estereoisómero o un derivado protegido de éste, donde R^1 es como se
lo define en la fórmula (I).

Formas cristalinas

En otro aspecto, en la invención se provee metil éster de ácido 4-(4-(((2-isopropil-1H-benzoimidazol-4-carbonil)amino]metil)-piperidin-1-ilmetil)piperidin-1-carboxílico en forma de base libre cristalina o solvato. Se han observado tres formas distinguibles de metil éster de ácido 4-(4-(((2-isopropil-1H-benzoimidazol-4-carbonil)amino]metil)-piperidin-1-ilmetil)piperidin-1-carboxílico cristalino (conocido como compuesto **1** de aquí en adelante).

La Forma cristalina I de la presente invención es una base libre cristalina. La Forma I se caracteriza por un patrón de difracción de rayos X en polvo (PXRD) que tiene dos o más picos de difracción en valores de 2θ seleccionados entre $15,08 \pm 0,20$, $15,41 \pm 0,20$, $19,00 \pm 0,20$, $19,70 \pm 0,20$ y

23,68 $\pm$ 0,20. En particular, la Forma I se caracteriza por un patrón de difracción de rayos X en polvo que tiene picos de difracción en valores de 2θ de 19,00 $\pm$ 0,20 y 19,70 $\pm$ 0,20.

Como es bien conocido en el campo de la difracción de rayos X en polvo, las posiciones de los picos en los espectros de PXRD son relativamente menos sensibles a los detalles experimentales, tales como los detalles de la preparación de la muestra y la geometría de los instrumentos, que las alturas de los picos. Por ende, en un aspecto, la Forma cristalina I se caracteriza por un patrón de difracción de rayos X en polvo donde las posiciones de los picos están sustancialmente de acuerdo con las indicadas en la Figura 1.

La Forma I ha sido caracterizada adicionalmente por análisis de difracción de rayos X de la estructura cristalina que proporcionan los siguientes parámetros de la red: la celda individual es ortorrómbica, con dimensiones $a = 16,9053 \text{ \AA}$, $b = 9,5172 \text{ \AA}$, $c = 15,4659 \text{ \AA}$; el espacio de grupo es $Pna2_1$; la densidad calculada es de $1,22 \text{ g/cm}^3$. La estructura molecular resultante confirma que la composición química es la del compuesto 1, y que la unidad asimétrica no contiene agua u otras moléculas de solvente. Los picos de difracción de rayos X en polvo predichos a partir de las posiciones atómicas derivadas concuerdan en gran medida con los resultados observados.

La Forma cristalina I de la presente invención también se caracteriza por su estabilidad térmica a temperatura elevada, como lo evidencia su perfil de calorimetría diferencial de barrido (DSC), que presenta un pico de flujo de calor endotérmico en el rango de entre aproximadamente 145°C y aproximadamente 155°C, típicamente entre aproximadamente 146 y 148°C, como se ilustra en la Figura 2, que puede identificarse como un punto de fusión. Además, el perfil de

análisis gravimétrico térmico (TGA) no presenta un suceso de cambio de peso significativo antes del inicio de la degradación, que ocurre a más de aproximadamente 240°C.

En aún otro aspecto, la presente forma cristalina se caracteriza por su espectro de absorción infrarroja, que presenta bandas de absorción significativas a aproximadamente 766, 1097, 1251, 1413, 1449, 1579, 1609, 1640 y 1696 cm^{-1} .

Se ha demostrado que la presente forma cristalina tiene un perfil de absorción/desorción reversible, con un nivel de higroscopicidad excepcionalmente bajo (es decir, una ganancia de peso de menos de aproximadamente 0,25% en el rango de humedad de entre 2% de humedad relativa y 90% de humedad relativa a temperatura ambiente), como se ilustra en la Figura 3.

Adicionalmente, se ha descubierto que la Forma cristalina I del compuesto 1 es estable al exponerla a una temperatura y una humedad elevada. Después de almacenarla por tres meses a 40°C y 75% de humedad relativa, no hubo cambios detectables en los perfiles de DSC, TGA o PXRD, en la pureza química determinada con un análisis de HPLC, y no se observaron cambios en su apariencia visual. Tampoco se detectaron cambios por DSC, TGA o PXRD después de triturar las partículas de la Forma I, desde un tamaño de partícula promedio basado en volumen de aproximadamente 470 micrones hasta un tamaño de partícula promedio basado en volumen de aproximadamente 15, aproximadamente 22 o aproximadamente 29 micrones.

La Forma cristalina II del compuesto 1 se caracteriza por los perfiles de DSC y TGA de la Figura 4. El análisis TGA indica un inicio de pérdida de peso

en el rango de temperatura de entre aproximadamente 95 y aproximadamente 105°C , típicamente a aproximadamente 100°C, en un perfil de pasos consistente con la pérdida de agua o solvente, mientras que el perfil de DSC presenta un pico en el flujo de calor endotérmico a entre aproximadamente 143 y aproximadamente 145°C, concurrente con el suceso de fusión. El patrón de PXRD de la Forma II es indistinguible del de la Forma I. Si bien no se ha realizado una identificación positiva, el perfil de TGA de la Forma II es consistente con la interpretación de la Forma II como solvato.

La tercera forma cristalina de la invención ha sido identificada como un monohidrato. La Forma III se caracteriza por un patrón de difracción de rayos X en polvo (PXRD) que tiene dos o más picos de difracción en valores de 2θ seleccionados entre $9,14 \pm 0,20$, $12,41 \pm 0,20$, $12,74 \pm 0,20$, $17,75 \pm 0,20$, $18,47 \pm 0,20$, $20,63 \pm 0,20$, $21,13 \pm 0,20$ y $27,05 \pm 0,20$, como se ilustra en la Figura 5. En particular, la Forma III se caracteriza por un patrón de difracción de rayos X en polvo que tiene picos de difracción en valores de 2θ de $9,14 \pm 0,20$ y $20,63 \pm 0,20$.

Los perfiles de DSC y TGA de la Forma III, indicados en la Figura 6, demuestran que el material tiene un perfil de pérdida de peso en pasos que comienza a una temperatura en el rango de entre aproximadamente 65 y aproximadamente 75°C, típicamente a aproximadamente 70°C, y presenta un pico de flujo de calor endotérmico a una temperatura entre aproximadamente 90 y aproximadamente 100°C, consistente con la pérdida de agua del monohidrato y la fusión subsiguiente. Además, la Forma III se caracterizó con un análisis de difracción de rayos X de su estructura cristalina con los siguientes parámetros de red: la celda individual es monoclinica, con

dimensiones $a = 14,8101 \text{ \AA}$, $b = 9,9985 \text{ \AA}$, $c = 17,9222 \text{ \AA}$; $\beta = 106,3020^\circ$, el espacio de grupo es $P2_1/n$; la densidad calculada es de $1,23 \text{ g/cm}^3$. La estructura molecular resultante confirma que la composición química es la del compuesto 1, y que la unidad asimétrica contiene una sola molécula de agua.

Las formas cristalinas separadas de la presente invención pueden obtenerse por medios reproducibles poniendo en práctica los siguientes procedimientos. La Forma cristalina I puede prepararse dispersando el compuesto 1 en un diluyente inerte seleccionado entre acetonitrilo, éter, ciclohexano y acetato de etilo, en una proporción de entre aproximadamente 15 mg y aproximadamente 25 mg de compuesto 1 por mililitro de diluyente, para formar una mezcla, y permitiendo que la mezcla se evapore a temperatura ambiente para obtener la formación de cristales.

Como alternativa, la Forma I puede obtenerse con un proceso de intercambio de solventes entre el compuesto 1 en bruto en solución, sin aislar primero el compuesto 1 amorfo, como se describe en el Ejemplo 216 más adelante. Típicamente, la reacción para preparar el compuesto 1 se realiza en un diluyente aprótico polar, tal como diclorometano, donde el compuesto es muy soluble. Para preparar la forma cristalina I, se agrega acetonitrilo mientras se destila al vacío el producto de reacción en bruto para eliminar el diclorometano. Se prepara una mezcla que tiene entre aproximadamente 1 y aproximadamente 200 mg de compuesto 1 por mililitro de acetonitrilo, típicamente entre aproximadamente 50 y aproximadamente 125 mg de compuesto 1 por mililitro de acetonitrilo, a partir del residuo remanente después de la destilación, y se la calienta a una temperatura de aproximadamente 75°C . Después, la mezcla se enfría a una temperatura no mayor que

aproximadamente 20°C para proporcionar la forma cristalina I, que se aísla de acuerdo con procedimientos convencionales. En un ejemplo de un proceso, la mezcla se enfría hasta que ocurre la nucleación; típicamente a una temperatura de entre aproximadamente 55 y aproximadamente 65°C, y se mantiene a dicha temperatura por aproximadamente una hora. Luego se enfría a una velocidad de entre aproximadamente 0,25 y aproximadamente 0,4°C por minuto, hasta una temperatura de aproximadamente 20°C. Para incrementar el rendimiento de la forma cristalina I, la mezcla puede enfriarse adicionalmente a una velocidad de entre aproximadamente 0,5 y aproximadamente 0,75°C por minuto, hasta una temperatura de entre aproximadamente 0 y aproximadamente 5°C.

Para preparar la Forma II, el compuesto 1 amorfo se dispersa en hexano a temperatura ambiente hasta una concentración final de aproximadamente 10 mg/ml, y la mezcla resultante se sonica. Después de aproximadamente 24 horas a temperatura ambiente, se obtienen sólidos cristalinos de la Forma II.

La Forma III se prepara disolviendo el compuesto 1 amorfo en una mezcla de solventes de 1:1 de etanol: agua, a temperatura ambiente, hasta una concentración final de aproximadamente 20 mg/ml, y sonicando la solución por aproximadamente 30 segundos. La solución se deja evaporar parcialmente en un recipiente sin tapa. Después de aproximadamente 24 horas, se obtienen sólidos cristalinos de la Forma III.

Composiciones farmacéuticas

Los compuestos de benzimidazol-carboxamida de esta invención se administran típicamente al paciente bajo la forma de una composición farmacéutica. Dichas composiciones farmacéuticas pueden administrarse al

paciente a través de cualquier ruta de administración aceptable, incluyendo, sin limitaciones, modos de administración oral, rectal, vaginal, nasal, por inhalación, tópica (incluyendo transdérmica) y parenteral.

Por consiguiente, en uno de los aspectos de la composición, esta invención se dirige a una composición farmacéutica que comprende un vehículo o excipiente aceptable para el uso farmacéutico, y una cantidad efectiva para el uso terapéutico de un compuesto de fórmula (I), o una sal o solvato del mismo aceptable para el uso farmacéutico. Opcionalmente, dichas composiciones farmacéuticas pueden contener otros agentes terapéuticos y/o de formulación, si se lo desea.

Las composiciones farmacéuticas de la invención típicamente contienen una cantidad eficaz para uso terapéutico de un compuesto de la presente invención o una sal aceptable para el uso farmacéutico del mismo. Típicamente, estas composiciones farmacéuticas contendrán entre aproximadamente 0,1 y aproximadamente 95% en peso del agente activo; incluyendo entre aproximadamente 5 y aproximadamente 70% en peso; y entre aproximadamente 10 y aproximadamente 60% en peso del agente activo.

Puede usarse cualquier vehículo o excipiente convencional en las composiciones farmacéuticas de la invención. La selección de un vehículo o excipiente particular, o de combinaciones de vehículos o excipientes, dependerá del modo de administración usado para tratar un paciente, o del tipo de condición médica o estado de enfermedad particular. Respecto de esto, la preparación de una composición farmacéutica adecuada para un modo de administración particular está dentro del alcance de los especialistas en la técnica farmacéutica. Adicionalmente, los ingredientes para dichas

composiciones están disponibles comercialmente, por ejemplo, en Sigma, P.O. Box 14508, St. Louis, MO 63178. A modo de ilustración adicional, se describen técnicas convencionales de formulación en *Remington: The Science and Practice of Pharmacy*, 20ª edición, Lippincott Williams & White, Baltimore, Maryland (2000); y H.C. Ansel et al., *Pharmaceutical Dosage Forms and Drug Delivery Systems*, 7ª edición, Lippincott Williams & White, Baltimore, Maryland (1999).

Los ejemplos representativos de materiales que pueden servir como vehículos aceptables para el uso farmacéutico incluyen, sin limitaciones, los siguientes: (1) azúcares, tales como lactosa, glucosa y sacarosa; (2) almidones, tales como almidón de maíz y almidón de papa; (3) celulosa y sus derivados, tales como carboximetilcelulosa de sodio, etil celulosa y acetato de celulosa; (4) tragacanto en polvo; (5) malta; (6) gelatina; (7) talco; (8) excipientes, tales como manteca de cacao y ceras para supositorios; (9) aceites, tales como aceite de maní, aceite de semilla de algodón, aceite de cártamo, aceite de sésamo, aceite de oliva, aceite de maíz y aceite de soja; (10) glicoles, tales como propilenglicol; (11) polioles, tales como glicerina, sorbitol, manitol y polietilenglicol; (12) ésteres, tales como oleato de etilo y laurato de etilo; (13) agar; (14) agentes amortiguadores, tales como hidróxido de magnesio e hidróxido de aluminio; (15) ácido alginico; (16) agua libre de pirógenos; (17) solución salina isotónica; (18) solución de Ringer; (19) alcohol etílico; (20) soluciones amortiguadoras de fosfato; y (21) otras sustancias no tóxicas compatibles empleadas en composiciones farmacéuticas.

Las composiciones farmacéuticas de la invención se preparan típicamente mezclando o combinando de forma exhaustiva e íntima un

compuesto de la invención con un vehículo aceptable para el uso farmacéutico y uno o más ingredientes opcionales. Si resulta necesario o se lo desea, puede luego moldearse la mezcla uniforme combinada resultante, o puede cargársela en tabletas, cápsulas, píldoras y semejantes usando procedimientos y equipos convencionales.

Las composiciones farmacéuticas de la invención preferentemente se envasan en formas de dosificación unitaria. El término "forma de dosificación unitaria" hace referencia a una unidad físicamente discreta adecuada para administrar una dosis a un paciente, es decir, que cada unidad contiene una cantidad predeterminada de agente activo calculada para producir el efecto terapéutico deseado, ya sea solo o en combinación con una o más unidades adicionales. Por ejemplo, dichas formas de dosificación unitaria pueden ser cápsulas, comprimidos, píldoras y similares.

En una realización preferida, las composiciones farmacéuticas de la invención son apropiadas para una administración oral. Las composiciones farmacéuticas apropiadas para administración oral pueden tomar la forma de cápsulas, tabletas, píldoras, pastillas, sachés, grageas, polvos, gránulos; o como una solución o una suspensión en un líquido acuoso o no acuoso; o como emulsiones líquidas de aceite en agua o agua en aceite; o como elixires o jarabes; y semejantes; donde cada uno contiene una cantidad predeterminada de un compuesto de la presente invención como ingrediente activo.

Cuando se desee una administración oral en una forma de dosificación sólida (es decir, como cápsulas, tabletas, píldoras y semejantes), las composiciones farmacéuticas de la invención comprenderán típicamente un

compuesto de la presente invención, como ingrediente activo, y uno o más vehículos aceptables para el uso farmacéutico, tales como citrato de sodio o fosfato dicálcico. Opcionalmente o como alternativa, dichas formas de dosificación sólidas pueden comprender también: (1) rellenos o extensores, tales como almidones, lactosa, sacarosa, glucosa, manitol y/o ácido silícico; (2) aglutinantes, tales como carboximetilcelulosa, alginatos, gelatina, polivinilpirrolidona, sacarosa y/o acacia; (3) humectantes, tales como glicerol; (4) agentes desintegradores, tales como agar-agar, carbonato de calcio, almidón de papa o tapioca, ácido alginico, determinados silicatos, y/o carbonato de sodio; (5) agentes que demoran la solubilización, tales como parafina; (6) aceleradores de la absorción, tales como compuestos de amonio cuaternario; (7) agentes humectantes, tales como alcohol cetílico y/o monoestearato de glicerol; (8) absorbentes, tales como caolín y/o arcilla bentonita; (9) lubricantes, tales como talco, estearato de calcio, estearato de magnesio, polietilenglicoles sólidos, lauril sulfato de sodio y/o mezclas de los mismos; (10) agentes colorantes; y (11) agentes amortiguadores.

También puede haber agentes de liberación, agentes humectantes, agentes de recubrimiento, edulcorantes, agentes saborizantes y perfumantes, conservantes y antioxidantes presentes en las composiciones farmacéuticas de esta invención. Los ejemplos de antioxidantes aceptables para el uso farmacéutico incluyen: (1) antioxidantes solubles en agua, tales como ácido ascórbico, clorhidrato de cisteína, bisulfato de sodio, metabisulfato de sodio, sulfito de sodio y semejantes; (2) antioxidantes solubles en aceite, tales como palmitato de ascorbilo, hidroxianisol butilado (BHA), hidroxitolueno butilado (BHT), lecitina, galato de propilo, alfa-tocoferol y semejantes; y (3) agentes

quelantes de metales, tales como ácido cítrico, ácido etilendiaminotetraacético (EDTA), sorbitol, ácido tartárico, ácido fosfórico y semejantes. Los agentes de recubrimiento para tabletas, cápsulas, píldoras y semejantes incluyen aquellos usados para recubrimientos entéricos, tales como ftalato de acetato de celulosa (CAP), ftalato de acetato de polivinilo (PVAP), ftalato de hidroxipropil metilcelulosa, copolímeros de éster de ácido metacrílico, trimetilato de acetato de celulosa (CAT), carboximetiletilcelulosa (CMEC), succinato de acetato de hidroxipropil metil celulosa (HPMCAS), y semejantes.

Si se lo desea, las composiciones farmacéuticas de la presente invención también pueden formularse para obtener la liberación lenta o controlada del ingrediente activo usando, a modo de ejemplo, hidroxipropil metil celulosa en proporciones variables; u otras matrices de polímeros, liposomas y/o microesferas.

Además, las composiciones farmacéuticas de la presente invención pueden contener opcionalmente agentes opacantes y pueden formularse de modo que liberen el ingrediente activo solamente, o con preferencia, en una determinada porción del tracto gastrointestinal, opcionalmente de una manera demorada. Los ejemplos de composiciones de embebido que pueden usarse incluyen sustancias poliméricas y ceras. El ingrediente activo también puede estar en una forma micro-encapsulada, si resulta apropiado, con uno o más de los excipientes descritos previamente.

Las formas de dosificación líquidas para administración oral incluyen, a modo de ilustración, emulsiones, microemulsiones, soluciones, suspensiones, jarabes y elixires aceptables para el uso farmacéutico. Dichas formas de dosificación líquidas comprenden típicamente el ingrediente activo y un

diluyente inerte, tal como, por ejemplo, agua u otros solventes, agentes solubilizadores y emulsionantes, tales como alcohol etílico, alcohol isopropílico, carbonato de etilo, acetato de etilo, alcohol bencílico, benzoato de bencilo, propilenglicol, 1,3-butilenglicol, aceites (especialmente aceite de semilla de algodón, maní, maíz, germen, oliva, castor y sésamo), glicerol, alcohol tetrahidrofurílico, polietilenglicoles y ésteres de ácidos grasos de sorbitán, y mezclas de los mismos. Las suspensiones, además del ingrediente activo, pueden contener agentes de suspensión, tales como, por ejemplo, alcoholes isoestearílicos etoxilados, polioxietilen sorbitol y ésteres de sorbitán, celulosa microcristalina, metahidróxido de aluminio, bentonita, agar-agar y tragacanto, y mezclas de los mismos.

Como alternativa, las composiciones farmacéuticas de la invención se pueden formular para una administración por inhalación. Las composiciones farmacéuticas adecuadas para administración por inhalación tomarán típicamente la forma de un aerosol o un polvo. Dichas composiciones se administran generalmente usando dispositivos de administración bien conocidos, tales como un nebulizador de inhalación, un inhalador de dosis medidas, un inhalador de polvo seco o un dispositivo de administración similar.

Cuando se las administra por inhalación usando un recipiente presurizado, las composiciones farmacéuticas de la invención comprenden típicamente el ingrediente activo y un propelente apropiado, tal como diclorodifluorometano, triclorofluorometano, diclorotetrafluoroetano, dióxido de carbono u otro gas apropiado.

Además, la composición farmacéutica puede tomar la forma de una cápsula o un cartucho (por ejemplo, de gelatina) que comprende un compuesto

de la invención y un polvo apropiado para usar en un inhalador de polvo. Las bases de polvo apropiadas incluyen, a modo de ejemplo, lactosa o almidón.

Los compuestos de la invención también pueden administrarse por vía transdérmica, usando sistemas de administración transdérmica y excipientes conocidos. Por ejemplo, puede mezclarse un compuesto de la invención con potenciadores de la permeabilidad, tales como propilenglicol, monolaurato de polietilenglicol, azacicloalcan-2-onas y semejantes, e incorporarse en un parche o un sistema de administración similar. Pueden usarse excipientes adicionales, incluyendo agentes gelificantes, emulsionantes y amortiguadores, en dichas composiciones transdérmicas, si se lo desea.

Las siguientes formulaciones ilustran composiciones farmacéuticas representativas de la presente invención:

Ejemplo de formulación A

Se preparan cápsulas de gelatina dura para administración oral como se indica a continuación:

Ingredientes	Cantidad
Compuesto de la invención	50 mg
Lactosa (secada por pulverización)	200 mg
Esterarato de magnesio	10 mg

Procedimiento representativo: Los ingredientes se mezclan exhaustivamente y luego se cargan en una cápsula de gelatina dura (260 mg de composición por cápsula).

