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I Comprobante de pago de la tasa de presentaciOn de la solicitud
D Comprobante de pago par reivindicaciOn de prioridad
E1 Documento que acredita la existencia representaciOn legal cuanclo el solicitante sea persona
Juridica
1 Poderes, Si fuera el caso
0 Documento de cesiOn del inventor al solicitante o a su causante
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I1 Copia de la solicitud Internacional (Resumen descripcion reivindicaciOn Dibujos)
1Z1 Traducción de la solicitud internacional al castellano (Resumen, descripciOn, reivindicacion
Dibujos)
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1. Notificacion de cambio
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(54)’I’itic: CRYSTALLINE FORM OF A QUINOLINONE-CARB()XAMIDE COMPOUND

(57) Abstract: The invention provides a crystalline hydrochloride salt of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-cat’hoxylic acid (IS,3R5R)-8-[(R)-2.hydroxy.3-(methanesull’onyl-rnethyl-aunino)propyl -8-azabicyclo(3.2. 1 loct-3-y.t (amtde or a solvate thereof. The invention also provides pharmaceutical compositions comprising such crystalline salt forms, methods of using such crystalline salt forms to treat diseases associated with 5 H’t’4 receptor activity, and processes useful for preparing such crystalline salt forms.

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CRYSTALLINE FORM OF A QUINOLINONE-CARBOXAMIDE COMPOUND

BACKGROUND OF THE INVENTION

15 Field ofthe Invention

The invention is directed to crystalline salt forms of a quinolinone-carboxamide compound which are useful as 5-HT4 receptor agonists. The invention is also directed to pharmaceutical compositions comprising such crystalline compounds, methods of using such compounds for treating medical conditions mediated by 5-HT4 receptor activity, and
20 processes useful for preparing such compounds.

State ofthe Art

Commonly-assigned U.S. Provisional Application No. 60/560,076, filed on April
7, 2004, and U.S. Patent Application No. 11/100,113, filed on April 6, 2005, disclose
25 novel quinolinone-carboxamide compounds that are expected to be useful for the
treatment of disorders ofreduced motility ofthe gastrointestinal tract. In particular, the compound 1 -isopropyl-2-oxo- 1,2-dihydroquinoline.-3 -carboxylic acid {(1 S,3R,SR)-8-{(R)- 2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabiCyclo[3.2.1]oct-3-yl} amide
is specifically disclosed in these applications as demonstrating 5-HT4 agonist activity.
30 The chemical structure of 1 -isopropyl-2-oxo- 1,2-dihydroquinollne-3-carboxylic acid {(1 S,3R,5R)-8-{(R)-2-hydroxy-3-(methanesulfonyl-methyl-amiflO)PrOPYI]-8-azabicyclo[3.2.floct-3-yl} amide is represented by formula I:

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To effectively use this compound as a therapeutic agent, it would be desirable to
have a solid-state salt form that can be readily manufactured and that has acceptable
5 chemical and physical stability. For example, it would be highly desirable to have a salt
form that is thermally stable, for example at temperatures exceeding about 200 °C, and is
not hygroscopic nor deliquescent, thereby facilitating processing and storage ofthe
material. Crystalline solids are generally preferred over amorphous forms, for enhancing purity and stability ofthe manufactured product.
10 No crystalline salt forms ofthe compound offormula I have previously been reported. Accordingly, a need exists for a stable, crystalline salt form ofthe compound of formula I that is neither hygroscopic nor deliquescent, and exhibits favorable thermal stability.

15 SUMMARY OF THE INVENTION

The present invention provides a crystalline hydrochloride salt of 1 -isopropyl-2-
oxo- 1,2-dihydroquinoline-3-carboxylic acid {(lS,3R,5R)-8-{(R)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl} amide or a solvate thereof. In one aspect, the crystalline salt form ofthe invention is a crystalline
20 hydrochloride salt ofthe compound of formula I. In another aspect, the crystalline salt
form ofthe invention is a crystalline hydrate ofthe hydrochloride salt ofthe compound of

formula I.

Surprisingly, the crystalline hydrochloride salt ofthe invention has been found to
be thermally stable at temperatures greater than about 200 °C and to exhibit a weight
25 change ofless than about 0.2 % when exposed to a range ofrelative humidity between
about 2 % and about 90 % at room temperature. Furthermore, neither the crystalline hydrochloride salt ofthe invention nor the hydrate thereof is deliquescent when exposed
to up to 90 % relative humidity at room temperature.

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Among other uses, the crystalline salt forms ofthe invention are expected to be
useful for preparing pharmaceutical compositions for treating disorders ofreduced
motility ofthe gastrointestinal tract. Accordingly, in another ofits composition aspects,
the invention provides a pharmaceutical composition comprising a pharmaceutically-
5 acceptable carrier and a crystalline hydrochloride salt of 1 -isopropyl-2-oxo- 1,2-
dihydroquinoline-3-carboxylic acid {(1S,3R,5R)-8-[(R)2-hydroxy-3(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo [3.2. 1]oct-3 -yl} amide or a solvate thereof.
The invention also provides a method oftreating a disease or condition associated
with 5-HT4 receptor activity, e.g. a disorder ofreduced motility ofthe gastrointestinal
10 tract, the method comprising administering to the mammal, a therapeutically effective
amount of a crystalline hydrochloride salt of 1 -isopropyl-2-oxo-I,2-dihydroquinoline-3-carboxylic acid {(lS,3R,5R)-8-[(R)-2-hydroxy-3-(metbanesulfonyl-methyl-amino)propyl]- 8-azabicyclo[3.2.1]oet-3-yl}amide or a solvate thereof.
in another method aspect, the invention provides a process for preparing a
15 crystalline hydrochloride salt ofthe invention, the process comprising contacting
1-isopropyl-2-oxo-I,2-dihydroquinolIne-3-carboxylic acid {(lS,3R,5R)-8-[(R)-2-hydroxy-3-(ruethanesulfonyl-methyl-amino)propyl]-S-azabicyclo[3.2.1]oct-3-yl} amide with hydrochloric acid to form a reaction mixture, and isolating the crystalline hydrochloride
salt from the reaction mixture.

20 The invention also provides a crystalline hydrochloride salt ofthe invention as described herein for use in therapy or as a medicament, as well as the use of a crystalline hydrochloride salt ofthe invention in the manufacture of a medicament especially for the

25

manufacture of a medicament for treating a disorder ofreduced motility ofthe gastrointestinal tract in a mammal.

BRIEF DESCRIPTION OF THE DRAWINGS

Various aspects ofthe present invention are illustrated by reference to the
accompanying drawings.
Figure 1 shows a powder x-ray diffraction (PXRD) pattern of a crystalline
30 hydrochloride salt of 1 -isopropyl-2-oxo- 1,2-dihydroquinoline-3 -carboxylic acid
{(1 S,3R,5R)-8-[(R)-2-hydroxy-3-(methanesulfonyl-methyl-atnhtlo)propylJ-8-azabicyclo[3.2.1 Joct-3-yl} amide ofthe invention.

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Figure 2 shows a differential scanning calorimetry (DSC) trace (bottom trace, right
side vertical axis) and a thermal gravirnetric analysis (TGA) trace (top trace, left side
vertical axis) for a crystalline hydrochloride salt of 1-isopropyl-2-oxo-1,2-dihydroquinolirte-3-carboxylic acid {(1 S,3R,5R-8.-[(R)-2-hydroxy-3-methanesulfony1-
5 methyl-amino)propyl]-8-azabicyclo [3.2.1]oct-3-yl} amide ofthe invention.
Figure 3 shows a dynamic moisture sorption (DM5) trace for a crystalline
hydrochloride salt of 1 -isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid
{(1 S,3R,5R)-8-[(R)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl}- 8-
azabicyclo[3.2, 1]oct-3 -yl} amide ofthe invention.
10 Figure 4 shows a powder x-ray diffraction (PXRD) pattern of a crystalline hydrate of a hydrochloride salt of 1 -isopropyl-2-oxo- 1,2-dihydroquinoline-3-carboxylic acid
{(1 S,3R,5R)-8-[(R)-2-hydroxy-3 -(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl} amide ofthe invention.
Figure 5 shows a differential scanning calorimetry (DSC) trace (top trace, left side
15 vertical axis) and a thermal gravimetric analysis (TGA) trace (bottom trace, right side
vertical axis) for a crystalline hydrate of a hydrochloride salt of 1 -isopropyl-2-oxo- 1,2-dihydroquinoline-3-carboxylic acid {(1 S,3R,5R)-8-{(R)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo [3.2.1 }oct-3-ylJ amide ofthe invention.
Figure 6 shows a dynamic moisture sorption (DM5) trace for a crystalline hydrate
20 of a hydrochloride salt of 1 -isopropyl-2-oxo- 1,2-dihydroquinoliue-3-carboxylic acid
{(l S,3R,5R)-8-[(R)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl3-8-
azabicyclo[3.2.1 oct-3-yl} ainide ofthe invention.

25 DETAILED DESCRIPTION OF THE INVENTION

The invention provides a crystalline hydrochloride salt of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(I S,3R,5R)-8-[(R)-2-hydroxy-3-(methanesulfonyl-xnethyl-amino)propyll-8-azabicyclo [3.2.1 }oct-3-yl} amide and solvates thereof.

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Definitions

When describing the compounds, compositions and methods ofthe invention, the following terms have the following meanings, unless otherwise indicated.
The term "therapeutically effective amount" means an amount sufficient to effect
5 treatment when administered to a patient in need oftreatment.
The tenn "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 ofthe disease, disorder, or medical condition in a
patient;
(c) suppressing the disease, disorder, or medical condition, i.e., slowing or
arresting the development ofthe disease, disorder, or medical condition in
15 a patient; or
(d) alleviating the symptoms ofthe disease, disorder, or medical condition in a
patient.
The term "solvate" means a complex or aggregate formed by one or more
molecules of a solute, i.e. a compound ofthe invention or a pharmaceutically-acceptable
20 salt thereof; and one or more molecules of a solvent. Such solvates are typically
crystalline solids having a substantially fixed molar ratio ofsolute 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 specifically termed a hydrate.
25 The term "crystalline hydrochloride salt" as used herein means a crystalline solid that does not include a substantially fixed molar fraction ofsolvent molecules in the
crystal lattice, i.e. one that is not a solvate. Solvates, or specifically hydrates, ofthe invention ar identified explicitly.
It must be noted that, as used in the specification and appended claims, the
30 singular forms "a", "an", "one", and "the’ may include plural references, unless the
content clearly dictates otherwise.
The term "amino-protecting group" means a protecting group suitable for
preventing undesired reactions at an amino nitrogen. Representative amino-protecting
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groups include, but are not limited to, formyl; acyl groups, for example alkanoyl groups,
such as acetyl; alkoxycarbonyl groups, such as tert-butoxycarbonyl (Boo); arylniethoxycarbonyl groups, such as benzyloxyoarbonyl (Cbz) and 9-fluorenylinethoxycarbonyl (Finoc); arylmethyl groups, such as benzyl (Bn), trityl (Tr),
5 and 1,1 -di-(4’-methoxyphenyl)methyl; silyl groups, such as trimethylsilyl (TMS) and tert-
butyldixnethylsilyl (TBDMS); and the like.