Ejemplo de formulación B

Se preparan cápsulas de gelatina dura para administración oral como se indica a continuación:

Ingredientes	Cantidad
Compuesto de la invención	20 mg
Almidón	89 mg
Celulosa microcristalina	89 mg
Esterarato de magnesio	2 mg

Procedimiento representativo: Los ingredientes se mezclan exhaustivamente y luego se hacen pasar por un tamiz U.S. de malla N° 45 y se cargan en una cápsula de gelatina dura (200 mg de composición por cápsula).

Ejemplo de formulación C

Se preparan cápsulas para administración oral de la siguiente forma:

Ingredientes	Cantidad
Compuesto de la invención	10 mg
Polioxietilen sorbitán monooleato	50 mg
Polvo de almidón	250 mg

Procedimiento representativo: Los ingredientes se mezclan exhaustivamente y luego se cargan en una cápsula de gelatina (310 mg de composición por cápsula).

Ejemplo de formulación D

Se preparan tabletas para administración oral como se indica a continuación:

Ingredientes	Cantidad
Compuesto de la invención	5 mg
Almidón	50 mg
Celulosa microcristalina	35 mg
Polivinilpirrolidona (10% en peso en agua)	4 mg
Carboximetil almidón de sodio	4,5 mg
Esterato de magnesio	0,5 mg
Talco	1 mg

Procedimiento representativo: Se hace pasar el ingrediente activo, el almidón y la celulosa a través de un tamiz US malla N° 45 y se mezcla exhaustivamente. La solución de polivinilpirrolidona se mezcla con los polvos resultantes, y esta mezcla se hace pasar posteriormente a través de un tamiz U.S. de malla N° 14. Los gránulos producidos de este modo se secan a 50-60°C y se hacen pasar a través de un tamiz U.S. de malla N° 18. Después se agrega el carboximetil almidón de sodio, el estearato de magnesio y el talco (previamente filtrado a través de un tamiz U.S. de malla N° 60) a los gránulos. Después de mezclar, se

comprime la mezcla en una máquina tableteadora para obtener una tableta con un peso de 100 mg.

Ejemplo de formulación E

Se preparan tabletas para administración oral como se indica a continuación:

Ingredientes	Cantidad
Compuesto de la invención	25 mg
Celulosa microcristalina	400 mg
Dióxido de silicio ahumado	10 mg
Ácido esteárico	5 mg

Procedimiento representativo: Los ingredientes se mezclan exhaustivamente luego se comprimen para formar comprimidos (440 mg de composición por comprimido).

Ejemplo de formulación F

Se preparan tabletas monorranuradas para administración oral de la siguiente forma:

Ingredientes	Cantidad
Compuesto de la invención	15 mg
Almidón de maíz	50 mg
Croscarmelosa sódica	25 mg
Lactosa	120 mg
Esterarato de magnesio	5 mg

Procedimiento representativo: Los ingredientes se mezclan exhaustivamente y se comprimen para formar un comprimido monorranurado (215 mg de composición por comprimido).

Ejemplo de formulación G

Se prepara una suspensión para administración oral de la siguiente forma:

Ingredientes Cantidad

Compuesto de la invención	0,1 g
Ácido fumárico	0,5 g
Cloruro de sodio	2,0 g
Metil parabeno	0,15 g
Propil parabeno	0,05 g
Azúcar granulada	25,5 g
Sorbitol (solución al 70%)	12,85 g
Veegum k (Vanderbilt Co.)	1,0 g
Saborizante	0,035 ml
Colorantes	0,5 mg
Agua destilada	c.s.p. 100 ml

Procedimiento representativo: Los ingredientes se mezclan para formar una suspensión que contiene 10 mg de ingrediente activo por cada 10 ml de suspensión.

Ejemplo de formulación H

Se prepara un polvo seco para administrar por inhalación de la siguiente forma:

Ingredientes	Cantidad
Compuesto de la invención	1,0 mg
Lactosa	25 mg

Procedimiento representativo: El ingrediente activo se microniza y luego se combina con lactosa. Esta mezcla combinada se carga luego en un cartucho de inhalación de gelatina. Se administran los contenidos del cartucho usando un inhalador de polvo.

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Ejemplo de formulación I

Se prepara un polvo seco para administración por inhalación en un inhalador de dosis medidas como se indica a continuación:

Procedimiento representativo: Se prepara una suspensión que contiene 5% en peso de un compuesto de la invención y 0,1% en peso de lecitina dispersando 10 g de compuesto activo, como partículas micronizadas con un tamaño promedio menor que 10 μm , en una solución formada a partir con 0,2 g de lecitina disueltos en 200 ml de agua desmineralizada. La suspensión se seca a chorro y se microniza el material resultante para obtener partículas con un diámetro promedio menor que 1,5 μm . Se cargan las partículas en cartuchos con 1,1,1,2-tetrafluoroetano a presión.

Ejemplo de formulación J

Se prepara una formulación inyectable como se indica a continuación:

Ingredientes	Cantidad
Compuesto de la invención	0,2 g
Solución amortiguadora de acetato de sodio (0,4 M)	40 ml
HCl (0,5 N) o NaOH (0,5 N)	cant. suf. hasta pH 4
Agua (destilada, estéril)	cant. suf. hasta 20 ml

Procedimiento representativo: Los ingredientes enumerados se mezclan y se ajusta el pH a $4 \pm 0,5$ usando HCl 0,5 N o NaOH 0,5 N.

Ejemplo de formulación K

Se preparan cápsulas para administración oral de la siguiente forma:

Ingredientes	Cantidad
Compuesto de la invención	4,05 mg
Celulosa microcristalina (Avicel PH 103)	259,2 mg

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Esterarato de magnesio	0,75 mg
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Procedimiento representativo: Los ingredientes se mezclan exhaustivamente y luego se cargan en una cápsula de gelatina (Tamaño N° 1, Blanca, Opaca) (264 mg de composición por cápsula).

Ejemplo de formulación L

Se preparan cápsulas para administración oral de la siguiente forma:

Ingredientes	Cantidad
Compuesto de la invención	8,2 mg
Celulosa microcristalina (Avicel PH 103)	139,05 mg
Esterarato de magnesio	0,75 mg

Procedimiento representativo: Los ingredientes se mezclan exhaustivamente y luego se cargan en una cápsula de gelatina (Tamaño N° 1, Blanca, Opaca) (148 mg de composición por cápsula).

Se debe tener en cuenta que se puede utilizar cualquier forma de los compuestos de la invención, (es decir, la base libre, una sal farmacéutica o un solvato) que sea adecuada para el modo de administración particular, en las composiciones farmacéuticas descritas precedentemente.

Utilidad

Los compuestos benzimidazol-carboxamida de la invención son agonistas del receptor 5-HT₄ y por lo tanto se espera que sean útiles para tratar condiciones médicas mediadas por receptores 5-HT₄ o asociadas con la actividad del receptor 5-HT₄, es decir condiciones médicas que mejoran por el tratamiento con un agonista del receptor 5-HT₄. Dichas condiciones médicas incluyen, sin limitarse, síndrome del intestino irritable (IBS), constipación

crónica, dispepsia funcional, retardo del vaciado gástrico, enfermedad de reflujo gastroesofágico (GERD), gastroparesis, íleo postoperatorio, pseudoobstrucción intestinal, y tránsito con retardo inducido por drogas. Además, se ha sugerido que algunos compuestos agonistas del receptor 5-HT₄ se pueden utilizar en el tratamiento de trastornos del sistema nervioso central incluyendo trastornos cognitivos, trastornos del comportamiento, trastornos del estado de ánimo, y trastornos de control de la función autónoma.

En particular, los compuestos de la invención aumentan la motilidad del tracto gastrointestinal (GI) y por lo tanto se espera que sean útiles para tratar trastornos del tracto GI a causa de la motilidad reducida en mamíferos, incluyendo humanos. Dichos trastornos de la motilidad GI incluyen, a modo de ilustración, constipación crónica, síndrome del intestino irritable con predominio de constipación (C-IBS), gastroparesis diabética e idiopática, y dispepsia funcional.

Por ello, en un aspecto en la invención se provee un método para incrementar la motilidad del tracto gastrointestinal en un mamífero, donde dicho método comprende la administración a dicho mamífero de una cantidad eficaz para uso terapéutico de una composición farmacéutica que comprende un vehículo aceptable para el uso farmacéutico y un compuesto de la invención.

Cuando se utilizan para tratar trastornos de motilidad reducida del tracto GI u otras condiciones mediadas por receptores 5-HT₄, los compuestos de la invención se administrarán típicamente por vía oral en una única dosis diaria o en varias dosis por día, aunque se pueden utilizar otras formas de administración. La cantidad de agente activo que se administra por dosis o la cantidad total que se administra por día será determinada típicamente por un

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médico, en base a las circunstancias relevantes, que incluyen la condición que se debe tratar, la ruta de administración seleccionada, el compuesto que se está administrando y su actividad relativa, la edad, peso, y respuesta del paciente individual, la gravedad de los síntomas del paciente, etcétera.

La dosis apropiada para tratar trastornos de motilidad reducida del tracto GI u otros trastornos mediados por receptores 5-HT₄ varía dentro de un rango entre aproximadamente 0,007 y aproximadamente 20 mg/kg/día de agente activo, incluyendo entre aproximadamente 0,0007 y aproximadamente 1mg/kg/día. Para un humano promedio de 70 kg, la misma podría ser de entre aproximadamente 0,05 y aproximadamente 70 mg por día de agente activo.

En un aspecto de la invención, los compuestos de la invención se utilizan para tratar la constipación crónica. Cuando se utilizan para tratar la constipación crónica, los compuestos de la invención se administrarán típicamente por vía oral en una única dosis diaria o en varias dosis por día. Preferentemente, la dosis para tratar la constipación crónica varía dentro del rango entre aproximadamente 0,05 y aproximadamente 70 mg por día.

En otro aspecto de la invención, los compuestos de la invención se utilizan para tratar el síndrome del intestino irritable. Cuando se utiliza para tratar el síndrome del intestino irritable con predominio de constipación, los compuestos de la invención se administrarán típicamente por vía oral en una única dosis diaria o en varias dosis por día. Preferentemente, la dosis para tratar el síndrome del intestino irritable con predominio de constipación varíe dentro del rango entre aproximadamente 0,05 y aproximadamente 70 mg por día.

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En otro aspecto de la invención, los compuestos de la invención se utilizan para tratar la gastroparesis diabética. Cuando se utilizan para tratar la gastroparesis diabética, los compuestos de la invención se administrarán típicamente por vía oral en una única dosis diaria o en varias dosis por día. Preferentemente, la dosis para tratar la gastroparesis diabética varíe dentro del rango entre aproximadamente 0,05 y aproximadamente 70 mg por día.

En aún otro aspecto de la invención, los compuestos de la invención se utilizan para tratar la dispepsia funcional. Cuando se utilizan para tratar la dispepsia funcional, los compuestos de la invención se administrarán típicamente por vía oral en una única dosis diaria o en varias dosis por día. Preferentemente, la dosis para tratar la dispepsia funcional varíe dentro del rango entre aproximadamente 0,05 y aproximadamente 70 mg por día.

La invención también provee un método para tratar un mamífero con una enfermedad o condición asociadas con la actividad del receptor 5-HT₄, donde dicho método comprende administrar al mamífero una cantidad efectiva para uso terapéutico de un compuesto de la invención o de una composición farmacéutica que comprende un compuesto de la invención.

Como se describió previamente, los compuestos de la invención son agonistas del receptor 5-HT₄. Por ello, en la invención se provee además un método de agonizar un receptor 5-HT₄ en un mamífero, donde dicho método comprende la administración de un compuesto de la invención a dicho mamífero. Además, los compuestos de la invención también son de utilidad como herramientas de investigación para investigar o estudiar sistemas o muestras biológicas que poseen receptores 5-HT₄ o para descubrir nuevos agonistas del receptor 5-HT₄. Más aún, como los compuestos de la invención

muestran selectividad de unión por los receptores 5-HT₄ en comparación con su unión a receptores de otros subtipos de 5-HT, en particular los receptores 5-HT₃, dichos compuestos son especialmente útiles para estudiar los efectos sobre el agonismo selectivo de los receptores 5-HT₄ en un sistema biológico o en una muestra. Cualquier sistema biológico o muestra apropiados con receptores 5-HT₄ se puede emplear en dichos estudios que se pueden llevar a cabo tanto *in vitro* como *in vivo*. Los sistemas biológicos o muestras apropiados para dichos estudios que son representativos incluyen, sin limitarse, células, extractos celulares, membranas plasmáticas, muestras de tejidos, mamíferos (como por ejemplo ratones, ratas, cobayos, conejos, perros, cerdos, etc.), etcétera.

En dicho aspecto de la invención, un sistema biológico o muestra que comprende un receptor 5-HT₄ se pone en contacto con una cantidad agonista del receptor 5-HT₄ de un compuesto de la invención. Luego se determinan los efectos sobre el agonismo del receptor 5-HT₄ utilizando procedimientos y equipos convencionales, como por ejemplo ensayos de unión de radioligandos y ensayos funcionales. Dichos ensayos funcionales incluyen cambios mediados por ligandos en el adenosina monofosfato cíclico (cAMP) intracelular, cambios mediados por ligandos en la actividad de la enzima adenilato ciclasa (que sintetiza el cAMP), cambios mediados por ligandos en la incorporación de análogos de guanosina trifosfato (GTP), como por ejemplo [³⁵S]GTPγS (guanosina 5'-O-(γ-tio)trifosfato) o GTP-Eu, en membranas aisladas mediante el intercambio de análogos de GTP catalizado por receptor por análogos de GDP, cambios de los iones calcio libres intracelulares mediados por ligandos (medidos, por ejemplo, con un lector de placas por imágenes de unión a

Clarke, Mc
COLON

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 07-127657- -00003-0000

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Act. 538 PETICION Folios: 2

SEÑORES
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES
E.S.D.

REF: EXPEDIENTE No. 07.127.657
SOLICITANTE: THERAVANCE, INC.

ASUNTO: SOLICITUD EXAMEN DE PATENTABILIDAD

DILIA RODRÍGUEZ D'ALEMAN identificada como aparece al pie de mi firma, domiciliada en Bogotá, en la Carrera 11 No. 86-53, piso 6, obrando como apoderada de la sociedad solicitante, pido a usted que se proceda a examinar si la invención, cuya patente se tramita en el expediente de la referencia, es patentable.

Adjunto al presente memorial el recibo No. 09-13,346 de 18 de febrero de 2009 por la suma de \$452.000.00, con lo cual queda cubierta la tasa oficial correspondiente a dicho estudio.

Señor Superintendente,

DILIA RODRIGUEZ D'ALEMAN
C.C. 41.654.882 de Bogotá
T.P. 20824 del C.S.J.

P-1029

DIVISIÓN DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. 07-127657

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **600** DE

FECHA **30 DE ENERO DE 2009** EL TÉRMINO HÁBIL SEÑALADO POR LA LEY

EXPIRA EL **29 DE ABRIL DE 2009**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI _____

NO X

Preparación 1: Síntesis de (piperidin-4-ilmetil)amida de ácido 2-isopropil-1H-benzoimidazol-4-carboxílico

a. Preparación de metiléster de ácido 2,3-diaminobenzoico

A una solución saturada de nitrógeno de metiléster de ácido 2-amino-3-nitrobenzoico (Chess GmbH, 50 g, 0,26 mol) en etanol absoluto (800 mL) se agregó hidróxido de paladio (Degussa, 20% p/p sobre carbono, 58,75% p/p agua, 10 g). La lechada se desgasificó luego se agitó vigorosamente bajo hidrógeno (4 atm) a temperatura ambiente durante 48 h. El catalizador se filtró y el filtrado se concentró al vacío para dar metiléster de ácido 2,3-diaminobenzoico como un aceite anaranjado oscuro que solidificó al reposar (43 g, 0,26 mol, 100%). (m/z): $[M-OCH_3]^+$ calculado para $C_8H_{10}N_2O_2$ 135,05; encontrado 135,3. 1H RMN (300 MHz, DMSO- d_6): δ (ppm) 3,74 (s, 3H), 4,80 (br s, 1H), 6,20 (br s, 1H), 6,38 (t, 1H), 6,70 (d, 1H), 7,06 (d, 1H).

b. Preparación de ácido 2-isopropil-1H-benzoimidazol-4-carboxílico

Una lechada de metiléster de ácido 2,3-diaminobenzoico (21,5 g, 0,13 mol) y ácido isobutírico (36,2 mL, 0,39 mol) en ácido clorhídrico acuoso (4M, 210 mL) se agitó bajo reflujo por 24 h para dar una solución homogénea. La solución se enfrió a 10 °C y el pH se elevó a 3,5 usando solución acuosa de hidróxido de sodio (4M, aproximadamente 210 mL), manteniendo la temperatura por debajo de 30 °C. La mezcla de reacción se agitó a temperatura ambiente durante 2 h, se enfrió a 10 °C, y el precipitado resultante se eliminó por filtración. La torta sólida se transfirió a un vaso y se agregó acetonitrilo (300 mL). La lechada se agitó a temperatura ambiente durante 1 h luego y se filtró para dar un sólido gris. El sólido se secó bajo vacío para dar el intermediario del título (23 g, 0,11

D1 divulga un compuesto de la fórmula (I) sus sales y sus composiciones, con potente actividad y selectividad sobre el receptor 5HT₄, útiles para tratar condiciones mediadas por dicho receptor.

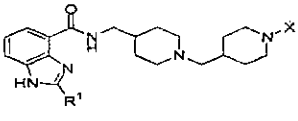
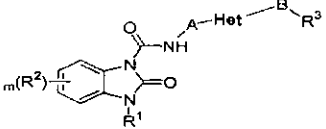
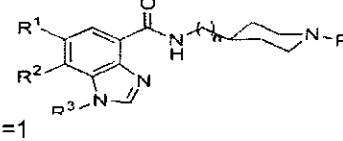
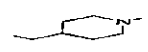
*D2 divulga un compuesto de la fórmula (I), derivado de Benzimidazoles, como modulador potente y selectivo del receptor 5HT₄, utilizado para tratar patologías como el desorden de la motilidad gastrointestinal. D1, www.espacenet.com y D2, Bioorganic and Medicinal Chemistry Letters

7. Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

La reivindicación 1 consiste en: un compuesto de formula (I).

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2
		
R1		
alquiloC3-5	No menciona. Es =O /	No menciona. Es H /
H	H	R1=H
H	H	R2=H
H	No menciona. R1= isopropil	R3=H
-CH2-	A= alquylene C1-4	n=1, es -CH2-
	B-R ³ = metilen-heterociclo= 	R=metil
X		
a) -C(O)OR ² grupo carboxyl	grupo carboxyl ✓	No menciona
b) -C(O)R ₃	No menciona	No menciona
c) -C(O)NR ₄ R ₅	No menciona	No menciona
d)-C(O)C(R ₆ R ₇)R ₈	No menciona	No menciona
e)-C(O)C(HR ₉)OR ₁₀	No menciona	No menciona
f)-S(O)2R ₁₁	No menciona	No menciona

El Estado de la Técnica más cercano es D1, que da a conocer compuestos derivados de Benzimidazolonas, como agonistas fuertemente selectivos del receptor 5HT₄, utilizado para tratar patologías como el desorden de la motilidad gastrointestinal (pg 3)

El compuesto, objeto de esta solicitud se diferencia de D1, en que R1 es un alquilo, la amida sustituida está en posición 4 sobre en el anillo benzimidazol y otros significados de X (b a f).

El efecto técnico logrado por estas diferencias es una actividad agonista de pEC₅₀ que varió entre 7.5 y 9.5, con concentraciones entre 10 pM y 100uM (folios 204-205, pgs 104 y 105).

Por lo tanto, el Problema Técnico objetivo es "cómo obtener otros compuestos Benzimidazoles, alternativos, a los ya divulgados en D1 con una actividad agonista de pEC₅₀ 7.5 y 9.5"

Pero D1, ya divulga que sus compuestos tienen una actividad agonista sobre el receptor 5-HT₄ (pg 38 y 39) a una concentración de 100nM (la cual está dentro del rango de concentración utilizado para los compuestos reclamados (10 pM y 100uM) y además, los compuestos del estado de la técnica, muestran una reducción de la prolongación QT (perfil cardíaco aceptable, pg3, líneas 14-17), al

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igual que la mencionada para los compuestos reivindicados (perfil cardíaco aceptable, en el folio 153, pg 53). Y adicionalmente ya D2 se muestra compuestos derivados de Benzimidazoles que tienen la sustitución sobre el anillo benzimidazol en la posición 4, al igual que los compuestos reclamados y que además presentan actividad sobre el receptor 5-HT4 y son útiles para tratar patologías como el desorden de la motilidad gastrointestinal (introducción), como la atribuida a los compuestos reclamados, al mencionar que son útiles en trastornos de motilidad reducida del tacto gastrointestinal.

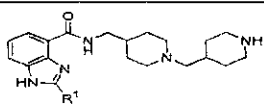
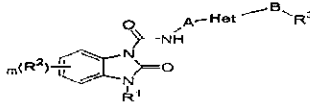
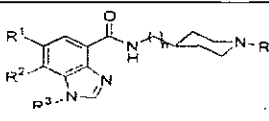
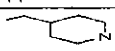
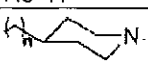
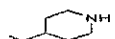
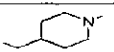
Lo anterior no permite evidenciar un efecto sorprendente sobre los compuestos divulgados que tienen estructura muy cercana y poseen la misma actividad como agonistas de 5-HT4, que son útiles para tratar los mismos trastornos.

Por lo tanto la reivindicación es obvia, porque la persona medianamente versada en la Materia enfrentada al problema de "cómo obtener compuestos Benzimidazoles, alternativos, a los ya divulgados en D1", con sus conocimientos y lo divulgado en D1 y D2, modificaría la posición de la amida sustituida sobre el anillo benzimidazol, como lo enseña D2 y realizaría sustituciones en X, logrando de esta forma el compuesto objeto de esta solicitud.

Se concluye que el compuesto de la Reivindicación 1, carece de Nivel Inventivo. Puesto que las reivindicaciones dependientes 1-19,24-25, no mencionan características inventivas adicionales, tampoco pueden ser consideradas inventivas.

La reivindicación 27 consiste en: un compuesto de formula (II)

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2
		 $n=1$
R1		
Alquilo C3-5	no menciona. Es =O	no menciona. Es H
H	H	R1=H
H	H	R2=H
H	no menciona. R1= es isopropil	R3=H
	A-Het, donde A es alquileno y Het es un heterociclo con un átomo de N	 $n=1$
	B-R ³ = metileno-heterociclo = 	R=metil

El Estado de la Técnica más cercano es D1, que da a conocer compuestos derivados de Benzimidazonas, (pg 4, formula (I), con el heterociclo de R³, sustituido o no; como agonistas fuertemente selectivos del receptor 5HT4, utilizado para tratar patologías como el desorden de la motilidad gastrointestinal (pg 3, líneas 5-17).

El compuesto, objeto de esta solicitud se diferencia de D1, en que el sustituyente de R1 es un radical alquilo y que la sustitución sobre el anillo benzimidazol, está en posición 4 y la 1-il-metilpiridina no esta sustituida.

El efecto técnico logrado por estas diferencias es una actividad agonista de pEC50 que varió entre 7.5 y 9.5, con concentraciones entre 10 pM y 100uM (folios 204-205, pgs 104 y 105).

d. Síntesis de (piperidin-4-ilmetil)amida de ácido 2-isopropil-1H-benzoimidazol-4-carboxílico

A una solución de *ter*-butiléster de ácido 4-[(2-isopropil-1H-benzoimidazol-4-carbonil)amino]metil]-piperidin-1-carboxílico (10,8 g, 27,0 mmol) disuelto en diclorometano (50 mL) a 0 °C se agregó lentamente ácido trifluoroacético (50 mL) en porciones de 5 mL. La solución se dejó subir la temperatura a temperatura ambiente, se agitó por otros 20 minutos luego se evaporó al vacío. Se eliminó el exceso de ácido trifluoroacético se eliminó mediante co-evaporación con tolueno. El residuo luego se disolvió en un volumen mínimo de diclorometano y se agregó lentamente a dietiléter (1 L) a 0 °C. La lechada resultante se agitó durante 2 h a temperatura ambiente luego se filtró para dar la sal bis-trifluoroacetato del compuesto del título como un sólido marrón claro (12,7 g, 24,0 mmol, 89%). (*m/z*): [M+H]⁺ calculado para C₁₇H₂₄N₄O 301,21; encontrado 301,5. Tiempo de retención (anal. HPLC: 2-50% MeCN/H₂O sobre 6 min) = 1,65 min. ¹H RMN (300 MHz, MeOD-*d*₃): δ (ppm) 1,59 (d, 6H), 1,60 (m, 1H), 2,03 (m, 2H), 2,04 (m, 1H), 3,00 (m, 2H), 3,43 (m, 2H), 3,45 (m, 2H), 3,63 (septeto, 1H), 7,63 (t, 1H), 7,90 (d, 1H), 7,96 (d, 1H), 9,04 (t, 1H).

Preparación 2: Síntesis de (1-piperidin-4-ilmetilpiperidin-4-ilmetil)amida de ácido 2-isopropil-1H-benzoimidazol-4-carboxílico

a. Preparación de *ter*-butiléster de ácido 4-(4-[(2-isopropil-1H-benzoimidazol-4-carbonil)amino]metil)-piperidin-1-ilmetil)piperidin-1-carboxílico

A una suspensión de bis-trifluoroacetato de (piperidin-4-ilmetil)amida de ácido 2-isopropil-1H-benzoimidazol-4-carboxílico (6,84 g, 12,95 mmol) en diclorometano (65 mL) a temperatura ambiente bajo nitrógeno se agregó en

$C_{28}H_{37}N_5O_3$ 492,30; encontrado 492,2. Tiempo de retención (anal. HPLC: 2-65% MeCN/H₂O sobre 4 min) = 1,68 min.

Ejemplo 6 {1-[1-(tiofeno-2-carbonil)piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-isopropil-1*H*-benzoimidazol-4-carboxílico; (*m/z*): [M+H]⁺ calculado para $C_{28}H_{37}N_5O_2S$ 508,28; encontrado 508,2. Tiempo de retención (anal. HPLC: 2-65% MeCN/H₂O sobre 4 min) = 1,94 min.

Ejemplo 7: Síntesis de {1-[1-(2-fluoro-5-trifluorometilbenzoil)piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-isopropil-1*H*-benzoimidazol-4-carboxílico

A una solución de ácido 2-fluoro-5-trifluorometilo benzoico (100 mg, 0,48 mmol) en dimetilformamida (4 mL) a temperatura ambiente se agregó hexafluorofosfato de O-(7-azabenzotriazol-1-il)-N,N,N',N'-tetrametiluronio (200 mg, 0,48mmol). La mezcla se agitó a temperatura ambiente durante 0,25 h, luego se agregaron (1-piperidin-4-ilmetilpiperidin-4-ilmetil)amida de ácido 2-isopropil-1*H*-benzoimidazol-4-carboxílico (210 mg, 0,48 mmol) y *N,N*-diisopropiletilamina (0,184 mL, 0,96 mmol) la agitación continuó por a otras 0,5 h. La solución se evaporó al vacío y el producto crudo se purificó por HPLC de fase inversa [gradiente de 5-10-25% (5-10% durante 10 min; 10-25% durante 50 min); velocidad de flujo 15 mL/min; detección a 280 nm] para dar la sal bis trifluoroacetato del compuesto del título como un sólido blanco (70 mg, 0,09 mmol, 18 %). (*m/z*): [M+H]⁺ calculado para $C_{31}H_{37}F_4N_5O_2$ 588,30; encontrado 588,2. Tiempo de retención (anal. HPLC: 2-60% MeCN/H₂O sobre 4 min) = 2,39 min.

Ejemplo 8: Síntesis de {1-[1-(2-fluoro-fenilcarbamoil)piperidin-4-ilmetil]piperidin-4-ilmetil}-amida de ácido 2-isopropil-1H-benzoimidazol-4-carboxílico

Se disolvió (1-piperidin-4-ilmetilpiperidin-4-ilmetil)amida de ácido 2-isopropil-1H-benzoimidazol-4-carboxílico (220 mg, 0,55 mmol) en *N,N*-dimetilformamida (2,0 mL) a temperatura ambiente. A esta solución se agregó *N,N*-diisopropiletilamina (143,2 mg, 1,1 mmol) seguido por *o*-fluorofenilisocianato (75,4 mg, 0,55 mmol). La mezcla resultante se agitó a temperatura ambiente durante la noche, se concentró al vacío y el residuo se purificó mediante HPLC preparativa de fase inversa para dar la sal bis trifluoroacetato del compuesto del título como un sólido blanco (92,6 mg, 0,12 mmol, 22 %). (*m/z*): [M+H]⁺ calculado para C₃₀H₃₉FN₆O₂ 535,32; encontrado 535,2. Tiempo de retención (anal. HPLC: 2-65% MeCN/H₂O sobre 4 min) = 2,09 min.

Ejemplo 9: Síntesis de [1-(1-metansulfonilpiperidin-4-ilmetil)piperidin-4-ilmetil]amida de ácido 2-isopropil-1H-benzoimidazol-4-carboxílico

Se disolvió (1-piperidin-4-ilmetilpiperidin-4-ilmetil)amida de ácido 2-Isopropil-1H-benzoimidazol-4-carboxílico (40 mg, 0,1 mmol) en *N,N*-dimetilformamida (1,0 mL) a temperatura ambiente. A esta solución se agregó *N,N*-diisopropiletilamina (0,175 mL, 1 mmol) seguido por cloruro de metansulfonilo (11,5 mg, 0,1 mmol). La mezcla se agitó a temperatura ambiente durante 16 h, luego se concentró al vacío y el residuo se purificó mediante HPLC preparativa de fase inversa para dar la sal bis trifluoroacetato del compuesto del título como un sólido blanco (27,2 mg, 0,04 mmol, 40 %). (*m/z*): [M+H]⁺ calculado para

$C_{24}H_{37}N_5O_3S$ 476,27; encontrado 476,2. Tiempo de retención (anal. HPLC: 2-65% MeCN/H₂O durante 4 min) = 1,66 min.

Ejemplo 10: Síntesis de metiléster de ácido 4-(4-(((2-*ter*-butil-1*H*-benzoimidazol-4-carbonil)-amino)-metil)piperidin-1-ilmetil)piperidin-1-carboxílico

Al producto crudo de la Preparación 3 (2,4 g, 5,8 mmol) en diclorometano (29 mL) a temperatura ambiente se agregó *N,N*-diisopropiletilamina (1,5 g, 11,6 mmol). La mezcla resultante se enfrió a 0 °C y se agregó cloroformato de metilo (660 mg, 6,98 mmol) por goteo. La reacción se dejó subir la temperatura a temperatura ambiente y se agitó por otros 10 min. La solución se concentró, se redisolvió en ácido acético al 50 % en agua, se filtró y se purificó por HPLC preparativa de fase inversa. El sólido resultante se disolvió en diclorometano y se lavó con hidróxido de sodio acuoso 1M. La capa acuosa se extrajo dos veces con diclorometano, y las capas orgánicas combinadas se lavaron con salmuera, se secó sobre sulfato de sodio, se filtró y se concentró para dar el compuesto del título como una espuma blanca (1,3 g, 2,8 mmol, 48 %). (*m/z*): [M+H]⁺ calculado para $C_{26}H_{39}N_5O_3$ 470,32, encontrado 470,6. ¹H RMN (300 MHz, MeOD-*d*₃): δ (ppm) 1,02-1,16 (m, 2H), 1,49 (s, 9H), 1,47-1,7 (m, 4H), 1,82-2,03 (m, 4H), 2,74-2,94 (m, 6H), 3,31-3,40 (m, 2H), 3,54-3,58 (m, 2H), 3,56 (s, 3H), 3,98-4,03 (m, 2H), 7,41-7,46 (m, 1H), 7,71-7,74 (m, 1H), 7,79-7,82 (m, 1H), 9,35 (brs, 1H).

Ejemplos 11-13

Usando procedimientos similares a los del Ejemplo 10, excepto reemplazando el clorofornato de metilo con el reactivo cloruro apropiado, se prepararon los compuestos de las Ejemplos 11-13.

Ejemplo 11 {1-[1-(2-fluoro-benzoil)-piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-*ter*-butil-1*H*-benzoimidazol-4-carboxílico; (*m/z*): [M+H]⁺ calculado para C₃₁H₄₀FN₅O₂ 534,33; encontrado 534,4. Tiempo de retención (anal. HPLC: 2-65% MeCN/H₂O durante 4 min) = 2,09 min.

Ejemplo 12 {1-[1-(3-metil-benzoil)-piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-*ter*-butil-1*H*-benzoimidazol-4-carboxílico; (*m/z*): [M+H]⁺ calculado para C₃₂H₄₃N₅O₂ 530,35; encontrado 530,42. Tiempo de retención (anal. HPLC: 2-65% MeCN/H₂O al cabo de 4 min) = 2,22.

Ejemplo 13 {1-[1-(4-fluorobenzoil)-piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-*ter*-butil-1*H*-benzoimidazol-4-carboxílico; (*m/z*): [M+H]⁺ calculado para C₃₁H₄₀FN₅O₂ 534,33; encontrado 534,4. Tiempo de retención (anal. HPLC: 2-65% MeCN/H₂O al cabo de 4 min) = 2,17.

Ejemplo 14. Síntesis de metiléster de ácido 4-[4-({[2-(1-hidroxi-1-metiletil)-1*H*-benzoimidazol-4-carbonil]amino}metil)piperidin-1-ilmetil]piperidin-1-carboxílico

a. Preparación de ácido 2-(1-hidroxi-1-metiletil)-1*H*-benzoimidazol-4-carboxílico

A una solución de metiléster de ácido 2,3-diaminobenzoico (1,5 g, 9,2 mmol) en 4 M HCl (50 mL) se agregó ácido 2-hidroxiisobutírico (2,87 g, 27,6 mmol). La mezcla se agitó a ~90°C durante 24 h. Se neutralizó mediante el uso de solución acuosa de hidróxido de sodio a pH~3, y se concentró a sequedad. El

residuo se suspendió en metanol, y se filtró a través de un filtro de papel. El filtrado se concentró y el residuo se enjuagó con éter. El residuo sólido restante se disolvió en acetato de etilo, y se lavó con solución de salmuera. Después de secar sobre MgSO_4 , la solución orgánica se evaporó al vacío, para dar el intermediario del título como un aceite amarillo pálido (~800 mg). El producto crudo se usó en el paso siguiente sin purificación adicional. (m/z): $[\text{M}+\text{H}]^+$ calculado para $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_3$ 221,09; encontrado 221,1. ^1H -RMN (CD_3OD) δ (ppm) 7,8 (dd, 1H), 7,7 (dd, 1H), 7,2 (m, 1H), 1,3 (s, 6H).

b. Síntesis de metiléster de ácido 4-[4-([2-(1-hidroxi-1-metiletil)-1H-benzoimidazol-4-carbonil]amino)metil]piperidin-1-ilmetil]piperidin-1-carboxílico

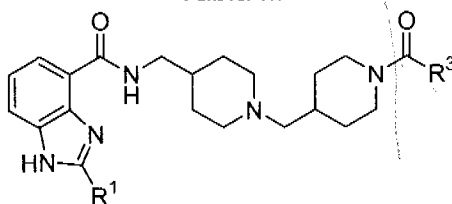
A una solución de el producto ácido benzoimidazol carboxílico del paso precedente (0,7 g, 3,18 mmol), se agregó el producto aminometilpiperidina de la Preparación 4 como la sal bis TFA (1,2 g, 3,13 mmol), y hidroxibenzotriazol (HOBt) (0,43 g, 3,18 mmol) en dimetilformamida (50 mL) se agregó trietilamina (1,3 mL, 9,3 mmol) y clorhidrato de *N*-Etil-*N'*-(3-dimetilaminopropil)carbodiimida (EDC) (0,67 g, 3,5 mmol). La mezcla se agitó a temperatura ambiente durante 12 h, y se concentró a sequedad al vacío. El residuo se particionó entre diclorometano (150 mL) y bicarbonato de sodio saturado. La capa orgánica se secó sobre MgSO_4 , y se evaporó a sequedad, para dar un residuo oleoso amarillo pálido. Se purificó por HPLC preparativa para dar la sal bis-trifluoroacetato del compuesto del título. (m/z): $[\text{M}+\text{H}]^+$ calculado para $\text{C}_{25}\text{H}_{37}\text{N}_5\text{O}_4$ 472,29; encontrado 472,5. Tiempo de retención (anal. HPLC: 5-30% MeCN/ H_2O durante 6 min) = 3,67 min. ^1H -RMN (CD_3OD) δ (ppm) 7,9-7,8

Ejemplo No.	R ¹	R ^{3a}	R ^{3b}	R ^{3c}	R ^{3d}	R ^{3e}	Fórmula molecular	[M+H] ⁺ calculado	[M+H] ⁺ encontrado
36	iPr	F			F		C ₃₀ H ₃₇ F ₂ N ₅ O ₂	538,29	538,2
37	iPr		F	F			C ₃₀ H ₃₇ F ₂ N ₅ O ₂	538,29	538,2
38	iPr	F	F				C ₃₀ H ₃₇ F ₂ N ₅ O ₂	538,29	538,2
39	iPr		F		F		C ₃₀ H ₃₇ F ₂ N ₅ O ₂	538,29	538,2
40	iPr	F		Cl			C ₃₀ H ₃₇ ClFN ₅ O ₂	554,26	554,4
41	iPr		CF ₃		CF ₃		C ₃₂ H ₃₇ F ₆ N ₅ O ₂	638,29	638,2
42	iPr	Cl		F			C ₃₀ H ₃₇ ClFN ₅ O ₂	554,26	554,2
43	iPr		Cl		Cl		C ₃₀ H ₃₇ Cl ₂ N ₅ O ₂	570,23	570,2
44	iPr			OCF ₃			C ₃₁ H ₃₈ F ₃ N ₅ O ₃	586,29	586,2
45	iPr		CF ₃	F			C ₃₁ H ₃₇ F ₄ N ₅ O ₂	588,29	588,2
46	iPr		Cl	F			C ₃₀ H ₃₇ ClFN ₅ O ₂	554,26	554,2
47	iPr	OCH ₃		Cl			C ₃₁ H ₄₀ ClN ₅ O ₃	566,28	566,2
48	iPr	CN					C ₃₁ H ₃₈ N ₆ O ₂	527,31	527,2
49	iPr	Cl		Cl			C ₃₀ H ₃₇ Cl ₂ N ₅ O ₂	570,23	570,2
50	iPr		F	CF ₃			C ₃₁ H ₃₇ F ₄ N ₅ O ₂	588,29	588,2
51	iPr			CN			C ₃₁ H ₃₈ N ₆ O ₂	527,31	527,2
52	iPr			OCHF ₂			C ₃₁ H ₃₉ F ₂ N ₅ O ₃	568,30	568,8
53	tBu	Cl		F			C ₃₁ H ₃₉ ClFN ₅ O ₂	568,28	568,2
54	tBu		Cl		Cl		C ₃₁ H ₃₉ Cl ₂ N ₅ O ₂	584,25	584,2
55	tBu		CN				C ₃₂ H ₄₀ N ₆ O ₂	541,32	541,4
56	tBu			OCF ₃			C ₃₂ H ₄₀ F ₃ N ₅ O ₃	600,31	600,2
57	tBu		CF ₃	F			C ₃₂ H ₃₉ F ₄ N ₅ O ₂	602,30	602,2
58	tBu		Cl	F			C ₃₁ H ₃₉ ClFN ₅ O ₂	568,28	568,2

Ejemplo No.	R ¹	R ^{3a}	R ^{3b}	R ^{3c}	R ^{3d}	R ^{3e}	Fórmula molecular	[M+H] ⁺ calculado	[M+H] ⁺ encontrado
59	tBu	OCH ₃		Cl			C ₃₂ H ₄₂ ClN ₅ O ₃	580,30	580,2
60	tBu	CN					C ₃₂ H ₄₀ N ₆ O ₂	541,32	541,4
61	tBu	Cl		Cl			C ₃₁ H ₃₉ Cl ₂ N ₅ O ₂	584,25	584,2
62	tBu		F	CF ₃			C ₃₂ H ₃₉ F ₄ N ₅ O ₂	602,30	602,2
63	tBu	F			CF ₃		C ₃₂ H ₃₉ F ₄ N ₅ O ₂	602,30	602,4
64	tBu			CN			C ₃₂ H ₄₀ N ₆ O ₂	541,32	541,2
65	tBu			OCHF ₂			C ₃₂ H ₄₁ F ₂ N ₅ O ₃	582,32	582,4
66	tBu						C ₃₁ H ₄₁ N ₅ O ₂	516,33	516,2
67	tBu	CH ₃					C ₃₂ H ₄₃ N ₅ O ₂	530,34	530,4
68	tBu	CF ₃					C ₃₂ H ₄₀ F ₃ N ₅ O ₂	584,31	584,4
69	tBu		F				C ₃₁ H ₄₀ FN ₅ O ₂	534,32	534,2
70	tBu		CF ₃				C ₃₂ H ₄₀ F ₃ N ₅ O ₂	584,31	584,4
71	tBu		Cl				C ₃₁ H ₄₀ ClN ₅ O ₂	550,29	550,2
72	tBu			CF ₃			C ₃₂ H ₄₀ F ₃ N ₅ O ₂	584,31	584,4
73	tBu			CH ₃			C ₃₂ H ₄₃ N ₅ O ₂	530,34	530,4
74	tBu			Cl			C ₃₁ H ₄₀ ClN ₅ O ₂	550,29	550,2
75	tBu			OCH ₃			C ₃₂ H ₄₃ N ₅ O ₃	546,34	546,4
76	tBu	F				F	C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552,31	552,4
77	tBu	F			F		C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552,31	552,2
78	tBu		F	F			C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552,31	552,2
79	tBu	F	F				C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552,31	552,4
80	tBu		F		F		C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552,31	552,4
81	tBu	F		F			C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552,31	552,2

Ejemplo No.	R ¹	R ^{3a}	R ^{3b}	R ^{3c}	R ^{3d}	R ^{3e}	Fórmula molecular	[M+H] ⁺ calculado	[M+H] ⁺ encontrado
82	tBu	F			Cl		C ₃₁ H ₃₉ ClFN ₅ O ₂	568,28	568,2
83	tBu		CF ₃		CF ₃		C ₃₃ H ₃₉ F ₆ N ₅ O ₂	652,30	652,2
84	iPr	OCF ₃					C ₃₁ H ₃₈ F ₃ N ₅ O ₃	586,29	586,2
85	iPr		OCF ₃				C ₃₁ H ₃₈ F ₃ N ₅ O ₃	586,29	586,2
86	tBu	OCF ₃					C ₃₂ H ₄₀ F ₃ N ₅ O ₃	600,31	600,2
87	tBu		OCF ₃				C ₃₂ H ₄₀ F ₃ N ₅ O ₃	600,31	600,2