Active Agt
The active agent in the present salt forms, i.e. the compound offormula I, is
10 designated 1 -isopropyl-2-oxo- 1,2-dihydroquinoline-3-carboxylic acid {(1S,3R,5R)-8-[(R)-
2-hydroxy-3-(methaxiesulfonyl-methyi-amino)propyl]-8-azabicyclo[3.2. l]oct-3-yl} ainide using the commercially-available AutoNom software (MDL Information Systems, GmbH, Frankfurt, Germany). The designation (1 S,3R,5R) describes the relative orientation ofthe bonds associated with the bicyclic ring system. The compound is alternatively denoted as
15 N- [(3-endo)-8- [(R)-2-hydroxy-3- (methanesulfonyl-rnethyl-axnino)propyl]-8-
azabicyclo[3.2.1]oct-3-yl]-I-(1 -methylethyl)-2-oxo- 1,2-dihydro-3-quinolinecarboxamide.

Salt Forms ofthe Invention

In one aspect, the invention provides crystalline 1 -isopropyl-2-oxo- 1,2-
20 dihydroquinoline-3-carboxylic acid {(1S, 3R,5R)-8-[(R)-2hydroxy-3-(methanesu1fouyl-
methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl} amide hydrochloride.
A crystalline hydrochloride salt ofthe invention typically contains between about
0.8 and about 1.2 molar equivalents of hydrochloric acid per molar equivalent ofthe compound of formula I, including between about 0.9 and about 1.1 molar equivalents of
25 hydrochloric acid per molar equivalent ofthe compound offormula I.
The molar ratio ofhydrochloric acid to the active agent can be readily determined
by methods available to those skilled in the art. For example, such molar ratios can be readily determined by titration with a standard solution ofsilver nitrate. Alternatively, elemental analysis, 1H NMR, and ion chromatography methods can be used to determine
30 the molar ratio,
In one aspect, the crystalline hydrochloride salt ofthe present invention is characterized by a powder x-ray diffraction (PXRD) pattern having two or more
diffraction peaks at 20 values selected from 4.41–0.2, 8.82–0.2, 9.08–0,2, 11.21–0.2,
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14.40–0.2, 16.42*0.2, 17.35–0.2, 17.61–0.2, 18.14–0.2, 19.04–0.2, 19.95–0.2,
20.20–0.2, 21.23–0.2, 22.13–0.2, 22.48–0.2, 22,8310.2, 24.16–0.2, 25.37+0.2,
25.56+0.2, 26.22–0.2, 27.33+0.2, 29.08+0.2, and 29.61–0.2. In particular, in this aspect, the crystalline form is characterized by a powder x-ray diffraction pattern having two or
5 more diffraction peaks at 20 values selected from 14.401-0.2, 17.35+0.2, 17.6110.2.
19.04–0.2, 21.23–0.2, and 22.13–0.2.
As is well known in the field ofpowder x-ray diffraction, peak positions of PXRD
spectra are relatively less sensitive to experimental details, such as details ofsample preparation and instrument geometry, than are the relative peak heights. Thus, in one
10 aspect, a crystalline hydrochloride salt ofthe compound offormula 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.
The crystalline hydrochloride salt ofthe present invention is also characterized by
high temperature thermal stability as evidenced by its differential scanning calorimetry
15 (DSC) trace which exhibits a peak in endothermic heat flow in the range of about 230 °C
to about 260 °C, as illustrated in Figure 2. Furthermore, the thermal gravimetric analysis (TGA) trace shows no significant thermal event below about 225 °C.
In yet another aspect a crystalline hydrochloride salt is characterized by its infrared absorption spectrum which shows significant absorption bands at about 758, 783, 795,
20 802, 949, 981, 1149, 1158, 1217, 1332, 1377, 1453, 1467, 1487, 1525, 1566, 1575, 1615,
1672, and 3197 cm’.
A crystalline hydrochloride salt ofthe compound offormula I has been
demonstrated to have a reversible sorption/desorption profile with an exceptionally low
level ofhygroscopicity (i.e., less than about 0.2 % weight gain in the humidity range of
25 2 % relative humidity to 90 % relative humidity at room temperature) as shown in
Figure 3.
Additionally, the crystalline hydrochloride salt ofthe compound of formula I has
been found to be stable upon exposure to elevated temperature and humidity for an extended period. For example, after storage for 24 weeks at 40°C and 75 % relative
30 humidity, analysis by HPLC showed no chemical degradation and there were no
detectable changes in the DSC, TGA, or PXR.D results
In another aspect, the invention provides a crystalline hydrate of a hydrochloride
salt ofthe compound offormula I.
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In one aspect, a crystalline hydrate of a hydrochloride salt ofthe present invention
is characterized by a powder x-ray diffraction (PXRD) pattern having two or more diffraction peaks at 28 values selected from 5.3010.2, 7.43–0.2, 8.72–0.2, 10.520.2, 13.85–0.2, 14.11–0.2, 15.80–0.2, 15.99–0.2, 17.26–0.2, 19.53–0.2, 20.0810.2,
5 21.06–0.2, 21.48–0.2, 21.92–0,2, 22.85–0.2, 23.91–0.2, 25.2810.2, 26.06–0.2,
27.34–0,2, 27.51–0.2, and 29.67–0.2. In particular, in this aspect, the crystalline form is characterized by a powder x-ray diffraction pattern having two or more diffraction peaks
at 20 values selected from 10.52–0.2, 13.85–0.2, 15.80–0.2, 17.26–0.2, and 21.064--0.2.
In another aspect, a crystalline hydrate of a hydrochloride salt ofthe compound of
10 formula I is characterized by a powder x-ray diffraction pattern in which the peak
positions are substantially in accordance with those shown in Figure 4.
The crystalline hydrate of a hydrochloride salt ofthe present invention is also
characterized by its differential scanning calorimetry (DSC) trace which exhibits a substantial peak in endothermic heat flow identified with melting ofthe crystal in the
15 range of about 225 °C to about 250 °C, with broad or weak endothernis at lower
temperatures as illustrated in Figure 5. Furthermore, the thermal gravirnetric analysis (TGA) trace shows the degradation temperature is above about 250 °C.
A crystalline hydrate of a hydrochloride salt ofthe compound offormula I has
been demonstrated to have a reversible sorption/desorption profile at room temperature
20 over the entire range of about 2 % to about 90 % relative humidity, as illustrated in

25

Figure 6. The crystalline hydrate exhibits less than about 0.25 % weight gain between about 40 % and about 75 % relative humidity.
These properties ofthe salt forms ofthis invention are further illustrated in the
Examples below.

yhetic Procedures
The active agent, 1-isopropyl-2-oxo- 1,2-dihydroquinoline-3-carboxylic acid {(1S,3R,5R)-8-[(R)-2-hydroxy-3-(rnethanesulfonyl-methyl-amino)propyl]-S.. azabicyclo[3.2.1]oct3-yl} amide, can be prepared from readily available starting materials
30 using the procedures described in the Examples below, or using the procedures described
in the commonly-assigned U.S. applications listed in the Background section ofthis application.

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To prepare a crystalline hydrochloride salt ofthe invention, the 1 -isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1 S,3R, 5R)- 8-[(R)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl}-8-azabicyclo [3.2.1]oct-3-yl} amide is typically contacted with about 1 to about 1.5 molar equivalents, including from about 1 to about
5 1.2 molar equivalents, of concentrated hydrochloric acid. Generally, this reaction is conducted in an inert diluent at a temperature ranging from about 20 °C to about 80 °C. Suitable inert diluents for this reaction include, but are not limited to, ethanol, methanol, isopropanol, ethyl acetate, acetonitrile, toluene, tetrahydrofliran, and combinations
thereof.

10 Upon completion ofthe reaction, a crystalline salt ofthe invention is isolated from
the reaction mixture by any conventional means, such as precipitation, concentration, centrifugation, and the like,
The crystalline hydrate may he prepared by dissolving the hydrochloride salt of
1 -isopropyl-2-oxo-1,2-dihydroquino1ine3-carboxylic acid {(l S,3R,5R)-8-{(R)-2hydroxy-
15 3.-(nethanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl} amide in water at a concentration above about 50 mg/niL, which provides a suspension from which the resulting crystalline hydrate maybe isolated by conventional means.

Pharmaceutical Comt,ositions
20 The crystalline hydrochloride salt forms ofthe 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.
25 Accordingly, in one ofits compositions aspects, the invention is directed to a
pharmaceutical composition comprising a pharmaceutically-acceptable carrier or
excipient and a therapeutically effective amount of a crystalline hydrochloride salt of a compound offormula I. Optionally, such pharmaceutical compositions may contain other therapeutic and/or formulating agents if desired.
30 The pharmaceutical compositions ofthe invention typically contain a
therapeutically effective amount of a crystalline salt ofthe present invention. Typically,
such pharmaceutical compositions will contain from about 0.1 to about 95% by weight of

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the active agent; including from about 1 to about 70% by weight, such as from about 5 to about 60% by weight ofthe active agent.
Any conventional carrier or excipient may be used in the pharmaceutical
compositions ofthe invention. The choice of a particular carrier or excipient, or
5 combinations of carriers or excipients, will depend on the mode of administration being
used to treat a particular patient or type ofmedical 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 ofthose skilled in the pharmaceutical arts. Additionally, the ingredients for such compositions are commercially-available from, for
10 example, Sigma, P.O. Box 14508, St. Louis, MO 63178. By way offurther illustration,
conventional formulation techniques are described in Remington: The Science and

Practice ofPharmacy, 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).
15 Representative examples ofmaterials 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;
20 (5) malt; (6) gelatin; (7) tale; (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
25 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 ofthe invention are typically prepared by
thoroughly and intimately mixing or blending a compound ofthe invention with a
30 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.