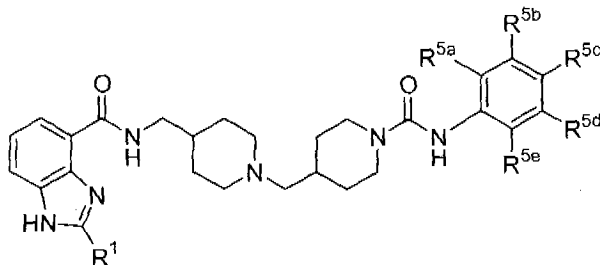
Tabla III



Ejemplo No.	R ¹	R ³	Fórmula molecular	[M+H] ⁺ calculado	[M+H] ⁺ encontrado
88	iPr	N(CH ₃) ₂	C ₂₆ H ₄₀ N ₆ O ₂	469,32	469,4
89	iPr	morfolin-1-ilo	C ₂₈ H ₄₂ N ₆ O ₃	511,33	511,4
90	iPr	CH ₃	C ₂₅ H ₃₇ N ₅ O ₂	440,30	440,2
91	iPr	tetrahidrofuran-2-ilo	C ₂₈ H ₄₁ N ₅ O ₃	496,32	496,4
92	iPr	-CH ₂ -tiofen-3-ilo	C ₂₉ H ₃₉ N ₅ O ₂ S	522,28	522,2
93	iPr	2,2-dimetilpropilo	C ₂₉ H ₄₅ N ₅ O ₂	496,36	496,4
94	iPr	-CH ₂ -tiofen-2-ilo	C ₂₉ H ₃₉ N ₅ O ₂ S	522,28	522,2
95	iPr	ciclohexilo	C ₃₀ H ₄₅ N ₅ O ₂	508,36	508,4
96	iPr	(S)-1-metilpropilo	C ₂₈ H ₄₃ N ₅ O ₂	482,34	482,4

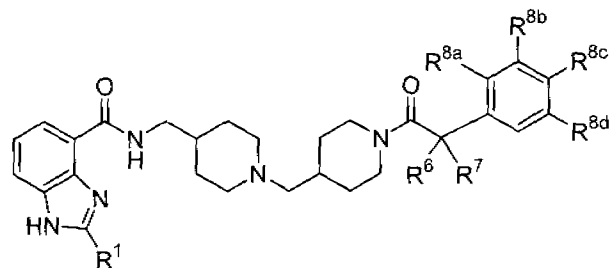
Ejemplo No.	R ¹	R ³	Fórmula molecular	[M+H] ⁺ calculado	[M+H] ⁺ encontrado
97	iPr	-CH ₂ -naft-1-ilo	C ₃₅ H ₄₃ N ₅ O ₂	566,34	566,4
98	iPr	ciclopentilo	C ₃₀ H ₄₅ N ₅ O ₂	508,36	508,4
99	iPr	(<i>R</i>)-tetrahidrofuran-2-ilo	C ₂₈ H ₄₁ N ₅ O ₃	496,32	496,4
100	tBu	furan-2-ilo	C ₂₉ H ₃₉ N ₅ O ₃	506,31	506,2
101	tBu	-CH ₂ -tiofen-3-ilo	C ₃₀ H ₄₁ N ₅ O ₂ S	536,30	536,2
102	tBu	2,2-dimetilpropilo	C ₃₀ H ₄₇ N ₅ O ₂	510,37	510,4
103	tBu	-CH ₂ -tiofen-2-ilo	C ₃₀ H ₄₁ N ₅ O ₂ S	536,30	536,2
104	tBu	(<i>S</i>)-1-metilpropilo	C ₂₉ H ₄₅ N ₅ O ₂	496,36	496,4
105	tBu	-CH ₂ -naft-1-ilo	C ₃₆ H ₄₅ N ₅ O ₂	580,36	580,4
106	tBu	(<i>R</i>)-tetrahidrofuran-2-ilo	C ₂₉ H ₄₃ N ₅ O ₃	510,34	510,4
107	tBu	(<i>S</i>)-4-oxo-azetidin-2-ilo	C ₂₈ H ₄₀ N ₆ O ₃	509,32	510,4
108	tBu	piridin-2-ilo	C ₃₁ H ₄₂ N ₆ O ₂	531,34	531,2

Tabla IV



Ejemplo No.	R ¹	R ^{5a}	R ^{5b}	R ^{5c}	R ^{5d}	R ^{5e}	Fórmula molecular	[M+H] ⁺ calculado	[M+H] ⁺ encontrado
109	iPr	CH ₃					C ₃₁ H ₄₂ N ₆ O ₂	531,34	531,4
110	iPr		F				C ₃₀ H ₃₉ FN ₆ O ₂	535,31	535,2
111	iPr	CF ₃					C ₃₁ H ₃₉ F ₃ N ₆ O ₂	585,31	585,2
112	iPr	OCF ₃					C ₃₁ H ₃₉ F ₃ N ₆ O ₃	601,30	601,2
113	iPr			OCHF ₂			C ₃₁ H ₄₀ F ₂ N ₆ O ₃	583,31	583,2
114	iPr						C ₃₀ H ₄₀ N ₆ O ₂	517,32	517,4
115	iPr	CH ₃				CH ₃	C ₃₂ H ₄₄ N ₆ O ₂	545,35	545,4
116	iPr			OCF ₃			C ₃₁ H ₃₉ F ₃ N ₆ O ₃	601,30	601,2
117	iPr	tBu					C ₃₄ H ₄₈ N ₆ O ₂	573,38	573,4
118	iPr	Cl					C ₃₀ H ₃₉ ClN ₆ O ₂	551,28	551,2
119	tBu	Cl					C ₃₁ H ₄₁ ClN ₆ O ₂	565,30	565,2
120	tBu	CH ₃					C ₃₂ H ₄₄ N ₆ O ₂	545,35	545,5
121	tBu	F					C ₃₁ H ₄₁ FN ₆ O ₂	549,33	549,2
122	tBu	CF ₃					C ₃₂ H ₄₁ F ₃ N ₆ O ₂	599,32	599,2
123	tBu	OCF ₃					C ₃₂ H ₄₁ F ₃ N ₆ O ₃	615,32	615,2
124	tBu			OCHF ₂			C ₃₂ H ₄₂ F ₂ N ₆ O ₃	597,33	597,4
125	tBu	F					C ₃₁ H ₄₁ FN ₆ O ₂	549,33	549,2
126	tBu						C ₃₁ H ₄₂ N ₆ O ₂	531,34	531,4
127	tBu	CH ₃				CH ₃	C ₃₃ H ₄₆ N ₆ O ₂	559,37	559,4
128	tBu			OCF ₃			C ₃₂ H ₄₁ F ₃ N ₆ O ₃	615,32	615,2
129	tBu	tBu					C ₃₅ H ₅₀ N ₆ O ₂	587,40	587,4

Tabla V



Ejemplo No.	R ¹	R ⁶	R ⁷	R ^{8a}	R ^{8b}	R ^{8c}	R ^{8d}	Fórmula molecular	[M+H] ⁺ calculado	[M+H] ⁺ encontrado
130	iPr		(S)-OH					C ₃₁ H ₄₁ N ₅ O ₃	532,32	532,2
131	iPr		oxo					C ₃₁ H ₃₉ N ₅ O ₃	530,31	530,2
132	iPr					Cl		C ₃₁ H ₄₀ ClN ₅ O ₂	550,29	550,2
133	iPr		(S)-CH ₃					C ₃₂ H ₄₃ N ₅ O ₂	530,34	530,4
134	iPr		-(CH ₂) ₂ -					C ₃₃ H ₄₃ N ₅ O ₂	542,34	542,4
135	iPr			F		F		C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552,31	552,2
136	iPr				F			C ₃₁ H ₄₀ FN ₅ O ₂	534,32	534,2

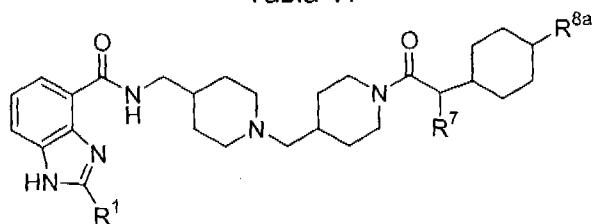
Ejemplo No.	R ¹	R ⁶	R ⁷	R ^{8a}	R ^{8b}	R ^{8c}	R ^{8d}	Fórmula molecular	[M+H] ⁺ calculado	[M+H] ⁺ encontrado
137	iPr				Cl			C ₃₁ H ₄₀ ClN ₅ O ₂	550,29	551,2
138	iPr				F	F		C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552,31	552,2
139	iPr				F		F	C ₃₁ H ₃₉ F ₂ N ₅ O ₂	552,31	553,2
140	iPr							C ₃₁ H ₄₁ N ₅ O ₂	516,33	516,4
141	tBu		(S)- OH					C ₃₂ H ₄₃ N ₅ O ₃	546,34	546,4
142	tBu					F		C ₃₂ H ₄₂ FN ₅ O ₂	548,33	548,2
143	tBu	oxo						C ₃₂ H ₄₁ N ₅ O ₃	544,32	544,4
144	tBu					Cl		C ₃₂ H ₄₂ ClN ₅ O ₂	564,30	565,2
145	tBu		CH ₃			2-metilpropilo		C ₃₇ H ₅₃ N ₅ O ₂	600,42	600,4
146	tBu		(S)- CH ₃					C ₃₃ H ₄₅ N ₅ O ₂	544,36	544,4
147	tBu	-(CH ₂) ₂ -						C ₃₄ H ₄₅ N ₅ O ₂	556,36	556,4
148	tBu			Cl		Cl		C ₃₂ H ₄₁ Cl ₂ N ₅ O ₂	598,26	599,2
149	tBu			F		F		C ₃₂ H ₄₁ F ₂ N ₅ O ₂	566,32	566,2

Ejemplo No.	R ¹	R ⁶	R ⁷	R ^{8a}	R ^{8b}	R ^{8c}	R ^{8d}	Fórmula molecular	[M+H] ⁺ calculado	[M+H] ⁺ encontrado
150	tBu				F			C ₃₂ H ₄₂ FN ₅ O ₂	548,33	548,4
151	tBu					CF ₃		C ₃₃ H ₄₂ F ₃ N ₅ O ₂	598,33	598,4
152	tBu				CF ₃			C ₃₃ H ₄₂ F ₃ N ₅ O ₂	598,33	598,2
153	tBu				Cl			C ₃₂ H ₄₂ ClN ₅ O ₂	564,30	564,2
154	tBu				F	F		C ₃₂ H ₄₁ F ₂ N ₅ O ₂	566,32	566,2
155	tBu				F		F	C ₃₂ H ₄₁ F ₂ N ₅ O ₂	566,32	566,4
156	tBu							C ₃₂ H ₄₃ N ₅ O ₂	530,34	530,4

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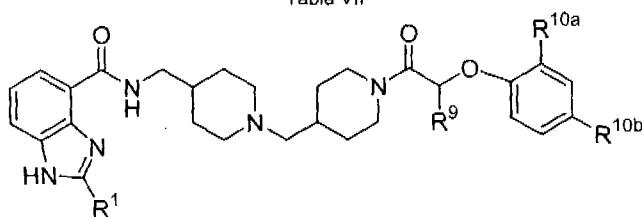
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Tabla VI



Ejemplo No.	R ¹	R ⁷	R ^{8a}	Fórmula molecular	[M+H] ⁺ calculado	[M+H] ⁺ encontrado
157	iPr	(R)-OH		C ₃₁ H ₄₇ N ₅ O ₃	538,37	538,4
158	iPr			C ₃₁ H ₄₇ N ₅ O ₂	522,37	522,4
159	tBu	(R)-OH		C ₃₂ H ₄₉ N ₅ O ₃	552,38	552,4
160	tBu			C ₃₂ H ₄₉ N ₅ O ₂	536,39	536,4

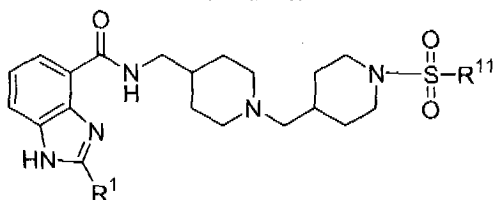
Tabla VII



Ejemplo No.	R ¹	R ⁹	R ^{10a}	R ^{10b}	Fórmula molecular	[M+H] ⁺ calculado	[M+H] ⁺ encontrado
161	iPr			CH ₃	C ₃₂ H ₄₃ N ₅ O ₃	546,34	546,4
162	iPr		Cl	Cl	C ₃₁ H ₃₉ Cl ₂ N ₅ O ₃	600,24	601,2
163	iPr	CH ₃		Cl	C ₃₂ H ₄₂ ClN ₅ O ₃	580,30	581,2
164	iPr	CH ₃			C ₃₂ H ₄₃ N ₅ O ₃	546,34	546,4

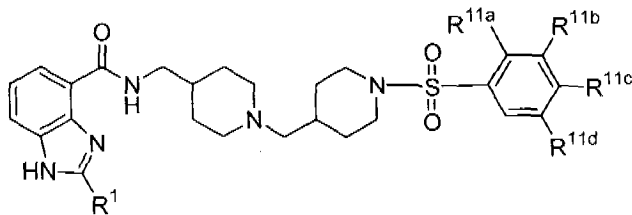
165	tBu			CH ₃	C ₃₃ H ₄₅ N ₅ O ₃	560,35	560,4
166	tBu		Cl	Cl	C ₃₂ H ₄₁ Cl ₂ N ₅ O ₃	614,26	615,2
167	tBu	CH ₃		Cl	C ₃₃ H ₄₄ ClN ₅ O ₃	594,31	595,2
168	tBu	CH ₃			C ₃₃ H ₄₅ N ₅ O ₃	560,35	560,4

Tabla VIII



Ejemplo No.	R ¹	R ¹¹	Fórmula molecular	[M+H] ⁺ calculado	[M+H] ⁺ encontrado
169	iPr	CH ₃	C ₂₄ H ₃₇ N ₅ O ₃ S	476,26	476,2
170	iPr	2,4-dimetilisoxazol-2-ilo	C ₂₈ H ₄₀ N ₆ O ₄ S	557,28	557,2
171	iPr	-CH ₂ -fenilo	C ₃₀ H ₄₁ N ₅ O ₃ S	552,29	552,2
172	tBu	2,4-dimetilisoxazol-2-ilo	C ₂₉ H ₄₂ N ₆ O ₄ S	571,30	571,2
173	tBu	-CH ₂ -fenilo	C ₃₁ H ₄₃ N ₅ O ₃ S	566,31	566,2

Tabla IX



Ejemplo No.	R ¹	R ^{11a}	R ^{11b}	R ^{11c}	R ^{11d}	Fórmula molecular	[M+H] ⁺ calculado	[M+H] ⁺ encontrado
174	iPr	CF ₃				C ₃₀ H ₃₈ F ₃ N ₅ O ₃ S	606,27	606,2

Ejemplo No.	R ¹	R ^{11a}	R ^{11b}	R ^{11c}	R ^{11d}	Fórmula molecular	[M+H] ⁺ calculado	[M+H] ⁺ encontrado
175	iPr	CN				C ₃₀ H ₃₈ N ₆ O ₃ S	563,27	563,2
176	iPr			OCH ₃		C ₃₀ H ₄₁ N ₅ O ₄ S	568,29	568,2
177	iPr		Cl			C ₂₉ H ₃₈ ClN ₅ O ₃ S	572,24	572,2
178	iPr	F				C ₂₉ H ₃₈ FN ₅ O ₃ S	556,27	556,2
179	iPr			CF ₃		C ₃₀ H ₃₈ F ₃ N ₅ O ₃ S	606,27	606,2
180	iPr			iPr		C ₃₂ H ₄₅ N ₅ O ₃ S	580,32	580,4
181	iPr	Cl				C ₂₉ H ₃₈ ClN ₅ O ₃ S	572,24	572,2
182	iPr	CH ₃			F	C ₃₀ H ₄₀ FN ₅ O ₃ S	570,28	570,2
183	iPr	Cl		F		C ₂₉ H ₃₇ ClFN ₅ O ₃ S	590,23	590,2
184	iPr	CH ₃	Cl			C ₃₀ H ₄₀ ClN ₅ O ₃ S	586,25	586,2
185	iPr			tBu		C ₃₃ H ₄₇ N ₅ O ₃ S	594,34	594,4
186	iPr	OCH ₃			Cl	C ₃₀ H ₄₀ ClN ₅ O ₄ S	602,25	602,2
187	iPr			Cl		C ₂₉ H ₃₈ ClN ₅ O ₃ S	572,24	572,2
188	iPr					C ₂₉ H ₃₉ N ₅ O ₃ S	538,28	538,2
189	iPr			F		C ₂₉ H ₃₈ FN ₅ O ₃ S	556,27	556,2
190	iPr		CH ₃			C ₃₀ H ₄₁ N ₅ O ₃ S	552,29	552,2
191	iPr			CH ₃		C ₃₀ H ₄₁ N ₅ O ₃ S	552,29	552,2
192	iPr		CF ₃			C ₃₀ H ₃₈ F ₃ N ₅ O ₃ S	606,27	606,2
193	iPr	CH ₃				C ₃₀ H ₄₁ N ₅ O ₃ S	552,29	552,2
194	tBu	CN				C ₃₁ H ₄₀ N ₆ O ₃ S	577,29	577,2
195	tBu			CF ₃		C ₃₁ H ₄₀ F ₃ N ₅ O ₃ S	620,28	620,2
196	tBu			iPr		C ₃₃ H ₄₇ N ₅ O ₃ S	594,34	594,4
197	tBu	Cl				C ₃₀ H ₄₀ ClN ₅ O ₃ S	586,25	586,2

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(38,4 mL, 1,1 eq) manteniendo la temperatura por debajo de 10°C. Cuando la adición se completó, la mezcla de reacción se calentó a 20-30°C durante 1 hora. Después de completarse la reacción, se agregó diclorometano (500 mL) a la mezcla de reacción. La capa orgánica se recolectó y se lavó con solución 1M de ácido fosfórico (200 mL), solución saturada de bicarbonato de sodio (200 mL) y solución saturada de cloruro de sodio (200 mL). La capa orgánica se secó sobre sulfato de sodio (50 g, 1 equivalente p/p) y luego se destiló bajo vacío para producir el intermediario del título. (67,0 g, 90% de rendimiento)

b. Preparación de metiléster de ácido 4-formilpiperidin-1-carboxílico

Se disolvió metiléster de ácido 4-hidroximetilpiperidin-1-carboxílico (34,7 g, 1,0 eq) en diclorometano y se enfrió a 0-10°C. Se agregó una solución de bicarbonato de sodio (2,35 g, 0,14 eq) y bromuro de sodio (2,40 g, 0,10 eq) en agua (100 mL) durante 15 min manteniendo la temperatura a 0-10 °C. Se agregó radical libre 2,2,6,6-tetrametil-1- piperidiniloxi (TEMPO) (0,32 g , 0,01 eq) a la mezcla, seguido por solución 10-13% p/v de hipoclorito de sodio (135 mL, 1,1 eq) durante 1 h con buena agitación manteniendo la temperatura a 0-10°C. después de completarse la reacción, las capas se separaron y la capa orgánica se lavó con agua (150 mL) y se secó sobre sulfato de sodio (30 g, 1 p/p eq). El solvente se eliminó por destilación para dar el intermediario del título. (31,0 g, 90% de rendimiento)

c. Preparación de (piperidin-4-ilmetil)amida de ácido 2-isopropil-1H-benzoimidazol-4-carboxílico

Se agregó ácido trifluoroacético (56,0 mL, 10 eq) a un recipiente que contiene una solución a ~5°C de ter-butiléster de ácido 4-[(2-isopropil-1H-

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benzoimidazol-4-carbonil)amino]metil]-piperidin-1-carboxílico (30,0 g, 1,0 eq) en diclorometano (300 mL) manteniendo la temperatura por debajo de 10°C. La mezcla resultante se agitó a 20-30 °C durante 2 h. Cuando la reacción se completó, se agregaron trietilamina (73,2 mL, 7,0 eq) y ácido acético (4,3 mL, 1,0 eq) para dar una solución de el intermediario del título con un pH aparente de aproximadamente 4 que se usó directamente en el paso siguiente.

d. Síntesis de metiléster de ácido 4-(4-[(2-isopropil-1H-benzoimidazol-4-carbonil)-amino]metil]piperidin-1-ilmetil)piperidin-1-carboxílico

Se agregó metiléster de ácido 4-formilpiperidin-1-carboxílico (25,7 g, 2,0 eq) a la solución preparada en el paso precedente manteniendo la temperatura a 20-30 °C. Después de agitar por 30 min, se agregó triacetoxiborohidruro de sodio (24,3 g, 1,5 eq) manteniendo la temperatura a 20-30°C. La mezcla de reacción se agitó a 20-30°C durante 30 min. Después de completarse la reacción, se agregó ácido clorhídrico 1 M (300 mL) para detener la reacción. La capa acuosa que contenía producto se recolectó y se lavó con diclorometano (150 mL). La capa acuosa se trató con carbono activado (Darco G60, 6 g, 20% p/p) para eliminar el color. La suspensión se agitó durante 1 hr, y luego se filtró a través de una almohadilla de Celite. Se agregó diclorometano (300 mL) a la solución acuosa y el producto de base libre usando hidróxido de sodio 4 N ajustando el pH de la capa acuosa a 12-13. La capa orgánica se recolectó y se lavó con agua (300 mL). La capa orgánica se destiló a 80 °C y el solvente se intercambió con acetonitrilo (2 x 300 mL), para eliminar diclorometano y trietilamina residual. Los sólidos se suspendieron en acetonitrilo (600 mL), y la mezcla se calentó hasta disolver los sólidos (~75°C). La solución se enfrió hasta ocurrir la nucleación (~55 - 65°C) y se mantuvo por 1 h. La lechada se

enfrió a 20°C durante 2 h, y luego a 0-5°C durante 30 min, seguido por agitación a 0-5°C durante 30 min. Los sólidos se filtraron y se lavó con acetonitrilo frío (60 mL). La torta húmeda se secó bajo vacío a 60 °C durante 6 h para dar el compuesto del título. (28,3 g, 85% de rendimiento).

Ejemplo 217: Síntesis de metiléster de ácido 4-(4-[[[2-isopropil-1H-benzoimidazol-4-carbonil)-amino]metil]piperidin-1-ilmetil]piperidin-1-carboxílico cristalino (Forma I)

Se dispersó metiléster de ácido 4-(4-[[[2-isopropil-1H-benzoimidazol-4-carbonil)-amino]metil]piperidin-1-ilmetil]piperidin-1-carboxílico en forma sólida amorfa, preparado de acuerdo con el proceso del Ejemplo 214 se dispersó en los diluyentes inertes listados en la Tabla X a continuación. La mezclas se expusieron a la atmósfera y se dejaron evaporar por completo. Los sólidos resultantes se caracterizaron mediante difracción de rayos-x en polvo. Todos los sólidos se demostró que eran cristalinos con un patrón de difracción de rayos-x en polvo consistente con el indicado a continuación en Ejemplo 220, que se obtuvo a partir de la muestra del Ejemplo 215.

Tabla X: Síntesis de formas cristalinas

Diluyente	Compuesto de Fórmula (l) (mg)	Volumen de diluyente (mL)
Éter	4,60	0,230
Ciclohexano	4,87	0,486
Acetato de etilo	5,67	0,284

Ejemplo 218: Síntesis de metiléster de ácido 4-(4-[[2-isopropil-1H-benzoimidazol-4-carbonil)-amino]metil]piperidin-1-ilmetil)piperidin-1-carboxílico cristalino (Forma II)

Se dispersó metiléster de ácido 4-(4-[[2-isopropil-1H-benzoimidazol-4-carbonil)-amino]metil]piperidin-1-ilmetil)piperidin-1-carboxílico en forma sólida amorfa (42,2 mg), se dispersó en hexano (4,22 mL) a temperatura ambiente a una concentración final de 10 mg/mL. La solución se sonicó para dispersar los sólidos más grandes. Después de 24 h a temperatura ambiente (aproximadamente 22 °C) la cristalización había ocurrido. Los sólidos cristalinos se aislaron mediante filtración al vacío, antes del análisis.

Ejemplo 219: Síntesis de metiléster de ácido 4-(4-[[2-isopropil-1H-benzoimidazol-4-carbonil)-amino]metil]piperidin-1-ilmetil)piperidin-1-carboxílico cristalino (Forma III)

Se disolvió metiléster de ácido 4-(4-[[2-isopropil-1H-benzoimidazol-4-carbonil)-amino]metil]piperidin-1-ilmetil)piperidin-1-carboxílico en forma sólida amorfa (38 mg) en una mezcla de solventes 1:1 de etanol:agua (1,9 mL) a temperatura ambiente a una concentración final de 20 mg/mL. La solución se sonicó por 30 segundos para asegurar una disolución completa. La solución luego se dejó evaporar lentamente en un frasco destapado. Después de 24 h a temperatura ambiente la cristalización había ocurrido. Los sólidos cristalinos se aislaron mediante filtración al vacío. La torta de filtrado se lavó una vez con una mezcla de solventes 1:1 de etanol:agua, antes del análisis.

Ejemplo 220: Difracción de rayos X en polvo

Se obtuvieron patrones de difracción de polvo en rayos X con un Difractómetro de Rayos X Thermo ARL Modelo X'TRA (Thermo ARL SA, Suiza), usando radiación de Cu K α a 1,542 Å (45 kV, 40 mA) con un detector Si(Li) de estado sólido. El análisis se efectuó típicamente a una velocidad de análisis de 2°/minuto, con un tamaño de paso de 0,03° por punto en un rango de entre 2 y 35° en ángulos dos theta. Las muestras, tal como se las recibió o trituradas hasta obtener un polvo fino, se envasaron con cuidado en un inserto de cuarzo de 7,8 mm de diámetro y 0,5 mm de profundidad, diseñado para que pudiera ser colocado en el recipiente de análisis de muestras del instrumento de carga superior. La calibración del instrumento en un ángulo dos theta de $\pm$ 0,02° se verificó cada semana con referencia a un matel siliconado. En la Figura 1 se ilustra un patrón de PXRD representativo para el compuesto cristalino del Ejemplo 215 (Forma I), que se trituró a mano hasta obtener un polvo. En la Figura 5 se ilustra un patrón de PXRD representativo de una muestra de la forma cristalina III, obtenida con un difractómetro Rigaku con radiación de Cu K α (30 kV, 15 mA).

Ejemplo 221: Análisis de la estructura por rayos X

a. Forma I

Un trozo del cristal producido en el Ejemplo 215, con dimensiones de 0,33 x 0,17 x 0,11 mm, se montó en una fibra de vidrio. Los datos de la estructura por rayos X se obtuvieron usando un detector de área de rayos SMART 6K CCD con un diámetro de ventana de 13,5 cm, controlado por software SMART versión 5.630 (Bruker, 2003) usando radiación Cu K α . La distancia entre la muestra y el detector fue de 5,039 cm. Los datos se

recolectaron a una temperatura de $-153 \pm 1^\circ\text{C}$, y se analizaron usando software SHELXS versión 6.14 (Bruker, 2003). Se obtuvieron los siguientes parámetros representativos: la celda individual es ortorrómbica, con dimensiones $a = 16,9053 \text{ \AA}$, $b = 9,5172 \text{ \AA}$, $c = 15,4659 \text{ \AA}$; el espacio de grupo es $Pna2_1$; la densidad calculada es de $1,22 \text{ g/cm}$. Los picos de difracción de rayos X en polvo predichos a partir de las posiciones atómicas derivadas concuerdan en gran medida con los resultados observados, como se describe en el Ejemplo 220 y se ilustra en la Tabla XI.

Tabla XI: Posiciones de los picos de PXRD

Observado 2θ (grados)	Predicho 2θ (grados)
$15,08 \pm 0,20$	$15,1 \pm 0,2$
$15,41 \pm 0,20$	$15,6 \pm 0,2$
$19,00 \pm 0,20$	$19,2 \pm 0,2$
$19,70 \pm 0,20$	$19,5 \pm 0,2$
$23,68 \pm 0,20$	$23,7 \pm 0,2$

b. Forma III

Un trozo del cristal producido con el proceso del Ejemplo 219, con dimensiones de $0,35 \times 0,12 \times 0,09 \text{ mm}$, se analizó con el método descrito con anterioridad. Se obtuvieron los siguientes parámetros de la red: la celda individual es monoclinica, con dimensiones $a = 14,8101 \text{ \AA}$, $b = 9,9985 \text{ \AA}$, $c = 17,9222 \text{ \AA}$; $\beta = 106,3020^\circ$, el espacio de grupo es $P2_1/n$; la densidad calculada es de $1,23 \text{ g/cm}^3$.

Ejemplo 222: Análisis térmico

La calorimetría diferencial de barrido (DSC) se realizó usando un módulo TA Instruments Modelo Q-100. Los datos se recolectaron y se analizaron

usando software TA Instruments Thermal Advantage para la serie Q™. Se pesó con precisión una muestra de aproximadamente 7 mg en una plancha de aluminio con tapa. La muestra se evaluó usando una pendiente de calentamiento lineal de 10°C/minuto, entre 5°C y aproximadamente 200°C. La celda de DSC se purgó con nitrógeno seco durante el uso.

El análisis termogravimétrico (TGA) se realizó usando un módulo TA Instruments Modelo Q-500. Los datos se recolectaron y se analizaron usando software TA Instruments Thermal Advantage para la serie Q™. Se colocó una muestra que pesaba aproximadamente 2 mg en un recipiente de aluminio en una base de paladio, y se la analizó entre la temperatura ambiente y aproximadamente 300°C, con una velocidad de calentamiento lineal de 10°C/minuto. Las recámaras de equilibrio y el horno se purgaron con nitrógeno durante el uso.

Se ilustran gráficas representativas de DSC y TGA de material de la forma cristalina I (preparada de acuerdo con el proceso del Ejemplo 216), la Forma II y la Forma III en las Figuras 2, 4, y 6, respectivamente.

Ejemplo 223: Evaluación de la absorción dinámica de humedad

La evaluación de absorción dinámica de humedad (DMS) se realizó a 25°C usando una microbalanza atmosférica VTI, sistema SGA-100 (VTI Corp., Hialeah, FL 33016). Se usó un tamaño de muestra de aproximadamente 5-10 mg y se fijó la humedad en el valor ambiental al comenzar el análisis. Un análisis típico de DMS consistió en tres barridos: humedad relativa (HR) ambiente hasta 2%, entre 2% de HR y 90% de HR, entre 90% de HR y 5% de HR a una velocidad de análisis de 5% de HR/paso. Se midió la masa cada dos minutos y se cambió la HR hasta el siguiente valor ($\pm 5\%$ de HR) cuando la

masa de la muestra se encontraba estable dentro de un 0,02 % para 5 puntos consecutivos. En la Figura 3 se ilustra una isoterma de DMS representativa del compuesto cristalino del Ejemplo 215 (Forma I).

El compuesto cristalino de la invención presenta un perfil de adsorción/desorción reversible, con un cambio de peso menor que 0,25% para el rango completo entre 2% y 90% de RH, y un cambio de peso menor que 0,1% para el rango de humedad crítico de entre 40% y 75% de RH.

Ejemplo 224: Análisis infrarrojo

El espectro de absorción infrarroja (IR) del compuesto cristalino del Ejemplo 215 (Forma I) se determinó para el rango de frecuencia de entre 4000 y 675 cm^{-1} , usando un espectrómetro Avatar 360 FT-IR equipado con un recipiente para muestra con reflexión total atenuada (ATR) Nicolet. Un espectro de absorción IR representativo para una muestra del compuesto cristalino de la invención presentó bandas de absorción significativas en 766 ± 1 , 1097 ± 1 , 1251 ± 1 , 1413 ± 1 , 1449 ± 1 , 1579 ± 1 , 1609 ± 1 , 1640 ± 1 y $1696 \pm 1 \text{ cm}^{-1}$.

Ejemplo 225: Evaluación de la estabilidad en estado sólido

Las muestras del compuesto cristalino de Forma I, preparadas de acuerdo con el proceso del Ejemplo 216, se almacenaron en múltiples recipientes de vidrio abiertos a 40°C y 75% de RH. A intervalos específicos, se retiraron los contenidos de un recipiente representativo y se los analizó por DSC, TGA, PXRD y HPLC, para determinar la pureza química. Después de tres meses de almacenamiento, no hubo un cambio detectable en los termogramas de DSC o TGA ni el patrón de PXRD. La pureza química de la muestra almacenada fue de 99,5 %.

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Ensayo 1: Ensayo de unión a radioligando en receptores 5-HT_{4(c)} humanos**a. Preparación de membranas con 5-HT_{4(c)}**

Se cultivaron células HEK-293 (riñón embrionario humano) transfectadas en forma estable con ADNc del receptor 5-HT_{4(c)} humano (B_{max} = aproximadamente 6,0 pmol/mg de proteína, determinado usando el ensayo de unión a radioligando en membrana con [³H]-GR113808) en frascos T-225 con medio de Eagle modificado por Dulbecco (DMEM), que contenía 4500 mg/l de D-glucosa y clorhidrato de piridoxina (GIBCO-Invitrogen Corp., Carlsbad CA: N° Cat 11965) suplementado con 10% de suero fetal bovino (FBS) (GIBCO-Invitrogen Corp.: N° Cat 10437), L-glutamina 2 mM y (100 unidades) penicilina (100 µg) estreptomicina/ml (GIBCO-Invitrogen Corp.: N° Cat 15140) en una incubadora húmeda con 5% de CO₂ a 37°C. Se cultivaron las células bajo una presión de selección continua agregando 800 µg/ml de geneticina (GIBCO-Invitrogen Corp.: N° Cat 10131) al medio.

Se cultivaron las células hasta alcanzar aproximadamente 60-80% de confluencia (< 35 de pasajes de subcultivo). 20-22 horas antes de la cosecha, se lavaron las células dos veces y se les agregó DMEM libre de suero. Todos los pasos de fabricación se realizaron en hielo. Se levantó la monocapa de células por agitación mecánica y se tituló con una pipeta de 25 ml. Se recolectaron las células por centrifugación a 1000 rpm (5 minutos).

Para la preparación de membrana, se suspendieron los precipitados celulares en ácido 4-(2-hidroxietil)-1-piperazin-etansulfónico 50 mM (HEPES), pH 7,4 (amortiguador de preparación de membrana) (40 ml/rendimiento celular total de 30-40 frascos T225) y se homogenizó usando un destructor de tejidos politron (con parámetros de 19, 2 x 10 segundos) en hielo. Los homogenatos

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resultantes se centrifugaron a 1200 g por 5 minutos a 4°C. Se descartó el precipitado y se centrifugó el sobrenadante a 40000 g (20 minutos). Se lavó el precipitado una vez resuspendiéndolo con amortiguador de preparación de membranas y centrifugándolo a 40000 g (20 minutos). Se suspendió nuevamente el precipitado final en HEPES 50 mM, pH 7,4 (amortiguador de ensayo) (equivalente a 1 frasco T225 /1 ml). Se determinó la concentración de proteínas en la suspensión de membrana empleando el método de Bradford (Bradford, 1976). Se almacenaron las membranas en alícuotas a -80°C.

b. Ensayos de unión a radioligandos

Se realizaron ensayos de unión a radioligandos en placas de ensayo de polipropileno de 96 cavidades profundas de 1,1 ml (Axygen), en un volumen de ensayo total de 400 µl, que contenían 2 µg de proteínas de membrana en HEPES 50 mM, pH 7,4, con 0,025% de albúmina de suero bovino (BSA). Se realizaron los estudios de saturación para determinar los valores de K_d del radioligando usando [3 H]-GR113808 (Amersham Inc., Bucks, Reino Unido: N° Cat TRK944; actividad específica de aproximadamente 82 Ci/mmol) a 8-12 concentraciones diferentes, que variaron entre 0,001 nM y 5,0 nM. Se realizaron los ensayos de desplazamiento para determinar los valores de pK_i de los compuestos con [3 H]-GR113808 0,15 nM y once concentraciones diferentes del compuesto, que variaron entre 10 pM y 100 µM.

Los compuestos de prueba se recibieron como soluciones madre 10 mM en DMSO, y se los diluyó hasta 400 µM en HEPES 50 mM, pH 7,4, a 25°C, que contenía 0,1% de BSA, y luego se realizaron diluciones seriales (1:5) en el mismo amortiguador. Se determinó la unión no específica en presencia de GR113808 1 µM sin marcar. Se incubaron los ensayos por 60 minutos a

temperatura ambiente, y luego se terminaron las reacciones de unión realizando una filtración rápida en placas de fibra de vidrio de 96 cavidades GF/B (Packard BioScience Co., Meriden, CT), empapadas previamente en 0,3% de polietilenimina. Se lavaron las placas de filtro tres veces con amortiguador de filtración (HEPES 50 mM helado, pH 7,4) para eliminar la radiactividad no unida. Se secaron las placas, se agregaron 35 µl de fluido de centelleo líquido Microscint-20 (Packard BioScience Co., Meriden, CT) a cada cavidad y se realizó el conteo de las placas en un contador de centelleo líquido Packard Topcount (Packard BioScience Co., Meriden, CT).

Se analizaron los datos de unión empleando análisis de regresión no lineal con el conjunto de software GraphPad Prism (GraphPad Software, Inc., San Diego, CA), usando el modelo de 3 parámetros para competición por un sitio. Se ajustó el BOTTOM (mínimo de la curva) de acuerdo con el valor de la unión no específica, determinado en presencia de GR113808 1 µM. Se calcularon los valores de K_i para los compuestos de prueba, en Prism, para los valores de IC_{50} de mayor ajuste, y el valor de K_d del radioligando, usando la ecuación de Cheng-Prusoff (Cheng y Prusoff, *Biochemical Pharmacology*, 1973, 22, 3099-108): $K_i = IC_{50} / (1 + [L]/K_d)$, donde $[L]$ = concentración [3H]-GR113808. Se expresan los resultados como el logaritmo decimal negativo de los valores de K_i , pK_i .

Los compuestos de prueba con mayores valores de pK_i en este ensayo presentan una mayor afinidad de unión por el receptor 5-HT₄. Los compuestos de la invención evaluados en este ensayo tuvieron un valor de pK_i que varió entre aproximadamente 7,0 y aproximadamente 10,0. ?

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**Ensayo 2: Ensayo de unión a radioligando en receptores 5-HT_{3A} humanos:
determinación de la selectividad por subtipos del receptor**

a. Preparación de membranas con 5-HT_{3A}

Se obtuvieron células HEK-293 (riñón embrionario humano) transfectadas en forma estable con ADNc del receptor 5-HT_{3A} humano del Dr. Michael Bruess (Universidad de Bonn, RDA) (B_{max} = aproximadamente 9,0 pmol/mg de proteína, determinado usando el ensayo de unión a radioligando en membrana con [³H]-GR65630) en frascos T-225 o fábricas de células con medio de Eagle modificado por Dulbecco (DMEM) al 50% (GIBCO-Invitrogen Corp., Carlsbad, CA: N° Cat 11965) y 50% de F12 de Ham (GIBCO-Invitrogen Corp.: N° Cat 11765) suplido con 10% de suero fetal bovino inactivado con calor (FBS) (Hyclone, Logan, UT: Cat Nro SH30070,03) y (50 unidades) penicilina (50 µg) estreptomicina/ml (GIBCO-Invitrogen Corp.: N° Cat 15140) en una incubadora húmeda con 5% de CO₂ a 37°C.

Se cultivaron las células hasta alcanzar aproximadamente 70-80% de confluencia (< 35 de pasajes de subcultivo). Todos los pasos de fabricación se realizaron en hielo. Para cosechar las células, se aspiraron los medios y se lavaron las células con solución salina amortiguada con fosfato de Dulbecco, libre de Ca²⁺ y Mg²⁺ (dPBS). Se levantó la monocapa de células aplicando una agitación mecánica suave. Se recolectaron las células por centrifugación a 1000 rpm (5 minutos). Los pasos subsiguientes de la preparación de membrana se realizaron de acuerdo con el protocolo descrito previamente para las membranas que expresaban receptores 5-HT_{4(c)}.

b. Ensayos de unión a radioligandos

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Se realizaron ensayos de unión a radioligandos en placas de ensayo de polipropileno de 96 cavidades, en un volumen de ensayo total de 200 μ l, que contenían 1,5-2 μ g de proteínas de membrana en HEPES 50 mM, pH 7,4, con 0,025% de amortiguador de ensayo de BSA. Se realizaron los estudios de saturación para determinar los valores de K_d del radioligando usando [3 H]-GR65630 (Perkin Elmer Life Sciences Inc., Boston, MA: N° Cat NET1011, actividad específica de aproximadamente 85 Ci/mmol) a doce concentraciones diferentes, que variaron entre 0,005 nM y 20 nM. Se realizaron los ensayos de desplazamiento para determinar los valores de pK_i de los compuestos con [3 H]-GR65630 0,50 nM y once concentraciones diferentes del compuesto, que variaron entre 10 pM y 100 μ M. Los compuestos se recibieron como soluciones madre 10 mM en DMSO (véase la sección 3,1), se diluyeron hasta 400 μ M en HEPES 50 mM, pH 7,4, a 25°C, que contenía 0,1% de BSA, y luego se realizaron diluciones en serie (1:5) en el mismo amortiguador. Se determinó la unión no específica en presencia de MDL72222 10 μ M sin marcar. Se incubaron los ensayos por 60 minutos a temperatura ambiente, y luego se terminaron las reacciones de unión realizando una filtración rápida en placas de fibra de vidrio de 96 cavidades GF/B (Packard BioScience Co., Meriden, CT), empapadas previamente en 0,3% de polietilenimina. Se lavaron las placas de filtro tres veces con amortiguador de filtración (HEPES 50 mM helado, pH 7,4) para eliminar la radiactividad no unida. Se secaron las placas, se agregaron 35 μ l de fluido de centelleo líquido Microscint-20 (Packard BioScience Co., Meriden, CT) a cada cavidad y se realizó el conteo de las placas en un contador de centelleo líquido Packard Topcount (Packard BioScience Co., Meriden, CT).

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Se analizaron los datos de unión empleando el procedimiento de regresión no lineal descripto previamente para determinar valores de K_i . Se ajustó el BOTTOM (mínimo de la curva) de acuerdo con el valor de la unión no específica, determinado en presencia de MDL72222 10 μ M. Se definió la cantidad [L] en la ecuación de Cheng-Prusoff como la concentración de [3 H]-GR65630.

Se calculó la selectividad de subtipos del receptor 5-HT₄ por subtipos del receptor 5-HT₃ como la relación $K_i(5\text{-HT}_{3A})/K_i(5\text{-HT}_{4(c)})$. Los compuestos de la invención evaluados en este ensayo presentaron una selectividad por el subtipo del receptor 5-HT₄/5-HT₃ que varió entre aproximadamente 4000 y más de 400000.