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The pharmaceutical compositions ofthe invention are preferably packaged in a
unit dosage form. The term "unit dosage form" refers to a physically discrte 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
5 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 ofthe invention are
suitable for oral administration. Suitable pharmaceutical compositions for oral administration may be in the form of capsules, tablets, pills, lozenges, cachets, dragees,
10 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 ofthe present invention as an
active ingredient.
When intended for oral administration in a solid dosage form (i.e., as capsules,
15 tablets, pills and the like), the pharmaceutical compositions ofthe invention will typically
comprise a compound ofthe 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 extenders, such as starches, microcrystalline cellulose, lactose, sucrose, glucose, mannitol,
20 and/or silicic acid; (2) binders, such as carboxymethylcellulose, alginates, gelatin,
polyvinyl pyrrolidone, sucrose and/or acacia; (3) huinectants, 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
as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7)
25 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.
Release agents, wetting agents, coating agents, sweetening, flavoring and
30 perfuming agents, preservatives and antioxidants can also be present in the
pharmaceutical compositions ofthe invention. Examples ofpharmaceutically-acceptable antioxidants include: (1) water-soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium rnetabisulfate sodium sulfite and the like; (2) oil-

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soluble antioxidants, such as ascorbyl palinitate, 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,
5 capsules, pills and like, include those used for enteric coatings, such as cellulose acetate
phthalate (CAP), polyvinyl acetate phthalate (PVAP), hydroxypropyl methylcellulose phthalate, methacrylic acidmethacrylic acid ester copolymers, cellulose acetate
trimellitate (CAT), carboxymethyl ethyl cellulose (CMEC), hydroxypropyl methyl
cellulose acetate sucoinate (HPMCAS), and the like.
10 If desired, the pharmaceutical compositions ofthe present invention may also be formulated to provide slow or controlled release ofthe active ingredient using, by way of example, hydroxypropyl methyl cellulose in varying proportions; or other polymer
matrices, liposomes and/or microspheres.
In addition, the pharmaceutical compositions ofthe present invention may
15 optionally contain opacifying agents and may be formulated so that they release the active
ingredient only, or preferentially, in a certain portion ofthe 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-encapsulated form, if appropriate, with one or more ofthe above-described excipients.
20 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 agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl
25 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 ofsorbitan, and mixtures thereof. Suspensions, in addition to the active ingredient, may contain suspending agents such as,
for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters,
30 microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth,

and mixtures thereof.

Alternatively, the pharmaceutical compositions ofthe invention are formulated for administration by inhalation. Suitable pharmaceutical compositions for administration by
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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
5 pharmaceutical compositions ofthe invention will typically comprise the active ingredient and a suitable propellant, such as dichiorodifluoromethane, trichiorofluoromethane, dichiorotetrafluoroethane, 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 ofthe invention and
10 a powder suitable for use in a powder inhaler. Suitable powder bases include, by way of
example, lactose or starch.
The compounds ofthe invention can also be administered transdermally using
known transdermal delivery systems and excipients. For example, a compound ofthe
invention can be admixed with permeation enhancers, such as propylene glycol,

40 15 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
ofthe present invention:
20

Formulation Example A
Hard gelatin capsules for oral administration are prepared as follows:

25 Ingredients Amount
Salt ofthe invention 50 mg
Lactose (spray-dried) 200 mg
. Magnesium stearate 10 mg

30

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

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Formulation Example B
Hard gelatin capsules for oral administration are prepared as follows:

Ingredients Amount
Salt ofthe invention 20 mg
Starch 89 mg
Microcrystalline cellulose 89 mg
10 Magnesium stearate 2 mg

15

Rpresentative 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).

Formulation Examp
Capsules for oral administration are prepared as follows:

20 Ingredients Amount
Salt ofthe invention 10mg
Polyoxyethylene sorbitan monooleate 50 mg
Starch powder 250 mg
25

Representative Procedure: The ingredients are thoroughly blended and then loaded into a gelatin capsule (3 10 mg of composition per capsule).

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Formulation Example P

Tablets for oral administration are prepared as follows:
Ingredients Amount
5 Salt ofthe invention 5 mg
Starch 50 mg
Microcrystalline cellulose 35 mg
Polyvinylpyrrolidone (10 wt. in water) 4 mg
Sodium carboxyniethyl starch 4.5 mg
10 Magnesium stearate 0.5 mg
Talc 1mg

Representative Procedure: The active ingredient, starch and cellulose are passed
15 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
passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at
50-60°C and passed through aNa. 18 mesh U.S. sieve. The sodium
carboxyinethyl starch, magnesium stearate and talc (previously passed through a
20 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.

Formulation Example E
Tablets for oral administration are prepared as follows:
25

Ingredients Amount
Salt ofthe invention 25 mg
Microcrystalline cellulose 400 mg
30 Silicon dioxide fumed 10 mg
Stearic acid 5 mg

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

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Formulation Example F

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

Ingredients Amount
Salt ofthe invention 15 mg
Cornstarch 50 mg
Croscarmellose sodium 25 mg
Lactose 120 mg
10 Magnesium stearate 5 trig

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

Formulation Exainpe Q
A suspension for oral administration is prepared as follows:

20 Ingredients Amount

Salt ofthe invention 0.1 g
Fumaric acid 0.5 g
Sodium chloride 2.0 g
25 Methyl paraben 0,15g
Propyl paraben 0.05 g
Granulated sugar 25.5 g
Sorbitol (70% solution) 12.85 g
Veegum ic (Vanderbilt Co.) 1.0 g
30 Flavoring 0.035 mL
Colorings 0.5 mg
Distilled water q.s. to 100 mL

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Representative Procedure: The ingredients are mixed to form a suspension

containing 10 mg of active ingredient per 10 mL ofsuspension.

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g.ulation Example H
A dry powder for administration by inhalation is prepared as follows:

Ingredients Amount
Salt ofthe invention 1.0 mg
Lactose 25 mg

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 ofthe cartridge are administered using a powder inhaler.

15 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 salt ofthe
invention and 0.1 wt. % lecithin is prepared by dispersing 10 g of active
20 compound as micronized particles with mean size less than 10 pm in a solution
formed from 0.2 g oflecithin dissolved in 200 mL ofdemineralized water. The
suspension is spray dried and the resulting material is micronized to particles
having a mean diameter less than 1.5 p.m. The particles are loaded into cartridges
with pressurized 1,1,1,2-tetrafluoroethane.
25

Formulation Example J
An injectable formulation is prepared as follows:

30 Ingredients Amount
Salt ofthe invention 0.2 g
Sodium acetate buffer solution (0.4 M) 40 mL
HCI (0.5 N) or NaOH (0.5N) q.s. to pH 4
35 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 HCI or 0.5 N NaOH.

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Formulation Example K
Capsules for oral administration are prepared as follows:
Ingredients Amount
5 Salt ofthe Invention 4.05 mg
Microcrystalline cellulose (Avicel PH 103) 259.2 rug
Magnesium stearate 0.75 mg

10 Rgpresentative 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
15 Capsules for oral administration are prepared as follows:
Ingredients Amount
Salt ofthe Invention 8.2 rug
Microcrystalline cellulose (Avicel PH 103) 139.05 mg
20 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
25 capsule).

Utility
The compound offormula I, 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic
acid {(1S,3R,5R)-8{(R)-2hydroxy-3-(methanesulfonyl-methyl-amiflO)PrOpYl]-8-
30 azabicyclo{3.2. 1]oct-3-yl}amide, is a 5-HT4 receptor agonist and therefore the present crystalline salt forms ofthe compound offormula I 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 conditions include, but are not limited to, irritable bowel syndrome
35 (1135), chronic constipation, functional dyspepsia, delayed gastric emptying, gastroesophageal reflux disease (GERD), gastroparesis, diabetic and idiopathic
gastropathy, post-operative ileus, intestinal pseudo-obstruction, and drug-induced delayed transit. In addition, it has been suggested that some 5-HT4 receptor agonist compounds

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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 salt forms ofthe invention increase motility ofthe
5 gastrointestinal (GI) tract and thus are expected to be useful for treating disorders ofthe
01 tract caused by reduced motility in mammals, including humans. Such GI motility disorders include, by way ofillustration, chronic constipation, constipation-predominant irritable bowel syndrome (C-IBS), diabetic and idiopathic gastroparesis, and functional dyspepsia.
10 in one aspect, therefore, the invention provides a method ofincreasing 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-acceptable carrier and a crystalline salt ofthe invention.
When used to treat disorders ofreduced motility ofthe GI tract or other conditions
15 mediated by 5-HT4 receptors, the salt forms ofthe invention will typically be
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 trpica1ly be determined by a physician, in
the light ofthe relevant circumstances, including the condition to be treated, the chosen
20 route of administration, the actual compound administered and its relative activity, the
age, weight, and response ofthe individual patient, the severity ofthe patient’s symptoms, and the like.

Suitable doses for treating disorders ofreduced motility ofthe GI tract or other
disorders mediated by 5-HT4 receptors are expected to range from about 0.0007 to about
25 20 mg/kg/day of active agent, including from about 0.0007 to about 1 mg/kg/day. For an
average 70 kg human, this would amount to from about 0.05 to about 70 mg per day of active agent.
In one aspect ofthe invention, the salt forms ofthe invention are used to treat
chronic constipation. When used to treat chronic constipation, the salts ofthe invention
30 will typically be administered orally in a single daily dose or in multiple doses per day.
The dose for treating chronic constipation is expected to range from about 0.05 to about
70 mg per day.