Ensayo 3: Ensayo de acumulación de AMPc en células completas en placas instantáneas, con células HEK-293 que expresan receptores 5-HT_{4(c)}

En este ensayo se determinó la potencia funcional de un compuesto de prueba midiendo la cantidad de AMP cíclico producido cuando se pusieron en contacto células HEK-293, que expresaban receptores 5-HT₄, con distintas concentraciones de compuesto de prueba.

a. Cultivo de las células

Se prepararon células HEK-293 (riñón embrionario humano) transfectadas en forma estable con ADNc clonado del receptor 5-HT_{4(c)} humano, que expresaban el receptor a dos densidades diferentes: (1) una densidad de aproximadamente 0,5-0,6 pmol/mg de proteína, determinada usando un ensayo de unión a radioligando [3 H]-GR113808 en membrana, y (2)

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una densidad de aproximadamente 6,0 pmol/mg de proteína. Se cultivaron las células en frascos T-225 con medio de Eagle modificado por Dulbecco (DMEM), que contenía 4500 mg/l de D-glucosa y clorhidrato de piridoxina (GIBCO-Invitrogen Corp., Carlsbad CA: N° Cat 11965) suplementado con 10% de suero fetal bovino (FBS) (GIBCO-Invitrogen Corp.: N° Cat 10437), L-glutamina 2 mM y (100 unidades) penicilina (100 µg) estreptomycin/ml (GIBCO-Invitrogen Corp.: N° Cat 15140) en una incubadora húmeda con 5% de CO₂ a 37°C. Se cultivaron las células bajo una presión de selección continua agregando geneticina (800 µg/ml; GIBCO-Invitrogen Corp.: GIBCO-Invitrogen Corp.: N° Cat 10131) al medio.

b. Preparación de las células

Se cultivaron las células hasta alcanzar aproximadamente 60-80% de confluencia. Entre veinte y veintidós horas antes del ensayo, se lavaron las células dos veces y se les agregó DMEM libre de suero, que contenía 4500 mg/l de D-glucosa (GIBCO-Invitrogen Corp.: N° Cat 11965). Para cosechar las células, se aspiraron los medios y se agregaron 10 ml de Versene (GIBCO-Invitrogen Corp.: Cat N° 15040) a cada recipiente T-225. Las células se incubaron por 5 minutos a temperatura ambiente, y luego se separaron del recipiente con agitación mecánica. Se transfirió la suspensión celular a un tubo de centrifuga que contenía un volumen igual de dPBS tibia (37°C), y se centrifugó por 5 minutos a 1000 rpm. Se descartó el sobrenadante y se suspendió nuevamente el precipitado en amortiguador de estimulación entibado con anterioridad (37°C) (equivalente a 10 ml por cada 2-3 frascos T-225). Se registró este momento y se lo anotó como cero. Se contaron las células con un contador Coulter (conteo superior a 8 µm, el rendimiento por

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frasco fue de $1-2 \times 10^7$ células/frasco). Se suspendieron nuevamente las células a una concentración de 5×10^5 células/ml, en amortiguador de estimulación entibado con anterioridad (37°C) (tal como se obtiene en el conjunto de elementos de placas instantáneas), y se preincubó a 37°C por 10 minutos.

Se realizaron ensayos de AMPc en un formato de radioinmunoensayo, usando el sistema de ensayo de activación de adenilil ciclase en placas instantáneas con ^{125}I -AMPc (SMP004B, Perkin Elmer Life Sciences Inc., Boston, MA), de acuerdo con las instrucciones del fabricante.

Las células se cultivaron y se las preparó como se describió previamente. Las concentraciones finales de las células en el ensayo fueron de 25×10^3 células/cavidad, y el volumen de ensayo final fue de 100 μl . Los compuestos de prueba se recibieron como soluciones madre 10 mM en DMSO, y se los diluyó hasta 400 μM en HEPES 50 mM, pH 7,4, a 25°C , que contenía 0,1% de BSA, y luego se realizaron diluciones seriales (1:5) en el mismo amortiguador. Se realizaron ensayos de acumulación de AMP cíclico con 11 concentraciones diferentes de compuesto, que variaron entre 10 pM y 100 μM (concentraciones finales de ensayo). Se incluyó una curva de concentración de 5-HT-respuesta (entre 10 pM y 100 μM) en cada placa. Se incubaron las células, con agitación, a 37°C por 15 minutos, y se terminó la reacción agregando 100 μl de amortiguador de detección helado (como se obtiene en el conjunto de elementos de placas instantáneas) a cada cavidad. Se sellaron las placas y se las incubó a 4°C durante la noche. Se cuantificó la radiactividad unida por espectroscopía de proximidad de centelleo usando el dispositivo Topcount (Packard BioScience Co., Meriden, CT).

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Se extrapoló la cantidad de AMPc producido por ml de reacción a partir de la curva de referencia de AMPc, de acuerdo con las instrucciones proporcionadas en el manual de usuario del fabricante. Se analizaron los datos realizando análisis de regresión no lineal con el conjunto de software GraphPad Prism, usando el modelo de respuesta a la dosis sigmoidal de 3 parámetros (pendiente restringida a la unidad). Se informan los datos de potencia como valores de pEC_{50} , el logaritmo decimal negativo del valor de EC_{50} , donde EC_{50} es la concentración efectiva para obtener un 50% de respuesta máxima.

Los compuestos de prueba con un mayor valor de pEC_{50} en este ensayo tienen una potencia de agonismo mayor sobre el receptor 5-HT₄. Los compuestos de la invención evaluados en este ensayo, por ejemplo, en la línea celular (1), con una densidad de aproximadamente 0,5-0,6 pmol/mg de proteína, presentaron un valor de pEC_{50} que varió entre aproximadamente 7,5 y aproximadamente 9,5.

Ensayo 4: Ensayo de pinza de tensión *en vivo* de la inhibición de la corriente de iones de potasio en células completas que expresan el canal de potasio cardiaco hERG

Se obtuvieron células CHO-K1 transformadas en forma estable con ADNc de hERG de Gail Robertson, en la Universidad de Wisconsin. Se mantuvieron las células en almacenamiento criogénico hasta utilizarlas. Se expandieron las células y se las sometió a un pasaje en medio Eagle modificado por Dulbecco/F12, suplementado con 10% de suero fetal bovino y 200 µg/ml de geneticina. Se sembraron las células en cubreobjetos de vidrio recubiertos con poli-D-lisina (100 µg/ml), en placas de 35 mm² (que contenían 2

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ml de medio), a una densidad que permitió seleccionar las células aisladas para realizar estudios de pinza de tensión. Se mantuvieron las placas en un ambiente humidificado con 5% de CO₂ a 37°C.

Se preparó solución extracelular al menos cada 7 días y se la almacenó a 4°C mientras no se la utilizaba. La solución extracelular contenía (mM): NaCl (137), KCl (4), CaCl₂ (1,8), MgCl₂ (1), glucosa (10), ácido 4-(2-hidroxietil)-1-piperazin-etansulfónico (HEPES) (10), pH 7,4, con NaOH. La solución extracelular, en ausencia o presencia de compuesto de prueba, se colocó en recipientes de reserva, desde los que se la hizo fluir hacia la cámara de registro a aproximadamente 0,5 ml/minuto. Se preparó la solución intracelular, se la separó en alícuotas y se la almacenó a -20°C hasta el día en que se la usó. La solución intracelular contenía (mM): KCl (130), MgCl₂ (1), sal de ácido etilen glicol-bis(beta-aminoetil éter) *N,N,N',N'*-tetra acético (EGTA) (5), MgATP (5), ácido 4-(2-hidroxietil)-1-piperazin-etansulfónico (HEPES) (10), pH 7,2, con KOH. Todos los experimentos se realizaron a temperatura ambiente (20-22°C). Se transfirieron los cubreobjetos donde se habían sembrado las células a una cámara de registro, y se los perfundió en forma continua. Se formaron sellos Gigaohm entre las células y los electrodos de patch. Una vez obtenido un patch estable, se comenzó el registro en el modo de pinza de tensión, con un potencial de retención inicial de -80 mV. Después de obtener una corriente de células completas estable, se expusieron las células al compuesto de prueba. El protocolo de pinza de tensión fue: un paso con un potencial de retención de entre -80 mV y +20 mV por 4,8 segundos, repolarización a -50 mV por 5 segundos y luego retorno al potencial de retención original (-80 mV). Se puso en práctica este protocolo de tensión cada 15 segundos (0,067 Hz). Se

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determinaron las amplitudes de las corrientes pico durante la fase de repolarización usando el software pClamp. Se perfundieron los compuestos de prueba, a una concentración de 3 μ M, sobre las células durante 5 minutos, a lo que siguió un período de lavado de 5 minutos en ausencia de compuesto. Finalmente, se agregó un control positivo (cisapride 20 nM) a la porción perfundida para determinar la función de las células. El paso de cambio entre -80 mV y +20 mV activa el canal hERG, lo que da como resultado una corriente hacia el exterior. El paso de retorno a -50 mV da como resultado una corriente hacia el exterior, a medida que el canal se recupera de la inactivación y se desactiva.

Se determinaron las amplitudes de las corrientes pico durante la fase de repolarización usando el software pCLAMP. Se exportaron los datos de los artículos control y de prueba a Origin® (OriginLab Corp., Northampton MA), donde se normalizaron la amplitudes de las corrientes individuales respecto de la amplitud de la corriente inicial en ausencia de compuesto. Se calcularon los promedios y los errores estándar para cada condición, y se los representó versus el transcurso del tiempo en el experimento.

Se realizaron comparaciones entre las inhibiciones observadas de la corriente de K^+ después de una exposición de cinco minutos al artículo de prueba o al vehículo control (usualmente DMSO al 0,3%). Se realizaron comparaciones estadísticas entre grupos experimentales usando una prueba de t independiente para dos poblaciones (Microcal Origin v. 6,0). Las diferencias se consideraron significativas para $p < 0,05$.

A menor porcentaje de inhibición de la corriente iónica en este ensayo, menor es el potencial de los compuestos de prueba de cambiar el patrón de

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repolarización cardíaca cuando se los usa como agentes terapéuticos. Por ejemplo, los compuestos de Ejemplos 1-14, evaluados en este ensayo con una concentración de 3 μM , presentaron una inhibición de la corriente de iones de potasio menor que aproximadamente 30%, incluyendo valores menores que aproximadamente 20%.

Ensayo 5: Modelo de biodisponibilidad oral *in vitro*: ensayo de permeabilidad Caco-2

Se realizó el ensayo de permeabilidad Caco-2 para modelar la capacidad de los compuestos de prueba de pasar a través del intestino y alcanzar el torrente sanguíneo después de una administración oral. Se determinó la velocidad a la que los compuestos de prueba en solución atravesaban una monocapa celular diseñada para imitar la unión estrecha entre las monocapas del intestino humano.

Se obtuvieron células Caco-2 (adenocarcinoma de colon humano) en la ATCC (Colección Americana de Tipos de Cultivo; Rockville, MD). Para el estudio de permeabilidad, se sembraron las células a una densidad de 63000 células/cm² en filtros de policarbonato con trans-cavidades humedecidas previamente (Costar; Cambridge, MA). Se formó una monocapa de células después de 21 días de cultivo. Después de realizar el cultivo de células en la placa con trans-cavidades, se retiró la membrana que contenía la monocapa celular de la placa con trans-cavidades y se la insertó en la cámara de difusión (Costar; Cambridge, MA). Se insertó la cámara de difusión en el bloque de calentamiento, que estaba equipado con un circuito externo de circulación de agua, con la temperatura regulada por un termostato a 37°C. El colector de aire

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administraba 95% de O₂/5% de CO₂ a cada mitad de una cámara de difusión, y creaba un patrón de flujo laminar a través de la monocapa celular, lo que resultó efectivo para reducir la capa límite indeseada.

Se realizó el estudio de permeabilidad con concentraciones de compuesto de prueba de 100 μ M, y con ¹⁴C-manitol para monitorear la integridad de la monocapa. Todos los experimentos se realizaron a 37°C por 60 minutos. Se tomaron muestras a los 0, 30 y 60 minutos de los lados del donante y el receptor de la cámara. Se analizaron las muestras por HPLC o conteo de centelleo líquido para determinar las concentraciones de compuesto y manitol. Se calculó el coeficiente de permeabilidad (K_p) en cm/segundo.

En este ensayo, se considera que un valor de K_p mayor que aproximadamente 10×10^{-6} cm/segundo indica una biodisponibilidad favorable. Los compuestos de la invención evaluados en este ensayo típicamente presentaron valores de K_p de entre aproximadamente 10×10^{-6} cm/segundo y aproximadamente 50×10^{-6} cm/segundo.

Ensayo 6: Estudio farmacocinético en rata

Se prepararon formulaciones en solución acuosa de los compuestos de pruebas en ácido láctico al 0,1%, a un pH de entre aproximadamente 5 y aproximadamente 6. Se dosificaron ratas Sprague-Dawley macho (cepa CD, Charles River Laboratories, Wilmington, MA) con los compuestos de prueba por dosificación intravenosa (IV), a una dosis de 2,5 mg/kg o por administración oral (PO), a una dosis de 5 mg/kg. El volumen de dosificación fue de 1 ml/kg para la administración IV y 2 ml/kg para la administración PO. Se extrajeron muestras de sangre en serie de los animales antes de la dosificación, y 2 (IV solamente), 5, 15 y 30 minutos, y 1, 2, 4, 8 y 24 horas después de la

dosificación. Se determinaron las concentraciones de compuestos de prueba en plasma sanguíneo por medio de un análisis de cromatografía líquida-espectrometría de masa (LC-MS/MS) (MDS SCIEX, API 4000, Applied Biosystems, Foster City, CA), con un límite de cuantificación inferior de 1 ng/ml. Se evaluaron los parámetros farmacocinéticos convencionales realizando análisis no compartimentados (modelo 201 para la dosificación IV y modelo 200 para la dosificación PO), usando WinNonlin (versión 4,0,1, Pharsight, Mountain View, CA). El máximo en la curva de concentración de compuesto de prueba en plasma sanguíneo versus tiempo se denota C_{max} . Se calculó el área bajo la curva de concentración versus tiempo desde el momento de la dosificación hasta la última concentración mensurable ($AUC(0-t)$) empleando la regla trapezoidal lineal. Se calculó la biodisponibilidad oral ($F(\%)$), es decir, la relación entre $AUC(0-t)$ para una administración PO y $AUC(0-t)$ para una administración IV, normalizada de acuerdo con la dosis, como se indica a continuación:

$$F(\%) = AUC_{PO}/AUC_{IV} \times Dosis_{IV}/Dosis_{PO} \times 100\%$$

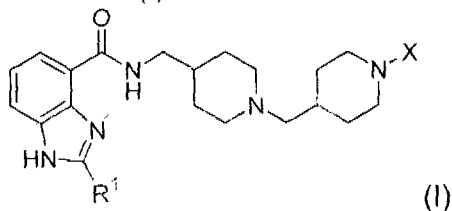
Se espera que los compuestos de prueba con mayores valores de los parámetros C_{max} , $AUC(0-t)$ y $F(\%)$ en este ensayo presenten una mayor biodisponibilidad cuando se los administren por vía oral. Los compuestos preferidos de la invención presentaron valores de C_{max} que típicamente variaron entre aproximadamente 0,06 y aproximadamente 0,8 $\mu\text{g/ml}$ y valores de $AUC(0-t)$ que típicamente variaron entre aproximadamente 0,14 y aproximadamente 1,2 $\mu\text{g}\cdot\text{hr/ml}$. A modo de ejemplo, el compuesto del Ejemplo 1 tuvo un valor de C_{max} de 0,8 $\mu\text{g/ml}$, un valor de $AUC(0-t)$ de 1,2 $\mu\text{g}\cdot\text{hr/ml}$ y una biodisponibilidad oral ($F(\%)$) en el modelo en rata de aproximadamente 75%.

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Si bien se ha descrito la presente invención con referencia a realizaciones específicas, los especialistas en la técnica deberán comprender que pueden efectuarse diversos cambios y pueden sustituirse elementos equivalentes, sin apartarse del espíritu verdadero y el alcance de la invención. Además, pueden efectuarse muchas modificaciones para adaptar una situación particular, un material, una composición relacionada, un proceso, uno o más pasos de un proceso al objetivo, el espíritu y el alcance de la presente invención. Todas estas modificaciones quedan dentro del alcance de las reivindicaciones adjuntas. Adicionalmente, todas las publicaciones, las patentes y los documentos de patentes citados en la presente documentación se incorporan por completo a modo de referencia en la presente documentación, en la misma extensión en que se incorpora cada uno individualmente a modo de referencia.

REIVINDICACIONES:

1. Un compuesto de fórmula (I):



en donde:

R^1 es C_{3-5} alquilo, opcionalmente sustituido con -OH; y

X se selecciona entre

(a) $-C(O)OR^2$ en donde R^2 es C_{1-4} alquilo o $-(CH_2)_n$ -fenilo en donde n es 0 o 1;

(b) $-C(O)R^3$ en donde R^3 se selecciona entre :

fenilo, opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre

C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, $-OCHF_2$, y $-CN$,

C_{1-5} alquilo,

C_{4-5} cicloalquilo, y

$-(CH_2)_m$ -A en donde m es 0 o 1 y A se selecciona entre amino, furanilo, tiofenilo, morfolinilo, tetrahydrofuranilo, piridinilo, naftalenilo, pirrolilo, tiomorfolinilo, pirrolidinilo, piperidinilo, oxoazetidinilo, tiazolidinilo, 1,1-dioxo isotiazolidinilo, y 2,4-dimetilisoxazolilo;

(c) $-C(O)NR^4R^5$ en donde R^4 es hidrógeno o C_{1-3} alquilo, y R^5 es fenilo opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, y $-OCHF_2$;

(d) $-C(O)C(R^6R^7)R^8$ en donde R^6 es hidrógeno o C_{1-3} alquilo y R^7 es hidrógeno, -OH, o C_{1-3} alquilo; o R^6 y R^7 juntos forman oxo o $-(CH_2)_2$; y R^8 es fenilo o

ciclohexilo, en donde fenilo o ciclohexilo se sustituyen opcionalmente con 1, 2, o 3 sustituyentes seleccionados entre C₁₋₄alquilo, halo, C₁₋₄alcoxi, -CF₃, -OCF₃, -OCHF₂, y -CN;

(e) -C(O)C(HR⁹)OR¹⁰ en donde R⁹ es hidrógeno o C₁₋₃alquilo, y R¹⁰ es fenilo opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C₁₋₄alquilo, halo, C₁₋₄alcoxi, -CF₃, -OCF₃, y -OCHF₂; y

(f) -S(O)₂R¹¹ en donde R¹¹ se selecciona entre C₁₋₃alquilo, -CH₂-fenilo, furanilo, tiofenilo, morfolinilo, tetrahydrofuranilo, piridinilo, naftalenilo, pirrolilo, tiomorfolinilo, pirrolidinilo, piperidinilo, oxoazetidinilo, tiazolidinilo, 1,1-dioxo isotiazolidinilo, 2,4-dimetilisoxazolilo, y fenilo opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C₁₋₄alquilo, halo, C₁₋₄alcoxi, -CF₃, -OCF₃, -OCHF₂, y -CN;

o una sal aceptable para uso farmacéutico o solvato o estereoisómero del mismo.

2. El compuesto de la reivindicación 1 en donde R¹ es C₃₋₅alquilo.
3. El compuesto de la reivindicación 2 en donde R¹ es isopropilo o *ter*-butilo.
4. El compuesto de la reivindicación 2 en donde X es -C(O)OR².
5. El compuesto de la reivindicación 4 en donde R² es C₁₋₃alquilo o fenilo.
6. El compuesto de la reivindicación 2 en donde X es -C(O)R³.
7. El compuesto de la reivindicación 6 en donde R³ es fenilo, opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C₁₋₄alquilo, halo, C₁₋₄alcoxi, -CF₃, -OCF₃, y -OCHF₂.
8. El compuesto de la reivindicación 2 en donde X es -C(O)NR⁴R⁵.
9. El compuesto de la reivindicación 2 en donde:

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R^1 es C_{3-4} alquilo; y

X se selecciona entre :

- (a) $-C(O)OR^2$ en donde R^2 es C_{1-3} alquilo o fenilo;
- (b) $-C(O)R^3$ en donde R^3 es fenilo, opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre C_{1-4} alquilo, halo, y $-CF_3$; furanilo; o tiofenilo;
- (c) $-C(O)NR^4R^5$ en donde R^4 es hidrógeno y R^5 es fenilo opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre C_{1-4} alquilo y halo;
- (d) $-C(O)C(R^6R^7)R^8$ en donde R^6 es hidrógeno y R^7 es hidrógeno, $-OH$, o metilo; o R^6 y R^7 juntos forman oxo o $-(CH_2)_2-$; y R^8 es fenilo o ciclohexilo, en donde fenilo o ciclohexilo se sustituyen opcionalmente con 1 o 2 sustituyentes seleccionados entre C_{1-4} alquilo y halo;
- (e) $-C(O)C(HR^9)OR^{10}$ en donde R^9 es hidrógeno o metilo y R^{10} es fenilo opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre C_{1-4} alquilo y halo; y
- (f) $-S(O)_2R^{11}$ en donde R^{11} es metilo o fenilo, opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre C_{1-4} alquilo y halo.

10. El compuesto de la reivindicación 9 en donde:

R^1 es isopropilo o *ter*-butilo; y

X se selecciona entre :

- (a) $-C(O)OR^2$ en donde R^2 es metilo o fenilo;
- (b) $-C(O)R^3$ en donde R^3 es fenilo, opcionalmente sustituido con 1 o 2 sustituyentes seleccionados entre metilo, cloro, fluoro, y $-CF_3$; furan-2-ilo; o tiofen-2-ilo; y
- (c) $-C(O)NR^4R^5$ en donde R^4 es hidrógeno y R^5 es fenilo opcionalmente sustituido con 1 fluoro o cloro.