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In another aspect ofthe invention, the salt forms ofthe invention are used to treat
irritable bowel syndrome. When used to treat constipation-predominant irritable bowel syndrome, the salts ofthe invention will typically be administered orally in a single daily dose or in multiple doses per day. The dose for treating constipation-predominant
5 irritable bowel syndrome is expected to range from about 0.05 to about 70 mg per day.
In another aspect ofthe invention, the salt forms ofthe invention are used to treat
diabetic and idiopathic gastroparesis. When used to treat diabetic and idiopathic gastroparesis, the salts ofthe invention will typically be administered orally in a single
daily dose or in multiple doses per day. The dose for treating diabetic gastroparesis is
10 expected to range from about 0.05 to about 70 mg per day.
In yet another aspect ofthe invention, the salt forms ofthe invention are used to
treat functional dyspepsia. When used to treat functional dyspepsia, the compounds ofthe invention will typically be administered orally in a single daily dose or in multiple doses
per day. The dose for treating functional dyspepsia is expected to range from about 0.05
15 to about 70 mg per day.
The invention also provides a method oftreating a mammal having a disease or
condition associated with 5-kIT4 receptor activity, the method comprising administering to the mammal a therapeutically effective amount of a salt form ofthe invention or of a pharmaceutical composition comprising a salt form ofthe invention.
20 As described above, salt forms ofthe invention are 5HT4 receptor agonists. The invention further provides, therefore, a method of agonizing a 5-HT4 receptor in a
mammal, the method comprising administering a salt form ofthe invention to the

mammal.

There properties, as well as the utility ofthe hydrochloride salt forms ofthe
25 invention, can be demonstrated using various in vitro and in viva assays well-known to
those skilled in the art. Representative assays are described in further detail in the following examples

30 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 ofthe invention.
In the examples below, the following abbreviations have the following meanings unless
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otherwise indicated. Abbreviations not defined below have their generally accepted
meanings.
J3oc tert-butoxycarbonyl
(Boc)20 di-tert-butyl dicarbonate
5 DCM dichioromethane
DMF NN-dimetbylforrnamide
DMSO dimethyl sulfoxide
EtOAc ethyl acetate
mCPBA ,n-chlorobenzoic acid
10 MeCN acetonitrile
MTBE tert-butyl methyl ether
PyBop benzotriazol-I-yloxytripyrrolidino-
phosphonium hexafluorophosphate
Rf retention factor
15 RT room temperature
TFA trifluoroacetic acid
THF tetrahydrofuran

Reagents (including secondary amines) and solvents were purchased from
20 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 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 ofreactions. Reaction
25 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. Characterization ofreaction products was routinely carried out by mass and ’H-NIvIIR spectrometry. For NMR measurement, samples were dissolved
30 in deuterated solvent (CD30D, CDC13, or DMSO-d6), 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. Water
35 content is determined by Karl Fischer titration using a Brinkmann (Westbury, NY)
Metrohm Karl Fischer Model 813 coulometer.

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Preparation 1: (1S,3R,5R)3-amino-8-azabicycoE3..2.1]oCtaUe-8-carbOXYtiC
acid tert-butyl ester
a. Prevaration of 8-benzy1-8-azabicc1o3.2.11octan-3-one
Concentrated hydrochloric acid (30 mL) was added to a heterogeneous solution of
5 2,5-dimethoxy tetrahydrofuran (82.2 g, 0.622 mol) in water (170 mL) while stirring. In a
separate flask cooled to OC (ice bath), concentrated hydrochloric acid (92 mL) was added slowly to a solution ofbenzyl amine (100 g, 0.933 mol) in water (350 mL). The 2,5-dimethoxytetrahydroftiran solution was stirred for approximately 20 min, diluted with
water (250 ml), and then the benzyl amine solution was added, followed by the addition
10 of a solution of 1,3-acetonedicarboxylic acid (100 g, 0.684 mol) in water (400 mL) and
then the addition ofsodium hydrogen phosphate (44 g, 0.31 mol) in water (200 mL). The
pH was adjusted from pH 1 to p1-I 4.5 using 40% NaOH. The resulting cloudy and pale
yellow solution was stirred overnight. The solution was then acidified to p1-I 3 from p1-I
7.5 using 50% hydrochloric acid, heated to 85 °C and stirred for 2 hours. The solution
. 15 was cooled to room temperature, basified to pH 12 using 40% NaOH, and extracted with
dichloromethane (3 x 500 mL). The combined organic layers were washed with brine,
dried (MgSO4), filtered and concentrated under reduced pressure to produce the crude title
intermediate as a viscous brown oil.
To a solution ofthe crude intermediate in methanol (1000 mL) was added di-tert-
20 butyl dicarbonate (74.6 g, 0.342 mol) at 0 °C. The solution was allowed to warm to room
temperature and stirred overnight. The methanol was removed under reduced pressure
and the resulting oil was dissolved in dichloromethane (1000 mL). The intermediate was extracted into 1 M H3PO4 (1000 niL) and washed with dichloromethane (3 x 250 mL)
The aqueous layer was basified to pH 12 using aqueous NaOH, and extracted with
25 dichloromethane (3 x 500 mL). The combined organic layers were dried (MgSO4),
filtered and concentrated under reduced pressure to produce the title intermediate as a viscous, light brown oil. ’H-NMR (ODd3) (ppm) 7.5-7.2 m, 5H, C6H5), 3.7 (s, 21-I, CH2Ph), 3.45 (broads, 2H, CH-NBn), 2.7-2.6 (dd, 2H, CH2CO), 2.2-2.1 (dd, 2H, CH2CO), 2.1-2.0 (m, 21-I, CH2CH2), 1.6 (in, 2H, CH2CH2). (,n/z): [M+Hr calcd for
30 C14}117N0 216.14; found, 216.0.

b. Preparation of 1Joctai8-crboxyljp acid Lert-butylster
To a solution of 8-benzyl-8-azabicyclo[3.2.1]octan-3-one (75 g, 0.348 mol) in EtOAc (300 mL) was added a solution of di-tert-butyl dicarbonate (83.6 g, 0.383 mol,
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1.1 eq) in EtOAc (300 mL). The resulting solution and rinse (100 mL EtOAc) was added
to a I L Parr hydrogenation vessel containing 23 g ofpalladium hydroxide (20 wt.% Pd,
dry basis, on carbon, -50% wet with water; e.g. Peariman’s catalyst) under a stream of nitrogen. The reaction vessel was degassed (alternating vacuum and N2 five times) and
5 pressurized to 60 psi of 112 gas. The reaction solution was agitated for two days and
recharged with H2 as needed to keep the 112 pressure at 60 psi until the reaction was complete as monitored by silica thin layer chromatography. The black solution was then filtered through a pad of Celitefi and concentrated under reduced pressure to yield the title intermediate quantitatively as a viscous, yellow to orange oil. It was used in the next step
10 without further treatment. ’I-I NMR (CDC13) (ppm) 4.5 (broad, 2H, CH-NBoe), 2.7
(broad, 2H, CH2CO), 2.4-2.3 (dd, 211, Ci-12CH2), 2.1 (broad in, 21-I, CH2CO), 1,7-1.6 (dd, 211, C112CH2), 1.5 (s, 911, (CH3)3COCON)).

c. Preparation of(lS,3R,5R).-3.-amino-8-azabicyclo{3.2. lloctane-8-carboxylic acid tert-
butyl ester
15 To a solution ofthe product ofthe previous step (75.4 g, 0.335 mol) in methanol (1 L) was added ammonium fonnate (422.5 g, 6.7 mol), water (115 mL) and 65 g of palladium on activated carbon (10% on dry basis, -50% wet with water; Degussa type E101NE/W) under a stream ofN2 while stirring via mechanical stirrer. After 24 and 48 hours, additional portions of ammonium formate (132g, 2.1 mol) were added each time.
20 Once reaction progression ceased, as monitored by anal. UPLC, Celitefi (>500g) was
added and the resulting thick suspension was filtered and then the collected solid was
rinsed with methanol (-500 mL). The filtrates were combined and concentrated under reduced pressure until all methanol had been removed. The resulting cloudy, biphasic solution was then diluted with IM phosphoric acid to a final volume of -1.5 to 2.0 L at
25 pH 2 and washed with dichtoromethane (3 x 700 niL). The aqueous layer was basifled to
pH 12 using 40% aq. NaOH, and extracted with dichioromethane (3 x 700 mL). The combined organic layers were dried over MgSO4, filtered, and concentrated by rotary evaporation, then high-vacuum leaving 52 g (70%) ofthe title intermediate, commonly N-Boc-endo-3-aminotropane, as a white to pale yellow solid. The isomer ratio of endo to
30 exo amine ofthe product was >99 based on ’H-NMR analysis (>96% purity by analytical
RPLC). ’H NMR (CDC13) 3 (ppm) 4.2-4.0 (broad d, 2H, CHNBoc), 3.25 (t, 11-I,
CEENH2), 2.1-2.05 (in, 4H), 1.9 (in, 21-I), 1.4 (s, 9H, (CH3)3000N), 1.2-1.1 (broad, 211).