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11. El compuesto de la reivindicación 2 en donde el compuesto se selecciona entre :

metiléster de ácido 4-(4-[[2-isopropil-1*H*-benzoimidazol-4-carbonil)-amino]metil)piperidin-1-ilmetil)piperidin-1-carboxílico;

feniléster de ácido 4-(4-[[2-isopropil-1*H*-benzoimidazol-4-carbonil)-amino]metil)piperidin-1-ilmetil)piperidin-1-carboxílico;

{1-[1-(2-clorobenzoil) piperidin-4-ilmetil] piperidin-4-ilmetil}amida de ácido 2-isopropil-1*H*-benzoimidazol-4-carboxílico;

{1-[1-(2,4-difluoro-benzoil)piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-isopropil-1*H*-benzoimidazol-4-carboxílico;

{1-[1-(furan-2-carbonil)-piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-isopropil-1*H*-benzoimidazol-4-carboxílico;

{1-[1-(tiofeno-2-carbonil)piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-isopropil-1*H*-benzoimidazol-4-carboxílico;

{1-[1-(2-fluoro-5-trifluorometilbenzoil)piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-isopropil-1*H*-benzoimidazol-4-carboxílico;

{1-[1-(2-fluoro-fenilcarbamoil)piperidin-4-ilmetil]piperidin-4-ilmetil}-amida de ácido 2-isopropil-1*H*-benzoimidazol-4-carboxílico;

metiléster de ácido 4-(4-[[2-*ter*-butil-1*H*-benzoimidazol-4-carbonil)-amino]metil)piperidin-1-ilmetil)piperidin-1-carboxílico;

{1-[1-(2-fluoro-benzoil)-piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-*ter*-butil-1*H*-benzoimidazol-4-carboxílico;

{1-[1-(3-metil-benzoil)-piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-*ter*-butil-1*H*-benzoimidazol-4-carboxílico;

{1-[1-(4-fluorobenzoyl)-piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-*ter*-butil-1*H*-benzoimidazol-4-carboxílico; y

sales y solvatos aceptables para uso farmacéutico y estereoisómeros del mismo.

12. El compuesto de la reivindicación 2 en donde el compuesto se selecciona entre :

metiléster de ácido 4-(4-[(2-isopropil-1*H*-benzoimidazol-4-carbonil)-amino]metil)piperidin-1-ilmetil)piperidin-1-carboxílico;

metiléster de ácido 4-(4-[(2-*ter*-butil-1*H*-benzoimidazol-4-carbonil)-amino]metil)piperidin-1-ilmetil)piperidin-1-carboxílico;

{1-[1-(2-fluoro-benzoyl)-piperidin-4-ilmetil]piperidin-4-ilmetil}amida de ácido 2-*ter*-butil-1*H*-benzoimidazol-4-carboxílico; y

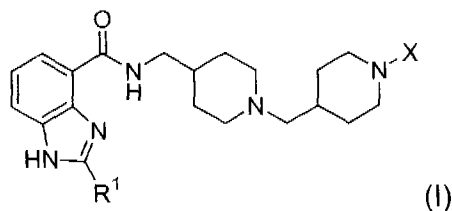
sales y solvatos aceptables para uso farmacéutico y estereoisómeros del mismo.

13. Metiléster de ácido 4-(4-[(2-isopropil-1*H*-benzoimidazol-4-carbonil)-amino]metil)piperidin-1-ilmetil)piperidin-1-carboxílico cristalino o un solvato del mismo.

— 14. El compuesto cristalino de la reivindicación 13, donde el compuesto cristalino se caracteriza por un patrón de difracción de rayos X en polvo que tiene dos o más picos de difracción en valores de 2θ seleccionados entre $15,08 \pm 0,20$, $15,41 \pm 0,20$, $19,00 \pm 0,20$, $19,70 \pm 0,20$ y $23,68 \pm 0,20$.

— 15. El compuesto cristalino de la reivindicación 14, donde el compuesto cristalino se caracteriza por un perfil de calorimetría diferencial de barrido que presenta un máximo en el flujo de calor endotérmico a una temperatura en el rango de entre aproximadamente 146°C y aproximadamente 148°C .

16. El compuesto cristalino de la reivindicación 13, donde el compuesto cristalino se caracteriza por un perfil de calorimetría diferencial de barrido que presenta un máximo en el flujo de calor endotérmico a una temperatura en el rango de entre aproximadamente 143°C y aproximadamente 145°C.
17. El compuesto cristalino de la reivindicación 13, donde el compuesto cristalino es un monohidrato.
18. El compuesto cristalino de la reivindicación 17 donde el compuesto cristalino se caracteriza por un patrón de difracción de rayos X en polvo que tiene dos o más picos de difracción en valores de 2θ seleccionados entre $9,14 \pm 0,20$, $12,41 \pm 0,20$, $12,74 \pm 0,20$, $17,75 \pm 0,20$, $18,47 \pm 0,20$, $20,63 \pm 0,20$, $21,13 \pm 0,20$ y $27,05 \pm 0,20$.
19. Una composición farmacéutica que comprende una cantidad eficiente para el uso terapéutico del compuesto de cualquiera de las reivindicaciones 1 a 18 y un vehículo aceptable para el uso farmacéutico.
20. Un compuesto como se reivindica en cualquiera de las reivindicaciones 1 a 18 para usar en terapia.
21. El uso de un compuesto de cualquiera de las reivindicaciones 1 a 18 para fabricar un medicamento.
22. El uso de la reivindicación 21, donde el medicamento es para el tratamiento de una condición médica en un mamífero asociada con la actividad del receptor 5-HT₄.
23. El uso de la reivindicación 22, donde la enfermedad o la condición es un trastorno de motilidad reducida del tracto gastrointestinal.
24. Un proceso para preparar un compuesto de fórmula (I),



en donde:

R^1 es C_{3-5} alquilo, opcionalmente sustituido con $-OH$; y

X se selecciona entre

(a) $-C(O)OR^2$ en donde R^2 es C_{1-4} alquilo o $-(CH_2)_n$ -fenilo en donde n es 0 o 1;

(b) $-C(O)R^3$ en donde R^3 se selecciona entre :

fenilo, opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre

C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, y $-OCHF_2$,

C_{1-5} alquilo,

C_{4-5} cicloalquilo, y

$-(CH_2)_m-A$ en donde m es 0 o 1 y A se selecciona entre furanilo, tiofenilo,

morfolinilo, tetrahidrofuranilo, piridinilo, naftalenilo, pirrolilo, tiomorfolinilo,

pirrolidinilo, piperidinilo, oxoazetidinilo, tiazolidinilo, 1,1-dioxo isotiazolidinilo, y

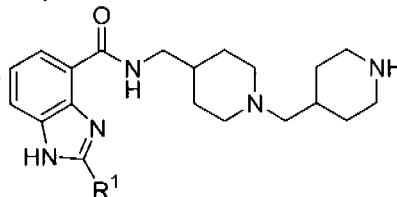
2,4-dimetilisoxazolilo;

(c) $-C(O)NR^4R^5$ en donde R^4 es hidrógeno o C_{1-3} alquilo, y R^5 es fenilo opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, y $-OCHF_2$;

(d) $-C(O)C(R^6R^7)R^8$ en donde R^6 es hidrógeno o C_{1-3} alquilo y R^7 es hidrógeno, $-OH$, C_{1-3} alquilo, o oxo; o R^6 y R^7 forman $-(CH_2)_2-$; y R^8 es fenilo o ciclohexilo, en donde fenilo o ciclohexilo se sustituyen opcionalmente con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, $-OCHF_2$, y $-CN$;

- (e) $-C(O)C(HR^9)OR^{10}$ en donde R^9 es hidrógeno o C_{1-3} alquilo, y R^{10} es fenilo opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, y $-OCHF_2$; y
- (f) $-S(O)_2R^{11}$ en donde R^{11} se selecciona entre C_{1-3} alquilo, $-CH_2$ -fenilo, furanilo, tiofenilo, morfolinilo, tetrahidrofuranilo, piridinilo, naftalenilo, pirrolilo, tiomorfolinilo, pirrolidinilo, piperidinilo, oxoazetidínilo, tiazolidínilo, 1,1-dioxo isotiazolidínilo, 2,4-dimetilisoxazolilo, y fenilo opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, $-OCHF_2$, y $-CN$;
- o una sal aceptable para uso farmacéutico o solvato o estereoisómero del mismo, el proceso comprende:

(i) hacer reaccionar un compuesto de fórmula (II):



(II)

con un compuesto de fórmula (III):



(III)

en donde L es grupo saliente;

(ii) hacer reaccionar un compuesto de fórmula (VIII):

con un compuesto de fórmula (XIII):

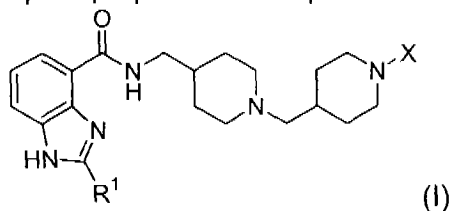
; o

(iii) hacer reaccionar un compuesto de fórmula (VI):

con un compuesto de fórmula (XIV):

para dar un compuesto de fórmula (I), o una sal o solvato aceptable para uso farmacéutico o estereoisómero del mismo.

25. Un proceso para preparar un compuesto de fórmula (I),



en donde:

R^1 es C_{3-5} alquilo; y

X se selecciona entre

(b) $-C(O)R^3$ en donde R^3 se selecciona entre :

fenilo, opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre

C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, y $-OCHF_2$,

C_{1-5} alquilo,

C_{4-5} cicloalquilo, y

$-(CH_2)_m-A$ en donde m es 0 o 1 y A se selecciona entre furanilo, tiofenilo,

morfolinilo, tetrahidrofuranilo, piridinilo, naftalenilo, pirrolilo, tiomorfolinilo,

pirrolidinilo, piperidinilo, oxoazetidinilo, tiazolidinilo, 1,1-dioxo isotiazolidinilo, y

2,4-dimetilisoxazolilo;

(d) $-C(O)C(R^6R^7)R^8$ en donde R^6 es hidrógeno o C_{1-3} alquilo y R^7 es hidrógeno, $-OH$,

C_{1-3} alquilo, o oxo; o R^6 y R^7 forman $-(CH_2)_2-$; y R^8 es fenilo o ciclohexilo, en

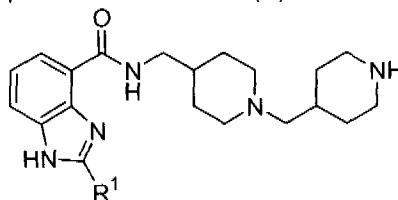
donde fenilo o ciclohexilo se sustituyen opcionalmente con 1, 2, o 3

sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, -

$OCHF_2$, y $-CN$; y

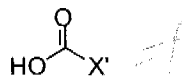
(e) $-C(O)C(HR^9)OR^{10}$ en donde R^9 es hidrógeno o C_{1-3} alquilo, y R^{10} es fenilo opcionalmente sustituido con 1, 2, o 3 sustituyentes seleccionados entre C_{1-4} alquilo, halo, C_{1-4} alcoxi, $-CF_3$, $-OCF_3$, y $-OCHF_2$; o una sal aceptable para uso farmacéutico o solvato o estereoisómero del mismo, el proceso comprende:

hacer reaccionar un compuesto de fórmula (II):



(II)

con un compuesto de fórmula (IV):

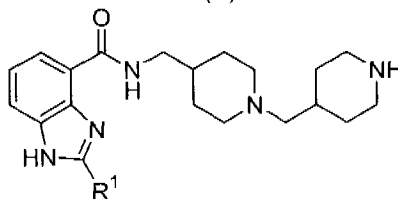


(IV)

en donde X' se selecciona entre R^3 , $C(R^6R^7)R^8$, y $C(HR^9)OR^{10}$ para dar un compuesto de fórmula (I) o una sal o solvato aceptable para uso farmacéutico o estereoisómero del mismo.

26. El producto preparado mediante el proceso de la reivindicación 24 o la reivindicación 25.

27. Un compuesto de fórmula (II):



(II)

en donde R^1 es C_{3-5} alquilo,

o una sal o estereoisómero o derivado protegido del mismo.

28. Un proceso para preparar metiléster de ácido 4-(4-[(2-isopropil-1H-benzoimidazol-4-carbonil)-amino]metil)piperidin-1-ilmetil)piperidin-1-carboxílico cristalino, el proceso comprende:

- (a) dispersar metiléster de ácido 4-(4-[(2-isopropil-1H-benzoimidazol-4-carbonil)-amino]metil)piperidin-1-ilmetil)piperidin-1-carboxílico en un diluyente inerte seleccionado entre acetonitrilo, éter, ciclohexano, y acetato de etilo en una proporción de entre aproximadamente 15 mg y 25 mg de metiléster de ácido 4-(4-[(2-isopropil-1H-benzoimidazol-4-carbonil)-amino]metil)piperidin-1-ilmetil)piperidin-1-carboxílico por mililitro de diluyente para formar una mezcla; y
- (b) dejar evaporar la mezcla para dar metiléster de ácido 4-(4-[(2-isopropil-1H-benzoimidazol-4-carbonil)-amino]metil)piperidin-1-ilmetil)piperidin-1-carboxílico cristalino.

29. Un proceso para preparar metiléster de ácido 4-(4-[(2-isopropil-1H-benzoimidazol-4-carbonil)-amino]metil)piperidin-1-ilmetil)piperidin-1-carboxílico cristalino, el proceso comprende:

- (a) hacer reaccionar (piperidin-4-ilmetil)amida de ácido 2-isopropil-1H-benzoimidazol-4-carboxílico con metiléster de ácido 4-formilpiperidin-1-carboxílico en un diluyente aprótico polar;
- (b) agregar acetonitrilo mientras ^{se} destila el producto del paso (a) para eliminar el diluyente aprótico polar del producto del paso (a);
- (c) preparar una mezcla del residuo a partir de la destilación del paso (b) en acetonitrilo a una concentración de entre aproximadamente 50 y aproximadamente 125 mg de metiléster de ácido 4-(4-[(2-isopropil-1H-benzoimidazol-4-carbonil)-amino]metil)piperidin-1-ilmetil)piperidin-1-carboxílico

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por mililitro de acetonitrilo a una temperatura suficiente para disolver el residuo;

y

(d) enfriar la mezcla de paso (c) a una temperatura de no más que aproximadamente 20 °C para dar metiléster de ácido 4-(4-[[[2-isopropil-1H-benzoimidazol-4-carbonil)-amino]metil]piperidin-1-ilmetil)piperidin-1-carboxílico cristalino.

30. El producto preparado mediante el proceso de la reivindicación 29 o la ~~reivindicación 30.~~ *no es un producto*

31. Un método para tratar un mamífero que tiene una condición médica asociada con la actividad del receptor 5-HT₄, donde el método comprende administrarle al mamífero una cantidad eficiente para el uso terapéutico de una composición farmacéutica que comprende un vehículo aceptable para el uso farmacéutico y un compuesto de cualquiera de las reivindicaciones 1 a 18.

32. El método de la reivindicación 31, donde la condición médica se selecciona entre síndrome del intestino irritable, constipación crónica, dispepsia funcional, retardo del vaciado gástrico, enfermedad de reflujo gastroesofágico, gastroparesis, íleo postoperatorio, pseudoobstrucción intestinal y tránsito con retardo inducido por drogas.

33. Un método para tratar un trastorno de motilidad reducida del tracto gastrointestinal en un mamífero, donde el método comprende administrarle al mamífero una cantidad eficiente para el uso terapéutico de una composición farmacéutica que comprende un vehículo aceptable para el uso farmacéutico y un compuesto de cualquiera de las reivindicaciones 1 a 18.

34. El método de la reivindicación 33, donde el trastorno de motilidad reducida se selecciona entre constipación crónica, síndrome del intestino

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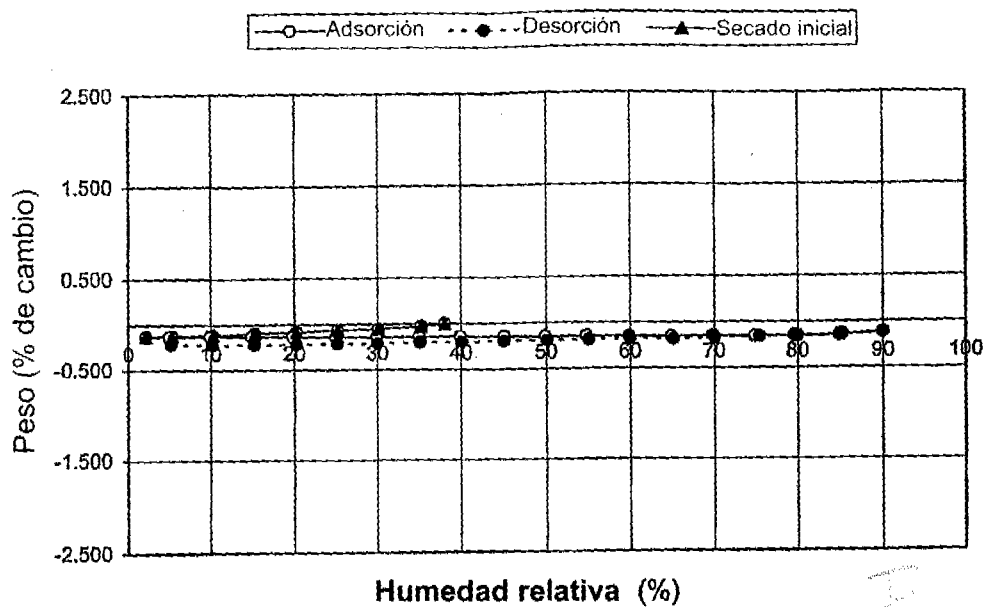


Figura 3

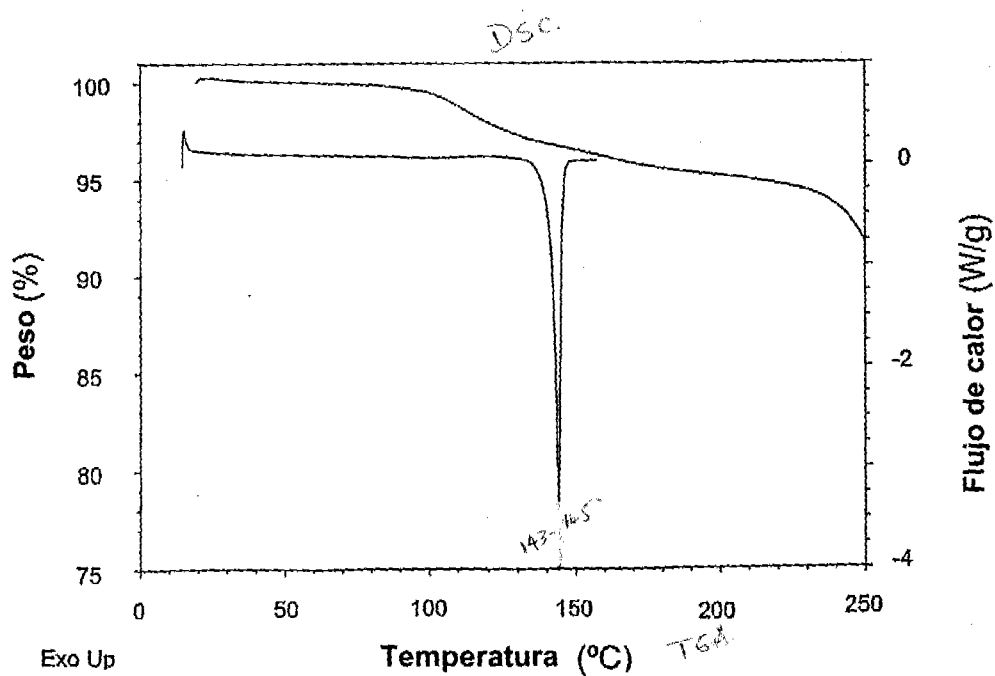


Figura 4

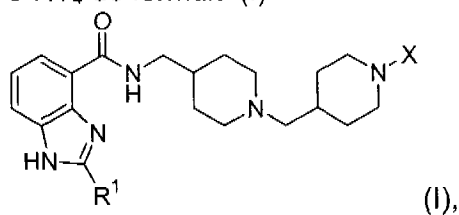
DSC II?

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**COMPUESTOS BENZIMIDAZOL-CARBOXAMIDA COMO AGONISTAS DEL
RECEPTOR 5-HT₄**

RESUMEN

La invención se relaciona con compuestos de benzimidazol-carboxamida agonistas del receptor 5-HT₄ de fórmula (I)



donde R¹ y X son como se los define en la memoria descriptiva, o sales, solvatos o estereoisómeros de éstos aceptables para el uso farmacéutico. La invención también se relaciona con composiciones farmacéuticas que comprenden dichos compuestos, métodos para usar dichos compuestos para tratar enfermedades asociadas con la actividad del receptor 5-HT₄, y procesos e intermediarios útiles para preparar dichos compuestos. Además, la invención se relaciona con formas cristalinas de un compuesto de fórmula (I).

P-212-PCT

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PCT REQUEST

Original (for SUBMISSION)

0	For receiving Office use only	
0-1	International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	
0-4	Form PCT/RO/101 PCT Request	
0-4-1	Prepared Using	PCT-SAFE [EASY model] Version 3.51.001.176 MT/FOP 20060101/0.20.4rc.2.7
0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	United States Patent and Trademark Office (USPTO) (RO/US)
0-7	Applicant's or agent's file reference	P-212-PCT
I	Title of Invention	BENZIMIDAZOLE-CARBOXAMIDE COMPOUNDS AS 5-HT4 RECEPTOR AGONISTS
II	Applicant	
II-1	This person is	applicant only
II-2	Applicant for	all designated States except US
II-4	Name	THERAVANCE, INC.
II-5	Address	901 Gateway Boulevard South San Francisco, California 94080 United States of America
II-6	State of nationality	US
II-7	State of residence	US
II-8	Telephone No.	(650) 808-6000
II-9	Facsimile No.	(650) 808-6078
III-1	Applicant and/or Inventor	
III-1-1	This person is	applicant and inventor
III-1-2	Applicant for	US only
III-1-4	Name (LAST, First)	MCKINNELL, Robert Murray
III-1-5	Address	375 4th Avenue Half Moon Bay, California 94019 United States of America
III-1-6	State of nationality	GB
III-1-7	State of residence	US

PCT REQUEST

Original (for SUBMISSION)

III-2	Applicant and/or inventor	applicant and inventor
III-2-1	This person is	US only
III-2-2	Applicant for	GENDRON, Roland
III-2-4	Name (LAST, First)	345 Franconia Street
III-2-5	Address	San Francisco, California 94110
		United States of America
III-2-6	State of nationality	CA
III-2-7	State of residence	US
III-3	Applicant and/or inventor	applicant and inventor
III-3-1	This person is	US only
III-3-2	Applicant for	JIANG, Lan
III-3-4	Name (LAST, First)	788 Santa Maria Lane
III-3-5	Address	Foster City, California 94404
		United States of America
III-3-6	State of nationality	CN
III-3-7	State of residence	US
III-4	Applicant and/or inventor	applicant and inventor
III-4-1	This person is	US only
III-4-2	Applicant for	CHOI, Seok-ki
III-4-4	Name (LAST, First)	525 Embarcadero Road
III-4-5	Address	Palo Alto, California 94301
		United States of America
III-4-6	State of nationality	KR
III-4-7	State of residence	US
III-5	Applicant and/or inventor	applicant and inventor
III-5-1	This person is	US only
III-5-2	Applicant for	LONG, Daniel D.
III-5-4	Name (LAST, First)	3837 21st Street
III-5-5	Address	San Francisco, California 94114
		United States of America
III-5-6	State of nationality	GB
III-5-7	State of residence	US

PCT REQUEST

Original (for SUBMISSION)

III-6	Applicant and/or inventor	
III-6-1	This person is	applicant and inventor
III-6-2	Applicant for	US only
III-6-4	Name (LAST, First)	FATHEREE, Paul R.
III-6-5	Address	921 Minnesota Street San Francisco, California 94107 United States of America
III-6-6	State of nationality	US
III-6-7	State of residence	US
III-7	Applicant and/or inventor	
III-7-1	This person is	applicant and inventor
III-7-2	Applicant for	US only
III-7-4	Name (LAST, First)	MARQUESS, Daniel
III-7-5	Address	2037 Touraine Lane Half Moon Bay, California 94019 United States of America
III-7-6	State of nationality	GB
III-7-7	State of residence	US
III-8	Applicant and/or inventor	
III-8-1	This person is	applicant and inventor
III-8-2	Applicant for	US only
III-8-4	Name (LAST, First)	DALZIEL, Sean M.
III-8-5	Address	201 Harrison Street #921 San Francisco, California 94105 United States of America
III-8-6	State of nationality	AU
III-8-7	State of residence	US
III-9	Applicant and/or inventor	
III-9-1	This person is	applicant and inventor
III-9-2	Applicant for	US only
III-9-4	Name (LAST, First)	PHIZACKERLEY, Kirsten M.
III-9-5	Address	10 McCoy Road San Rafael, California 94901 United States of America
III-9-6	State of nationality	US
III-9-7	State of residence	US

PCT REQUEST

Original (for SUBMISSION)

IV-1	Agent or common representative; or address for correspondence The person identified below is hereby/ has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name (LAST, First)	HAGENAH, Jeffrey A.
IV-1-2	Address	THERAVANCE, INC. 901 Gateway Boulevard South San Francisco, California 94080 United States of America
IV-1-3	Telephone No.	(650) 808-6406
IV-1-4	Facsimile No.	(650) 808-6078
IV-1-6	Agent's registration No.	35,175
IV-2	Additional agent(s)	agent
IV-2-1	Name (LAST, First)	EBERLE, Shelley
IV-2-2	Address	THERAVANCE, INC. 901 Gateway Boulevard South San Francisco, California 94080 United States of America
IV-2-3	Telephone No.	(650) 808-4010
IV-2-4	Facsimile No.	(650) 808-6078
IV-2-5	e-mail	
IV-2-6	Agent's registration No.	31,411
IV-3	Additional agent(s)	agent
IV-3-1	Name (LAST, First)	SAXON, Roberta P.
IV-3-2	Address	THERAVANCE, INC. 901 Gateway Boulevard South San Francisco, California 94080 United States of America
IV-3-3	Telephone No.	(650) 808-3764
IV-3-4	Facsimile No.	(650) 808-6078
IV-3-5	e-mail	
IV-3-6	Agent's registration No.	43,087
V	DESIGNATIONS	
V-1	The filing of this request constitutes under Rule 4.9(a), the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.	

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PCT REQUEST

Original (for SUBMISSION)

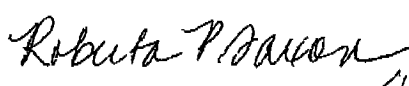
V-3	Identification of parent application or patent grant		
V-3-1	Designation	US	
V-3-1-1	Kind of parent application or grant	continuation-in-part	
V-3-1-2	Parent application or grant No.	60/684,466	
V-3-1-3	Parent application or grant date	25 May 2005 (25.05.2005)	
VI-1	Priority claim of earlier national application		
VI-1-1	Filing date	25 May 2005 (25.05.2005)	
VI-1-2	Number	60/684,466	
VI-1-3	Country	US	
VI-2	Priority claim of earlier national application		
VI-2-1	Filing date	25 May 2005 (25.05.2005)	
VI-2-2	Number	60/684,478	
VI-2-3	Country	US	
VI-3	Priority claim of earlier national application		
VI-3-1	Filing date	08 December 2005 (08.12.2005)	
VI-3-2	Number	60/748,415	
VI-3-3	Country	US	
VI-4	Priority document request The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1, VI-2, VI-3	
VII-1	International Searching Authority Chosen	European Patent Office (EPO) (ISA/EP)	
VIII	Declarations	Number of declarations	
VIII-1	Declaration as to the identity of the inventor	-	
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	-	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	-	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	-	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	-	

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PCT REQUEST

Original (for SUBMISSION)

IX	Check list	number of sheets	electronic file(s) attached
IX-1	Request (including declaration sheets)	7	✓
IX-2	Description	73	-
IX-3	Claims	11	-
IX-4	Abstract	1	✓
IX-5	Drawings	3	-
IX-7	TOTAL	95	
	Accompanying items	paper document(s) attached	electronic file(s) attached
IX-8	Fee calculation sheet	✓	-
IX-17	PCT-SAFE physical media	-	✓
IX-18	other	Transmittal Letter to the US Receiving Office	
IX-18	other	10 General Power of Attorney documents	
IX-18	other	Receipt Postcard	
IX-19	Figure of the drawings which should accompany the abstract		
IX-20	Language of filing of the international application	English	
X-1	Signature of applicant, agent or common representative	 43,087	
X-1-1	Name (LAST, First)	SAXON, Roberta P.	
X-1-2	Name of signatory		
X-1-3	Capacity		

FOR RECEIVING OFFICE USE ONLY

10-1	Date of actual receipt of the purported international application	
10-2	Drawings:	
10-2-1	Received	
10-2-2	Not received	
10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/EP
10-6	Transmittal of search copy delayed until search fee is paid	

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PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/JS2006/020085

International filing date (day/month/year)
24.05.2006

Priority date (day/month/year)
25.05.2005

International Patent Classification (IPC) or both national classification and IPC
INV. C07D401/14 C07D405/14 C07D409/14 A61K31/4545 A61P1/00

Applicant
THERAVANCE, INC.

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

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

2. FURTHER ACTION

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

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

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

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Date of completion of
this opinion

see form
PCT/ISA/210

Authorized Officer

Papathoma, Sofia

Telephone No. +49 89 2399-7536



**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2006/020085

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed
 - ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 28.1 (b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - ☐ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☐ on paper
 - ☐ in electronic form
 - c. time of filing/furnishing:
 - ☐ contained in the international application as filed.
 - ☐ filed together with the international application in electronic form.
 - ☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2006/020085

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

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

☐ the entire international application

☒ claims Nos. 31-35

because:

☒ the said international application, or the said claims Nos. 31-35 relate to the following subject matter which does not require an international search (*specify*):

see separate sheet

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

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

☒ no international search report has been established for the whole application or for said claims Nos. 31-35

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

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13ter.1(a) or (b).

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-bis of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.

☐ See Supplemental Box for further details

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2006/020085

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

1. Statement

Novelty (N)	Yes: Claims	<u>1-35</u>
	No: Claims	
Inventive step (IS)	Yes: Claims	
	No: Claims	<u>1-35</u>
Industrial applicability (IA)	Yes: Claims	<u>1-30</u>
	No: Claims	

2. Citations and explanations

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/US2006/020085

Re Item I

Basis of the report

This Written Opinion is based on the following **application documents**:

Description, Pages:

1-73 as originally filed

Claims, Numbers:

1-35 as originally filed

Figures, Sheets:

1/3-3/3 as originally filed

The present application refers to benzimidazole-carboxamide compounds of the formula (I) suitable as 5-HT₄ receptor agonists.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 31-35 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

- D1: LOPEZ-RODRIGUEZ MARIA L ET AL: "3-D-QSAR/CoMFA and recognition models of benzimidazole derivatives at the 5-HT₄ receptor" BIOORGANIC AND MEDICINAL CHEMISTRY LETTERS, vol. 11, no. 21, 5 November 2001 (2001-11-05), pages 2807-2811, XP002434296 ISSN: 0960-894X
- D2: JP 2004 277318 A (DAINIPPON PHARMACEUTICAL CO) 7 October 2004 (2004-

10-07)

D3: WO 2005/021539 A (PFIZER JAPAN INC [JP]; PFIZER [US]; IGUCHI SATORU [JP]; KATSU YASUHIRO) 10 March 2005 (2005-03-10)

D4: WO 2004/026868 A (PFIZER PHARMA [JP]; KATSU YASUHIRO [JP]; KON-I KANA [JP]; MORITA MIKIO) 1 April 2004 (2004-04-01)

1) Article 33(2) PCT

Document D1 discloses benzimidazole-carboxamide derivatives showing binding affinity to the 5-HT₄ receptor. The entities disclosed therein, and in particular the compounds 5 and 6 differ from the claimed entities in that the piperidinyl moiety is substituted by a methyl or a methylsulfonylaminoethyl group and not by a piperidinylmethyl group as presently claimed. Document D2 discloses compounds with a piperidinyl-methyl-piperidinyl-methyl-amino-carbonyl moiety. In these compounds, however, the Ar is defined as phenyl (Ar-1) or a benzofuran (Ar-2) and not as benzimidazole as in the present case.

Further, documents D3 and D4 disclose (D3: example 7) or claim a piperidinyl-methyl-piperidinyl-methyl-amino-carbonyl substitution of benzimidazolones (D3) or imidazopyridines (D4).

In view of the above the claimed subject-matter fulfills the requirements of Article 33(2) PCT.

2) Article 33(3) PCT

The technical problem underlying the present application is the provision of further compounds suitable as 5-HT₄ agonists.

As solution to the above technical problem the present application claims compounds of the formula (I).

The piperidinyl-methyl-amino-carbonyl substitution of different aromatic-/ heteroaromatic-rings is one of the characterising features of 5-HT₄ agonists. In particular:

a) document D3 referring to 5-HT₄ agonists discloses example 7 in which the R³ substituent is defined as piperidin-1-yl-ethyl,

b) document D4 discloses the piperidinyl-methyl-piperidinyl-methyl-amino-carbonyl substitution

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/US2006/020085

of aryl (Ar-1: phenyl) or heteroaryl (Ar-2: benzofuran) rings as one of the essential features of compounds having strong affinity to 5-HT₄ receptor.

On the other hand document D1 shows that benzimidazole-piperidin-4-yl-methyl carboxamides also show high affinities to the 5-HT₄ receptor.

On the basis of the information given by the prior art the structural design of the claimed entities is not regarded to be based on any inventive ingenuity.

The claimed subject-matter is therefore considered as not to fulfill the requirements of Article 33(3) PCT.

3) Article 34(4)(a)(i) PCT / Rule 67.1(iv) PCT

For the assessment of the present claims 31-35 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VII

Certain defects in the international application

- a) The relevant background art is missing from the description (Rule 5.1(a)(ii) PCT).
- b) When entering the regional phase before the EPO, the expression "the spirit of the invention" mentioned in page 73, line 14 can not be allowable under Rule 34 EPC, as it may imply a possible extension of the protection in some vague and not precisely defined way.
- c) Claim 25 includes all the features of the option (i) of claim 24 and should therefore be dependent on claim 24.

d) Claim 30 should refer to claims 28 and 29 and not to claims 29 and 30 as presently drafted (see objections under Re Item VIII, d)).

Re Item VIII

Certain observations on the international application

a) The limited variations in the substitution pattern of the claimed compounds in the disclosed examples do not justify the breadth of claims (**Article 6 PCT: fully supported by the description**).

The applicant is entitled to claim only obvious modifications of what was described (close related variations), since the claims should represent a reasonable generalisation of the examples given in the description: the technical features stated in the description/examples as being essential features of the invention described must be the same as those used to define the invention in the claims. The reason for that is that the problem to be solved **should be solved by the whole scope of the claimed subject matter** (every compound falling within its scope) and not just by individual compounds- especially since **the problem may be seen in the provision of compounds acting on a biological system** (i.e. on a system depending upon very different parameters). If this were not the case, an invention could arbitrarily be broadened to any limit without consideration, whether the compounds are actually solving the problem underlying the invention. On that basis, broader variations would be allowed only in the case that it is made credible that the claimed effect is retained.

b) In the independent claims 14 and 18 an attempt is made to define the crystal form merely through the presence of peaks in the X-ray powder diffractogram without, however, indicating the relative intensities of these peaks.

This practice is insufficient to characterise a crystal form and not according to the common general practice in the field. The USP general chapter on x-ray diffraction states that identity is established if scattering angles on the ten strongest reflections obtained for an analyte agree to within ± 0.20 degrees with that of the reference material and if the relative intensities of these reflections do not vary by more than 20 percent.

Hence the claimed crystal forms are not clearly defined in the present claims 14 and 18 contrary to the requirements of Article 6 PCT. Such an unclear definition cannot be allowed in a claim if the definition is essential having regard to the invention.

c) Claims 15 and 16 refer both to the same crystalline compound; the difference at the temperature range of the maximum of the endothermic heat flow in these two claims has to be clarified.

d) Claims 26 and 30 refer to the product obtained by the processes of claims 24 and 25 and 28 and 29 respectively. Product-by-process claims can only be allowed when the product obtained by the process can not be defined in any other way.

Furthermore, the applicant's attention is drawn to the fact, that by entering the European phase the Article 64(2) EPC applies, according to which the protection conferred by a patent, whose subject matter is a process, shall extend to the products directly obtained by this process.

e) With respect to claims 22, 31 and 35 it must be also mentioned, that these claims can not be considered:

i) as industrial applicable as the affinity to the 5-HT₄ receptor can not be considered in itself as a therapeutic application. The discovery, that a substance shows affinity to the 5-HT₄ receptor, even if representing an important piece of scientific knowledge, still needs to find a practical application in the form of a defined, real treatment of any pathological condition in order to make a technical contribution to the art and

ii) as clear (Article 6 PCT), since no instructions, in the form of any testable criteria, are available from the patent documents or from the common general knowledge allowing the skilled person to recognise, which conditions fall within the functional definition e.g. any condition associated with 5-HT₄ receptor activity, and accordingly within the scope of the claim.

The reason for the necessity of this clarification is, that a claim referring to a condition to be treated, which is functionally defined, would not be limited to the treatment of said specified condition (here gastrointestinal disorders etc.), but by contrast embraces an undefined number of conditions all allegedly capable of being improved or prevented by the affinity to the 5-HT₄ receptor. Under these circumstances the independent claim can only be regarded as clear if means are available to the skilled person for assessing whether or not an additional condition not expressly filed in the application but nevertheless affected by the administration of the said

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Bitte beachten Sie, dass angeführte Nichtpatentliteratur (wie z. B. wissenschaftliche oder technische Dokumente) je nach geltendem Recht dem Urheberrechtsschutz und/oder anderen Schutzarten für schriftliche Werke unterliegen könnte. Die Vervielfältigung urheberrechtlich geschützter Texte, ihre Verwendung in anderen elektronischen oder gedruckten Publikationen und ihre Weitergabe an Dritte ist ohne ausdrückliche Zustimmung des Rechtsinhabers nicht gestattet.

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XS CPRTENFRDE

PATENT COOPERATION TREATY

RECEIVED

JUN 18 2007

PCT THERAVANCE, INC.
LEGAL DEPARTMENT

From the INTERNATIONAL SEARCHING AUTHORITY

To:

THERAVANCE, INC.
Attn: Saxon, Roberta P.
901 Gateway Boulevard
South San Francisco, CA 94080
ETATS-UNIS D'AMERIQUE

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

(PCT Rule 44.1)

Date of mailing (day/month/year)	11/06/2007
Applicant's or agent's file reference P-212-PCT	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/US2006/020085	International filing date (day/month/year) 24/05/2006
Applicant THERAVANCE, INC.	

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

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international Application (see Rule 48):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the International Search Report.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes
1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 338.82.70

For more detailed instructions, see the notes on the accompanying sheet.

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

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

- ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.
☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Reminders

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

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

Within 19 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise, the applicant must, within 20 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 19 months.

See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the PCT Applicant's Guide, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the International Searching Authority

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

Saskia Stark

Article 19 Amend 8-11-07

Written Opinion (see notes on accompanying sheet)
9-11-07

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report and the written opinion of the International Searching Authority, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only (see *PCT Applicant's Guide*, Volume I/A, Annexes B1 and B2).

The attention of the applicant is drawn to the fact that amendments to the claims under Article 19 are not allowed where the International Searching Authority has declared, under Article 17(2), that no international search report would be established (see *PCT Applicant's Guide*, Volume I/A, paragraph 296).

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference P-212-PCT	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No. PCT/US2006/020085	International filing date (day/month/year) 24/05/2006	(Earliest) Priority Date (day/month/year) 25/05/2005
Applicant THERAVANCE, INC.		

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

This international search report consists of a total of 6 sheets.

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

1. **Basis of the report**
 - a. With regard to the language, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed
☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
 - b. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.
2. ☒ Certain claims were found unsearchable (See Box No. II)
3. ☐ Unity of invention is lacking (see Box No. III)
4. With regard to the title,

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

☒ the text is approved as submitted by the applicant
☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority
6. With regard to the drawings,
 - a. the figure of the drawings to be published with the abstract is Figure No. _____

☐ as suggested by the applicant
☐ as selected by this Authority, because the applicant failed to suggest a figure
☐ as selected by this Authority, because this figure better characterizes the invention
 - b. ☒ none of the figures is to be published with the abstract

INTERNATIONAL SEARCH REPORT

national application No
PCT/US2006/020085

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D401/14 C07D405/14 C07D409/14 A61K31/4545 A61P1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LOPEZ-RODRIGUEZ MARIA L ET AL: "3-D-QSAR/CoMFA and recognition models of benzimidazole derivatives at the 5-HT4 receptor" BIOORGANIC AND MEDICINAL CHEMISTRY LETTERS, vol. 11, no. 21, 5 November 2001 (2001-11-05), pages 2807-2811, XP002434296 ISSN: 0960-894X the whole document in particular compounds 5 and 6	1-35
Y	JP 2004 277318 A (DAINIPPON PHARMACEUTICAL CO) 7 October 2004 (2004-10-07) abstract; tables 3-8 ----- -/--	1-35

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

21 May 2007

Date of mailing of the international search report

11/06/2007

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

Papathoma, Sofia

INTERNATIONAL SEARCH REPORT

national application No
PCT/US2006/020085

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005/021539 A (PFIZER JAPAN INC [JP]; PFIZER [US]; IGUCHI SATORU [JP]; KATSU YASUHIRO) 10 March 2005 (2005-03-10) abstract; claims 1-19; examples 1-16	1-35
A	WO 2004/026868 A (PFIZER PHARMA [JP]; KATSU YASUHIRO [JP]; KON-I KANA [JP]; MORITA MIKIO) 1 April 2004 (2004-04-01) abstract; claims 1-16; examples 7-9,19,20,22	1-35

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 31-34 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.

Although claim 35 is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2006/020085

Box 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.: 31-35
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 III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

25A

INTERNATIONAL SEARCH REPORT

Information on patent family members

national application No

PCT/US2006/020085

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
JP 2004277318	A	07-10-2004	NONE	
WO 2005021539	A	10-03-2005	AU 2004268840 A1	10-03-2005
			BR PI0414105 A	21-11-2006
			CA 2537127 A1	10-03-2005
			CN 1845922 A	11-10-2006
			EP 1664036 A1	07-06-2006
			IS 8289 A	09-02-2006
			KR 20060087540 A	02-08-2006
			MX PA06002550 A	20-06-2006
			NL 1026959 C2	23-01-2006
			NL 1026959 A1	07-03-2005
WO 2004026868	A	01-04-2004	AU 2003259482 A1	08-04-2004
			BR 0314584 A	09-08-2005
			CA 2499494 A1	01-04-2004
			EP 1543004 A1	22-06-2005
			JP 2006502180 T	19-01-2006
			MX PA05003065 A	27-05-2005