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(m/z): [M+H] caled for C,H22N202 227.18; found, 227.2. Analytical HPLC (isocratic method; 2:98 (A:B) to 90:10 (A:B) over 5 mm): retention time = 2.14 mm.
Preparation 2: 1-isopropyl-2-oxo-12-dihydroquinoflne-3-carboxyltc acid
First, acetone (228.2 mL, 3.11 mol) was added to a stirred suspension of 2-
5 aminophenylinethanol (255.2 g, 2.07 mol) and acetic acid (3.56 mL, 62 mmol) in water (2
L) at room temperature. After 4 h, the suspension was cooled to 0 °C and stirred for an additional 2.5 h and then filtered. The solid was collected and washed with water and the
wet solid cooled and dried by lyophilisation to yield 2,2,-dimethyi-1,4-dihydro-2FT-benzo[1,3joxazine (332.2 g, 98 %) as an off-white solid. H NMR (CDC13; 300MHz):
10 1.48 (s, 61-I, C(C3)2), 4.00 (bs, 1H, NH), 4.86 (s, 2H, Cj), 6.66 (d, IH, ArU), 6.81 (t,
IH, ArH), 6.96 (d, 1H, ArH), 7.10 (t, 1H, Ar).
A solution of2,2,-dimethyl-1,4-dihydro-2H-benzo[1,3]oxazine (125 g, 0.77 mol)
in THF (1 L) was filtered through a scintillation funnel and then added dropwise via an addition funnel, over a period of 2.5 h, to a stirred solution of 1.0 M LiAIH4 in THF
15 (800 rnL) at 0 °C. The reaction was quenched by slow portioriwise addition of
Na2S0410H20 (110 g), over a period of 1.5 h, at 0’C. The reaction mixture was stirred overnight, filtered and the solid salts were washed thoroughly with THF. The filtrate was concentrated under reduced pressure to yield 2-isopropylaminophenylniethanol (120 g, 95
%) as a yellow oil. 1H NMR (CDCI3; 300MHz): 1.24 (d, 6H, CH(CH3)2), 3.15 (bs, IH,
20 O), 3.61 (sept, 111, CJj(C113)2), 4.57 (a, 2H, C), 6.59 (t, lI-I, ArH), 6.65 (d, 1H, ArH),
6.99 (d, ill, Arli), 7.15 (t, IH, ArH),
Manganese dioxide (85 % 182.6 g, 1.79 mol) was added to a stirred solution of 2-isopropylaminophenylmethanol (118 g, 0.71 mol) in toluene (800 niL) and the reaction mixture was heated to 117 °C for 4 h. The reaction mixture was allowed to cool to room
25 temperature overnight and then filtered through a pad of Celite which was eluted with
toluene. The filtrate was concentrated under reduced pressure to yield 2-isopropylaminobeazaldehyde (105 g, 90 %) as an orange oil. ’H NMR (CDCI3;
300MHz): 1.28 (d, 611, CH(C3)2), 3,76 (sept, 1H, CH(CH02), 6.65 (t, iii, ArH), 6.69 (d,
1H, ArH), 7.37 (d, 111, Ark), 7.44 (t, 11-i, ArE), 9.79 (s, iii, CEO).
30 2,2-Dimethyl-{1,3]dioxane-4,6-dione, commonly Meidrum’s acid, (166.9 g, 1.16 mol)
was added to a stirred solution of 2-isopropylaminobenzaldehyde (105 g, 0.64 mol), acetic acid
(73.6 mL, 1.29 mol) and ethylenediamine (43.0 mL, 0.64 mol) in methanol (1 L) at 0’C. The

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reaction mixture was stirred for 1 h at 0 °C and then at room temperature overnight. The resulting suspension was filtered and the solid washed with methanol and collected to yield the title intermediate, 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (146 g, 98 %) as an off-white solid. ijj NMR (CDC13; 300MHIz): 1.72 (d, 6H, CH(C)), 5.50 (bs, 111, Cj(CH3)2), 7.44 (t, IH, Ar), 7.75-7.77 (in, 2H, A4), 7.82 (d, 1H, ArH), 8.89 (s, IH, CH), Example 1: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinoUne-3-carboxylic
acid (1S,3R,5R)-8- (R)-2-bydroxy-3-(methanesulfonyl-metbyl-amino)propyl-8-aza-bicyc1o[3.2.1)oct-3-yl} amide
a. Preparation of (l,3R,5R-3-f 1-jpyi-2-oxo-1dihydroguino1ine-3-
10 carbonylaminQ3-8-azabicyclo[3.211octane-8-carboxv1ic acj4ert-buty1 ester
In a 3 L flask, 1-isopropyl-2-oxo-1,2-dibydroquinoline-3-carboxylic acid (112.4 g,
0.486 mol, 1.1 eq) was suspended in toluene (1 L). The mixture was heated to 85 °C and thionyl chloride (86.74 g, 0.729 mol) was added dropwise over 70 The mixture was heated at 95 °C for 1.5 li with stirring and then allowed to cool to room temperature.
15 Ina separate 12 L flask, (1S,3R,5R)-3-amino-8-azabicyclo[3.2.ljoctane-8-
carboxylic acid tert-butyl ester (100.0 g, 0,442 mol, 1 eq) was suspended in toluene (1 L) and 3 M NaOH (4 eq) was added. The mixture was stirred at room temperature for 10
min and then cooled to about 5 °C. The acid chloride solution was added slowly with stirring over 40 min keeping the internal temperature below 10 °C. The mixture was
20 stirred at 3-5 °C for 30 min and the layers were allowed to separate overnight. The toluene layer (-2.5 L) was collected, concentrated to about half(-1.2 L) by rotary evaporation, and used directly in the next step.

b. Preparation of 1-isopropy1-2-xo1,2-dihydroguino1une-3-carboxy1ic acid {L1S,3R,5B 8-azabicyclof3.2. lloct-3-y1} anide
25 To the toluene solution prepared in the previous step (-1.2 L) was added
trifluoroacetic acid (200 mL) over 20 min at 20 °C with stirring. The mixture was stirred
at 20°C for 2 ii. Water (1.55 L) was added and the mixture was stirred for 30 min at
20 °C. After 30 min, the mixture separated into three layers. The bottom layer
(-350 niL), a viscous brown oil, contained the crude intermediate.
30 To a 12 L flask charged with MTB7 (2.8 L), the crude brown oil was added over
I h at 1-2 °C with stirring. The suspension was stirred at the same temperature for I h and then filtered. The filtrate was washed with MTI3E (2 x 300 niL) and dried under vacuum

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at room temperature for 4 days to provide the trifluoroacetate salt ofthe title intermediate (163.3 g) as a pale yellow powder.
C. PreDaration ofN-methyl-N-[(@-2-oxiran-2-yjmethyl}methanesulfonaxnide
A 12 L flask was charged with water (1 L) followed by the addition NaOH (50 %
5 in water, 146.81 g, 1.835 mol), The beaker containing NaOH was washed with water
(2 x 500 mL) and the washings were added to the flask. The mixture was stirred at room temperature for 10 min and cooled to -8 °C. (N-methyl)methanesulfonamide (200.2 g, 1.835 mol) in water (500 mL) was added over 5 min. The mixture was stirred for 1 h at
4 °C and (S)-2-chloromethyloxirarie (339.6 g, 3.67 mol) was added. The mixture was
10 stirred for 20 h at 3-4 °C. Dichioromethane (2 L) was added and the mixture was stirred
for 30 min at 5-10 °C. The two layers were allowed to separate over 10 min and
collected. The organic layer (2.5 L) was added back to the 12 L flask and washed with
1 M H3PO4 (800 mL) and brine (800 rnL). Dichioromethane was removed by rotary evaporation. To the crude product, toluene (400 mL) was added and removed by rotary
15 evaporation. After three additional cycles ofthe toluene process, the title intermediate
was obtained (228,2 g) which was used without further purification in the next step.

d. Synthesis of 1 -isoDrovyl-2-oxo- 1,2-dihydrociuinoline-3-carboxylic acid (1S,3R,5R)-8--2-hydroxy-3-(piethanesulfonyl-tnethyl-amino)propy11-8-aza-bicyc1o13.2. 11ot
yl} amide
20 in a 3 L flask, 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1S,3R,5R)-8-azabieyelo{3.2. ljoct-3-yl} amide trifluoroacetate (105.0 g, 0.232 mol) was suspended in absolute ethanol (400 mL). To this suspension, NaOH (50 % in water,
0.243 mol. 1.05 eq) dissolved in absolute ethanol (100 mL) was added at room
temperature. The beaker containing the NaOH was washed with ethanol (2 x 50 mL) and
25 the washings were added to the reaction mixture. After 30 min ofstirring, a solution of
N-methyl-N-[(S)-2-oxiran-2-ylmethylmethanesulfonamide (62.0 g, 1.5 eq) in absolute ethanol (100 mL) was added. The mixture was refluxed for 2 h, cooled to room
temperature and seed crystals ofthe title compound were added. After about 5 min of stirring a white solid formed. The mixture was cooled to 3-5 °C and stirred for 2 h. The
30 white solid was filtered and the wet cake was washed with cold absolute ethanol
(3 x 50 mL). The solid was dried under vacuum at 30 °C for 60 h to provide the title compound (93.8 g, water content by Karl Fischer method 2.03 %). ’H NMR (CDCI3)
8 ppm 10.52 (d, 1H), 8,83 (s, 1H), 7.75 (d, 2H), 7.64-7.60 (m, 211), 7.28-7.26 in, 111),
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4.33-4.26 (m, 2H), 3.78-3.75 (m, 1H), 3.27-3.20 (in, 4H), 3.01. (s, 3H), 2.88 (s, 3H), 2.58-
253 (m, 1H), 2.30-1.81(m, ILEI), 1.68 (d, 6H).
The seed crystals were obtained from a previous preparation ofthe title compound
by the method ofthis example at smaller scale, in which crystallization occurred
5 spontaneously.

Example 2: Synthesis of crystalline hydrochloride salt of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1S,3R,SR)-8-I(R)-2-bydroxy-3-(metbanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo3.2.i] oct-3-y1 amide
In a 1 L flask, 1. -isopropyl-2-oxo- 1,2-dihydroquinoline-3-carboxylic acid
10 {(1S,3R,5R)-8-[(R)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-.
bicyclo[3.2.1]oct-3-yl}amide (34.7 g, 0,069 mol) was suspended in absolute ethanol
(210 mL). Concentrated HCl (1.1 eq) was added at room temperature with stirring. The mixture was stirred at reflux for 30 min and cooled to room temperature and stirred for
2 h. The solid was filtered and the wet cake was washed with cold absolute ethanol
15 (3 x 50 mL). The solid was dried under vacuum at 30 °C for 48 h to provide the title
compound (34.5 g, 93.7 % yield, water content by Karl Fischer method 0.13 %).

Example 3: Synthesis of crystalline hydrate of hydrochloride salt of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1S,3R,5R)-8-[(R)-2-bydroxy-3-(methanesulfonyl-metbyl-amino)propyl]-8-aza-bicyclo[3.2.1] oct-3-
20 yl}amlde
1 -Isopropyl-2-oxo-I,2-dihydroquinolin.e-3-carboxylic acid {(1S,3R,5R)-8-[(R)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl}-8-aza-bicyclo3.2.1 Joct-3-yl} amide hydrochloride (139 mg, 0.28 mniol) was dissolved in sterilized water for injection (2 ML). Over a few hours, the solution became a cloudy suspension. The suspension was stirred
25 and allowed to sit overnight at ambient temperature resulting in a white precipitate. The
solid was collected by filtration and dried for 2 min at ambient conditions (approximately 40-50 % relative humidity) to provide the title compound (130 mg, 91 % yield).

Examples 4 9: Properties of Salt Forms ofthe Invention
Samples ofthe crystalline hydrochloride salt of 1-isopropyl-2-oxo- 1,2-
30 dihydroquinoline-3-carboxylic acid {(1S,3R,5R)-8- [(R)-2-hydroxy-3-(methanesult’onyl-
methyl-amino)propyl-8-aza-bicyclo[3.2. 1]oct-3-yl} amide (compound of formula 1), prepared as in Example 2 and ofthe crystalline hydrate ofthe hydrochloride salt ofthe compound offormula I, prepared as in Example 3, were analyzed by powder x-ray

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diffraction (PXRD), differential scanning calorimetry (DSC), therrnogravimetric analysis (TGA) infrared spectroscopy (IR) and elemental analysis.

Example 4: Powder X-Ray Diffraction
Powder x-ray diffraction patterns were obtained with a Thermo ARL X-Ray
5 Diffraetoineter Model X’TRA (Thermo ARL SA, Switzerland) using Cu Ka radiation at
1.542 A (45 kV, 40 mA) with a Si(Li) solid-state detector. The analysis was typically performed at a scan rate of21/mm with a step size of 0.031 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 custom small-volume insert designed to fit into the instrument top-
10 loading sample cup for analysis. The instrument calibration to within d0,02° two-theta
angle was verified weekly by comparison with a silicon metal standard. Representative PXRD patterns for samples ofthe crystalline hydrochloride salt and ofthe hydrate ofthe hydrochloride salt ofthe invention are shown in Figures 1 and 4, respectively.

15 Example 5: 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 ScriesTM software. A sample of about 1-10 mg was accurately weighed into an aluminum pan with lid, The sample was evaluated using a linear heating ramp of
20 10 °C/min from 5 °C to, typically, 265 °C. The DSC cell was purged with thy nitrogen
during use. Representative DSC traces for samples ofthe crystalline hydrochloride salt
and ofthe crystalline hydrate of a hydrochloride salt of compound I are shown in Figures
2 and 5, respectively.
Therinogravimetric analysis (TGA) was performed using a TA Instruments Model
25 Q-500 module. Data were collected and analyzed using TA Instruments Thermal
Advantage for Q SeriesTM software. A sample weighing about 1-5 mg was placed in an aluminum pan on a platinum cradle and scanned from ambient temperature to 300 °C
with a linear heating rate of 10 ’C/min. The balance and furnace chambers were purged with nitrogen during use. Representative TGA traces for samples of a crystalline
30 hydrochloride salt and of a crystalline hydrate of a hydrochloride salt of compound I are
also shown in Figures 2 and 5, respectively.

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The DSC trace in Figure 2 demonstrates that a hydrochloride salt ofthe present
invention has excellent thermal stability with a maximum in endothermic heat flow in the range of about 230 IC to about 260 °C and no significant thermal events below about
225 °C. Comparison ofthe DSC and TGA traces indicates that a hydrochloride salt ofthe
5 present invention undergoes simultaneous melting and decomposition at temperatures

above about 230 T.

The DSC trace in Figure 5 for the present hydrate form exhibits a substantial peak
in endothermic heat flow identified with melting ofthe crystal in the range of about
225 °C to about 250 °C and broad or weak endotherms at lower temperatures.
10 Comparison ofthe DSC and TGA traces indicates that decomposition ofthe hydrate
crystalline form is not significant at the temperature ofthe melt transition.

Example 6: Dynamic Moisture Sorption Assessment
Dynamic moisture sorption (DMS) assessment was performed at 25 °C using a
15 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 ofthe analysis. A typical DMS analysis consisted ofthree scans:
ambient to 2% relative humidity (RH), 2% RH to 90% RH, 90% RH to 5% RH at a scan
rate of 5 % R14/step. The mass was measured every two minutes and the RH was changed
20 to the next value + 5 %RH) when the mass ofthe sample was stable to within 0.02 % for
5 consecutive points, Representative DMS traces for samples of a crystalline
hydrochloride salt and of a crystalline hydrate of a hydrochloride salt of compound I are shown in Figures 3 and 6, respectively.
The hydrochloride salt exhibits a reversible sorption/desorption profile with a
25 weight change ofless than 0.2 % over the entire range of 2 % to 90 % RH. The hydrate
form exhibits a reversible sorption/desorption profile with about a 2.3 % weight loss as
the sample was dried from ambient to 2 % RH which was regained as the dried specimen was exposed from 2 % RH to 40 % RH. The hydrate form had less than about 0.25 %

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weight gain in the range of 40 % RH to 75 % RH.

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Example 7: Infrared Analysis
The infrared (IR) absorption spectrum was determined over the frequency range
4000 to 675 cmt using an Avatar 360 FT-IR spectrometer equipped with a Nicolet
attenuated total reflection (ATR) sample holder. A representative IR absorption spectrum
5 for a sample of a crystalline hydrochloride salt ofthe invention had significant absorption
bands at 758–1, 783–1, 795–1, 802+1, 949–1, 981–1, 1149–1, 1158–1, 1217–1, 1332–1, 1377–1, 1453–1, 1467–1, 1487–1, 1525+1, 1566–1, 1575–1, 1615–1, 1672–1, and
3197–1 cm. -

10 Example 8: Solid State Stability Assessment
Samples ofthe hydrochloride salt ofthe invention 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 24 weeks ofstorage, there was no detectable change in the DSC. or TGA
15 thermograms nor in the PXRD pattern. The chemical purity ofthe stored sample was
99.6%.

Example 9: Determination of Counterion Molar Ratio
The counterion molar ratio ofhydrochloric acid (HA) to 1-isopropyl-2-oxo-1,2-
20 dihydroquinoline-3-carboxylic acid {(1 S,3R,5R)-8-[(R)-2hydroxy-3 -(methanesulfonyl-
methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl} ainide (compound offormula I) was calculated according to the following formula:
Counterion Ratio = (WpjMWi.) I (W11\4W1)
where WHA is the weight percentage ofHCI in the sample, MWHA is the molecular weight
25 of HCI, MW1 is the molecular weight ofthe compound offormula 1(504.6 amu) and Wi
is the weight percentage of compound offormula I in the sample, calculated according to
the formula:
Wi - 100 - WHX W140 - W5
where W1120 is the weight percentage water content, WRS is the weight percentage residual
30 solvent, under the assumption that compound I h.s no impurities.
The molar ratio ofhydrochloric acid to compound I for a sample of a crystalline hydrochloride salt ofthe invention was calculated as 0.94:1 using the weight percentage ofHCl (W) of 6.3 % and the values WH20 0.26 % and W1s = 0.47%. The HCl content

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was determined by titration with a standard solution ofsilver nitrate, the water content
W120 was determined by coulometric Karl Fisher titration and the residual solvent content
WRS was determined by gas chromatography.

5 Comparative Example 1: Synthesis of citric acid salt of 1-isopropyl-2-oxo-1,2-
dihydroquinoline-3-carboxylic acid ((IS,3R,5R)-8-[(.R)-2-bydroxy-3-(metbanesulfonyt-methyl-amino)propyl]-8-aza-bicyclo[3.2. 1] oct-3-yl} amicle
1 -Isopropyl-2-oxo-I,2-dihydroquinoline-3-carboxylic acid {(1 5, 3R, 5R)- 8- [(R)-2-hydroxy-3-(methanesu1forjyl-niethylamino)propyl]-8-aza-bieyclo[3.2.1 Joct-3-yl} amide
10 (0.1 g, 0.2 mmol) was suspended in ethanol (1 mL). To this suspension was added a 1M
solution of citric acid in ethanol (0.072 mL, 0.072 mmol, 0.33 eq). The mixture was
briefly sonicated until clarity, capped, and then allowed to sit overnight. The cap was
then removed and the mixture was allowed to evaporate under ambient conditions until solids were observed. The mixture was then recapped and allowed to sit for 72 h. The
15 resulting solid was filtered and washed with cold ethanol to give the title compound as a
solid (74.3 mg).

Comparative Example 2: Synthesis of acid salts of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(IS,3R,5R)-8-1(R)-2-bydroxy-3-(methanesulfony1-methyl-amino)propy1]-8-aa-bicyc1o[3.2.1] oct-3-yl} amide
20 Following the procedure of Comparative Example 1, the following acid salts of
1 -isopropyl-2-oxo- 1,2-dihydroquinoline-3 -carboxylie acid {(1 S,3R,5R)-8.-[(R)-2-hydroxy-3-(methanesulfonyl-rnethyl-amino)propylj-8-aza-bicyclo[3.2.1]oct-3 -yl} amide were prepared in solid form using the indicated equivalents of acid (product weight in second parentheses): adipic (0.5 eq) (48.5 mg); phosphoric (0.5 eq) (86.6 mg); sulfuric (0.5 eq)
25 (27.0 mg); tartaric (0.5 eq) (66.3 mg); malic (0.5 eq) (25.3 mg); and hydrobromic (1 eq)
(62.9 mg).

Comparative Example 3: Synthesis of methanesulfonic acid salt of 1-isopropyl-2-oxo-1,2-dthydroquinoline-3-carboxylic acid {(1S,3R,5R)-8- [(R)-2-hydroxy-3-(methanesulfouyl-metbyl-amino)propylj-8-aza-bicyclo[3.2.1] oct-3-
30 yl}am!de
To a solution of 1 -isopropyl-2-oxo- 1,2-dihyclroquinoline-3-carboxylic acid {(1S,3R,5R)-8-[R)-2-hydroxy-3-methanesulfony1-methyl-amino)propy-8-aza-bicyclo[3.2.1]oct-3-yl}an-iide (0.1 g, 0.2 mmol) in 50 % acetonitrile/water (1 mL) was
added a 1M solution ofmethanesulfonic acid in ethanol (0.2 mL, 0.2 mmol, 1 eq). The

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mixture was then frozen and lyophilized to dryness overnight. The resulting solid was dissolved in isopropanol (1 mL) with gentle warming and allowed to cool. The resulting solid was collected by filtration and washed with cold isopropanol to give the title compound as a solid (90 mg).

Comparative Example 4: Synthesis of acid salts of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1S,3R,5R)-8-[(R)-2-hydroxy-3-(methanesulfonyl-metbyl-amino)propylj-8-aza-bicycto[3.2.1]oct-3yl} amide
Following the procedure of Comparative Example 3, the following acid salts of 1-isopropyl-2-oxo- 1,2-dihydroquinoline-3 -carboxylic acid {(1S,3R,5R)-8-(R)-2-hydroxy-3-
10 (methanesulfonyl-metbyl-amino)propyl]-8aza-bicyclo[3.2.1 Joct-3-yl} amide were prepared in solid form using the indicated equivalents of acid (product weight in second parentheses): fumaric (1 eq) (107.2 mg); benzoic (1 eq) (105.2 nig); and (R)-rnandelic
(1 cci) (96.1 mg).

Comparative Example 5: Properties of Compounds of Comparative
15 Examples 1-4.
The crystalline acid salts ofthe compound offormula I ofComparative Examples
1-4 were analyzed by PXRD, DSC, and TGA. In all cases, a weight loss was observed by TGA at temperatures below about 100 °C, which may most likely be attributed to loss of solvent. The temperature(s) at which endotherruic heat flow was observed by DSC,
20 exclusive ofthe low temperature features attributed to solvent loss, along with confirmation of crystallinity by PXRD are summarized in Table I where the stoichiometry
is indicated by the number of equivalents of acid used to prepare the acid salt.

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Table I: Comparison Salts

[Acid Equivalents of
acid

DSC endotherm rXJW

citric 0.33 -425 °C, -460-180°C crystalline
adipic 0.5 -1500C crystalline
phosphoric 0.5 -215-220 °C crystalline
sulfuric 0.5 -150"C, -190-205 °C,
--240 °C

crystalline

tartaric 0.5 -.440 °C, ’485 Cc crystalline
malic 0.5 -110 °C, -485 °C I crystalline
hydrobromic 1 -’155 °C, ’-210°C crystalline
methanesulfonic I -475 °C, -235 °C crystalline
furnaric 1 -125 °C crystalline
benzoic 1 <150 °C 11 crystalline
(R)-mandelic 1 -120 °C crystalline
* multiple polymorphs, exotherm observed
onset ofmelt and decomposition

5 Assay 1: Radioligaud Binding Assay on 5-HT4() Human Receptors
a. Membrane Preparation 5-HT
HEK-293 (human embryonic kidney) cells stably-transfected with human 5-HT4()
receptor cDNA (Bmax = - 6.0 pmollmg protein, as determined using [311]-GR1 13808 membrane radioligand binding assay) were grown in T-225 flasks in Dulbecco’s Modified
10 Eagles Medium (DMEM) containing 4,500 mg/L D-glucose and pyridoxine hydrochloride
(GIBCO-lnvitrogen Corp., Carlsbad CA: Cat #11965) supplemented with 10% fetal
bovine serum (FBS) (GIBCO-Invitrogen Corp.: Cat 410437), 2 mM L-glutainine and
(100 units) penicillin-(100 jig) streptomycin/ml (GIBCO-Invitrogen Corp.: Cat #15140) in
a 5% CO?., humidified incubator at 37 °C. Cells were grown under continuous selection
15 pressure by the addition of 800 g/mL geneticin (G]BCO-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. All steps ofthe membrane preparation were performed on ice. The cell monolayer was
20 lifted by gentle mechanical agitation and trituration with a 25 xnL pipette. Cells were
collected by centrifugation at 1000 rpm (5

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For the membrane preparation, cell pellets were resuspended in ice-cold 50 mM 4-(2-hydroxyethyl)-1 -piperazineethanesuiphonic acid (HEPES), pH 7.4 (membrane 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 The resultant homogenates were
5 centrifuged at 1200 g for 5 min at 4°C. The pellet was discarded and the supernatant
centrifuged at 40,000 g (20 nun). The pellet was washed once by resuspension with membrane preparation buffer and centrifugation at 40,000 g (20 ruin), The final pellet
was resuspended in 50 mM HEPES, pH 7.4 (assay buffer) (equivalent 1
T225 flask/i mL). Protein concentration ofthe membrane suspension was determined by
10 the method ofBradford (Bradford, 1976). Membranes were stored frozen in aliquots at
-80 °C.

b. Radioljgand Binding Assays
Radioligand binding assays were performed in 1.1 mL 96- deep well
polypropylene assay plates (Axygen) in a total assay volume of 400 L containing 2 g
S15 membrane protein in 50 mM HEPES pH 7.4, containing 0.025% bovine serum albumin
(BSA). Saturation binding studies for determination ofKd values ofthe radioligand were
performed using [3H]-GR1 13808 (Amersham Inc., Bucks, UK: Cat #TRK944; specific
activity 82 Ci/nunol) at 8-12 different concentrations ranging from 0.001 nM - 5.0 nM.
Displacement assays for determination of pK1 values of compounds were performed with

20 [3fl] -GR1 13808 at 0,15 nM and eleven different concentrations of compound ranging
from 10pM- 100 PM.
Test compounds were received as 10 mM stock solutions in DMSO and diluted to
400 pM 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
25 of 1 4M unlabeled GR1 13808, Assays were incubated for 60 ruin at room temperature,
. and then the binding reactions were terminated by rapid filtration over 96-well GFLB glass
fiber filter plates (Packard BioScience Co., Meriden, CT) presoaked in
0.3% polyethyleneiniine. Filter plates were washed three times with filtration buffer (ice-
cold 50mM HEPES, p117.4) to remove unbound radioactivity. Plates were dried, 35 jtL
30 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).

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Binding data were analyzed by nonlinear regression analysis with the GraphPad
Prism Software package (GrapbPad Software, Inc., San Diego, CA) using the 3 -parameter
model for one-site competition. The BOTTOM (curve minimum) was fixed to the value
for nonspecific binding, as determined in the presence of 1.LM GR1 13808. Ki values for
5 test compounds were calculated, in Prism, from the best-fit IC50 values, and the Kd value
ofthe radioligand, using the Cheng-Prusoff equation (Cheng and Prusoff, Biochemical Pharmacology, 1973, 22, 3099-108): K1 = IC 1(1 + [LVK) where [L] = concentration [3H1-GR1 13808. Results are expressed as the negative decadic logarithm ofthe
K1 values, pK.
10 Test compounds having a higher pKi value in this assay have a higher binding affinity for the 5-liT4 receptor. The compound offormula I had a pKi value greater than about 7,5 in this assay.

Assay 2: Radioligand Binding Assay on 5-RT3A Human Receptors:
Determination of Receptor Subtype Selectivity
15 a. Membrane Preparation 5 -HT
HEK-293 (human embryonic kidney) cells stably-transfected with human 5-HT3A
receptor cDNA were obtained from Dr. Michael Bruess (University ofBonn, GDR)
(Bmax = - 9.0 pmollmg protein, as determined using [311]-GR65630 membrane
radioligand binding assay). Cells were grown in T-225 flasks or cell factories in 50%
20 Dulbecco’s Modified Eagles Medium (DMEM) (GJBCO-Jnvitrogen Corp., Carlsbad, CA:
Cat #11965) and 50% Ham’s P12 (GIBCO-Invitrogen Corp.: Cat 411765) supplemented
with 10% heat inactivated fetal bovine serum (PBS) (Hyclone, Logan, UT: Cat #SH30070.03) and (50 units) penicillin-(50 jg) streptomycin/ml (G1BCO-lnvitrogen
Corp.: Cat #15 140) in a 5% CO2. humidified incubator at 37 °C.
25 Cells were grown to roughly 7080% confluency (< 35 subculture passages). All
’ steps ofthe membrane preparation were performed on ice, To harvest the cells, the media
was aspirated and cells were rinsed with Ca"’, Mg"’-free Dulbecco’s phosphate buffered
saline (dPBS). The cell monolayer was lifted by gentle mechanical agitation. Cells were
collected by centrifugation at 1000 rpm (5 mm). Subsequeut steps ofthe membrane
30 preparation followed the protocol described above for the membranes expressing 5-HT4()
receptors.

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b. Radiolignd Binding Assays
Radioligand binding assays were performed in 96-well polypropylene assay plates
in a total assay volume of 200 LL containing 1.5-2 ig membrane protein in
50 mM HEPES pH 7.4, containing 0.025% BSA assay buffer. Saturation binding studies
5 for determination ofKd values ofthe radioligand were performed using [3H]-GR65630
(PerkinEhuer Life Sciences Inc., Boston, MA: Cat #NET101 1, specific activity -85 Ci/mmol) at twelve different concentrations ranging from 0.005 nM to 20 nM.
Displacement assays for determination ofpKi values of compounds were performed with {3H1-GR65630 at 0.50 nM and eleven different concentrations of compound ranging from
10 10 pM to 100 pM. Compounds were received as 10 mM stock solutions in DMSO (see
section 3. 1), diluted to 400 i.tM 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 was determined in the presence of 10[LM unlabeled MDL72222. Assays were incubated
for 60 min at room temperature, then the binding reactions were terminated by rapid
15 filtration over 96-well GFIB glass fiber filter plates (Packard BioScience Co.,
Meriden, CT) presoaked in 03% polyethyieneiniine. Filter plates were washed three
times with filtration buffer (ice-cold 50mM HEPES, pH7.4) to remove unbound
radioactivity. Plates were dried, 35 iL Microscint-20 liquid scintillation fluid (Packard BioScience Co., Meriden, CT) was added to each well and plates were counted in a
20 Packard Topeount liquid scintillation counter (Packard BioScience Co., Meriden, CT).
Binding data were analyzed using the non-linear regression procedure described
above to determine Ki values. The BOTTOM (curve minimum) was fixed to the value for nonspecific binding, as determined in the presence of 10 tM MDL72222. The quantity
[L] in the Cheng-Prusoff equation was defined as the concentration {3H}-GR65630.
25 Selectivity for the 5-HT4 receptor subtype with respect to the 5-UT3 receptor subtype was calculated as the ratio Ki(5-HT3A)LKj(5-HT4()). The compound offormula I
had a 5-HT4/5-HT3 receptor subtype selectivity greater than about 1000 in this assay.

Assay 3: Whole-cell CAMP Accumulation Flasliplate Assay with IIEK-293
cells expressing human 5-JiT4(C) Receptors
30 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-UT4 receptors were contacted with different concentrations oftest compound.

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a. Cell Culture
HEK-293 (human embryonic kidney) cells stably-transfected with cloned human
5-HT4() receptor eDNA were prepared expressing the receptor at two different densities:
(1) at a density of about 0,5-0.6 pmollmg protein, as determined using a [3H]-GR1 13808
5 membrane radioligand binding assay, and (2) at a density of about 6.0 pmollrng protein.
The cells were grown in T-225 flasks in Dulbecco’s Modified Eagles Medium (DMEM) containing 4,500 mg/L Dglucose (GIBCO-Invitrogen Corp.: Cat #11965) supplemented with 10% fetal bovine serum (PBS) (GIBCO-Jnvitrogen Corp.: Cat #10437) and (100
Units) penicillin-(100 g) streptomycin/ml (GIBCO-Invitrogen Corp.: Cat #15140) in a
10 5% CO2. humidified incubator at 37°C. Cells were grown under continuous selection
pressure by the addition of geneticin (800.ig/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
15 prior to assay, cells were washed twice, and fed, with serum-free DMEM containing
4,500 mg/L D-glucose (GIBCO-Invitrogen Corp.: Cat #11965). To harvest the cells, the media was aspirated and 10 mL Versene (G]13CO-h1vitrogen 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
20 containing an equal volume ofpre-warmed (37°C) dPBS and centrifuged for 5 ruin at
1000 rpm. The supernatant was discarded and the pellet was re-suspended in pre-warmed
(3 7°C) stimulation buffer (lOmL 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
m, flask yield was 1-2 x

107 cells/flask). Cells were resuspended at a concentration of
25 5 x 105cells/mi in pre-warmed (37°C) stimulation buffer (as provided in the flashplate
kit) and preincubated at 37°C for 10 mm.
cAMP assays were performed in a radioinimunoassay format using the Flashplate
Adenylyl Cyclase Activation Assay System with 1251-CAMP (SMPO04B, PerkinElmer Life Sciences Inc., Boston, MA), according to the manufacturer’s instructions.
30 Cells were grown and prepared as described above. Final cell concentrations in

the assay were 25 x 103 cells/well and the final assay volume was 100 jiL. Test
compounds were received as 10 mM stock solutions in DMSO, diluted to 400 jiM into

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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 ii different concentrations of compound ranging from 10 pM to 100 iM (final assay concentrations). A 5 -HT concentration-response curve (10 pM to 100 M) was included
on every plate. The cells were incubated, with shaking, at 37°C for 15 min and the
reaction terminated by addition of 100.tl 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).
10 The amount of cA1\lP produced per mL ofreaction was extrapolated from the 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 pEC50 values, the negative decadic
15 logarithm ofthe EC50 value, where EC50 is the effective concentration for a 50 %
maximal response.
Test compounds exhibiting a higher pEC50 value in this assay have a higher
potency for agonizing the 5-FIT4 receptor. The compound offormula I which was tested
in this assay in the cell line (1) having a density of about 0.5-0.6 pmol]mg protein, had a
20 pEC50 value greater than about 7.5.

Assay 4: In vitro Voltage Clamp Assay ofInhibition of Potassium Ion Current
in Whole Cells Expressing the bERG Cardiac Potassium Channel
CHO-K1 cells stably transfected with bERG eDNA were obtained from Gail
Robertson at the University ofWisconsin. Cells were held in cryogenic storage until
25 needed. Cells were expanded and passaged in Dulbecco’s Modified Eagles MediumIF12
supplemented with 10 % fetal bovine serum and 200 g/mL geneticin. Cells were seeded onto poly-D-lysine (100.tg/niL) coated glass coverslips, in 35 mm2 dishes (containing 2
inL 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.
30 Extracellular solution was prepared at least every 7 days and stored at 4°C when not in use. The extraceliular solution contained (mM): NaC1 (137), KCI (4), CaCl2 (1.8), MgCl2 (1), Glucose (10), 4-(2-hydroxyethyl)-1-piperazineethanesulphothc acid (HEPES)

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(10), pH 7.4 with NaOH. The extracellular solution, in the absence or presence oftest compound, was contained in reservoirs, from which it flowed into the recording chamber
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): KCI (1130),
5 MgCl2 (1), ethylene glycol-bis(beta-aminoethyl ether) N,N,N’,N’-tetra acetic acid salt
(EGTA) (5), MgATP (5), 4-(2-hydroxyethyl)-1-piperazineethanesulpbonic acid (HEPES) (10), pH 7.2 with KOH. All experiments were performed at room temperature (20-220C). 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
10 patch electrode. Once a stable patch was achieved, recording commenced in the voltage
clamp mode, with the initial holding potential at -80 mY. After a stable whole-cell
current was achieved, the cells were exposed to test compound. The standard voltage protocol was: step from the holding potential of-80 mV to +20 mV for 4.8 see, repolarize
to -50 mV for 5 sec and then return to the original holding potential (-80 mV), This
15 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 M were perfused over the cells for 5 minutes, followed by a 5 -minute washout period in the absence of compound. Finally a positive control (cisapride, 20 nM) was added to the perfusate to test the function ofthe cell. The step from -80 mV to +20
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 Originfi (OriginLab
25 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
30 five-minute exposure to either the test article or vehicle control (usually 0.3 % DMS0).
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.

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The smaller the percentage inhibition ofthe 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. The compound offormula I was tested in this assay at a concentration of 3.tM and exhibited an inhibition ofthe potassium ion current ofless
5 than about l5%.

Assay 5: In vitro Model of Oral Bioavailability: Caco-2 Permeation Assay
The Caco-2 permeation assay was performed to model the ability oftest
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
10 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
15 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 IC water for temperature
20 control. The air manifold delivered 95% 02/5% 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
100.iM and with 14C-mannitoi to monitor the integrity ofthe monolayer. All
25 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 ofthe chamber. Samples were analyzed by HPLC or liquid scintillation counting for test compound and marinitol concentrations.
The permeation coefficient (Kr) in cm/sec was calculated.
In this assay, a Kp value greater than about 10 x 106 cmIse is considered
30 indicative offavorable bioavailability. The compound of formula I was tested in this
assay and exhibited a Kp value of greater than about 20 x 10-6 era/see.

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Assay 6: Pharmacokinetic Study in the Rat
Aqueous solution formulations oftest compounds were prepared in 0.1 % lactic
acid at a pH ofbetween about 5 and about 6. Male Sprague-Dawley rats (CD strain,
Charles River Laboratories, Wilmington, MA) were dosed with test compounds via,
5 intravenous administration (1V) at a dose of2.5 mg/kg or by oral gavage (P0) at a dose of
5 mg/kg. The dosing volume was I mL/kg for IV and 2 mL/kg for P0 administration.
Serial blood samples were collected from animals pre-dose, and at 2 (IV only), 5, 15, and
30 and at 1, 2, 4, 8, and 24 hours post-dose. Concentrations oftest compounds in blood plasma were determined by liquid chromatography-mass spectrometry analysis
10 (LC-MS/MS) (MDS SCIEX, API 4000, Applied Biosystems, Foster City, CA) with a
lower limit of quantitation of 1 uglmL.
Standard pharmacokinetic parameters were assessed by non-compartmental
analysis (Model 201 for IV and Model 200 for P0) using WinNonlin (Version 4.0.1, Pharsight, Mountain View, CA). The maximum in the curve oftest compound
15 concentration in blood plasma vs. time is denoted The area under the concentration
vs. time curve from the time of dosing to the last measurable concentration (AUC(0-t))
was calculated by the linear trapezoidal rule. Oral bioavailability (F(%)), i.e. the dose-normalized ratio ofAUC(0-t) for P0 administration to AUC(0-t) for IV administration,
was calculated as:
20 F(%) = AUCo/AUC x Dosew/Dosepo x 100%
Test compounds which exhibit larger values ofthe parameters C, AUC(0-t),
and F(%) in this assay are expected to have greater bioavailability when administered
orally. The compound offormula I had a C1 value of 0.16 j.ig/mL, an AUC(0-t) value of 0.46..Lghr/mL and oral bioavailability (F(%)) in the rat model of about 19 %.
25 While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various
changes may be made and equivalents maybe substituted without departing from the true spirit and scope ofthe invention. In addition, many modifications may be made to adapt a particular situation, material, composition ofmatter, process, process step or steps, to the
30 objective, spirit and scope ofthe present invention. All such modifications are intended
to be within the scope ofthe 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.
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WHAT IS CLAIMED IS:

1. A crystalline salt form which is the hydrochloride salt of 1-isopropyl-2-
oxo.-I,2-dihydroquinoline-3-carboxylie acid {(1 S,3R,5R)-8-[(R)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oet-3-yl} amide or a solvate
5 thereof.

2. The crystalline salt form ofClaim I wherein the crystalline salt form is a
crystalline hydrochloride salt.

10 3. The crystalline salt forin ofClaim 2, wherein the crystalline salt form is
characterized by a powder x-ray diffraction pattern having two or more diffraction peaks
at 20 values selected from 4.41–0.2, 8.82–02, 9.08–0.2, 11.21–0.2, 14.40–0.2,
16.42–0.2, 17.35–0.2, 17.61–0.2, 18.14+0,2, 19.04–0.2, 19.95–0.2,20.20–0.2,
21.23+0.2, 22.13+0.2, 22.484-0.2, 22.83–0.2, 24.16–0.2, 25.37–0.2, 25.56–0.2,
15 26.22–0.2, 27.33–0.2, 29.08–0.2, and 29.61–0.2.

20

4. The crystalline crystalline salt form ofClaim 3 wherein the powder x-ray
diffraction pattern comprises two or more diffraction peaks at 20 values selected, from 14.40–0.2, 17.35–0,2,17.61:0-2, 19.04–0.2, 21.23*0.2, and 22.13–0.2.

5. The crystalline salt form of Claim 2, wherein the crystalline salt form is
characterized by a powder x-ray diffraction pattern in which the peak positions are substantially in accordance with the peak positions ofthe pattern shown in Figure 1.

25 6. The crystalline salt form ofClaim 2, wherein the crystalline salt form is
characterized by a differential scanning calorimetry trace which shows a maximum in endothermic heat flow at a temperature greater than about 230 °C.

7. The crystalline salt form ofClaim 2, wherein the crystalline salt form is
30 characterized by a differential scanning calorimetry trace substantially in accordance with
that shown in Figure 2.

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8. The crystalline salt form ofClaim 1 wherein the crystalline salt form is a
hydrate.

9. The crystalline salt form of Claim 8 wherein the crystalline salt form is
5 characterized by a powder x-ray diffraction pattern having two or more diffraction peaks
at 20 values selected from 5.30–0.2, 7.43–0,2, 8.72–0.2, 10.52–0.2, 13.85–0.2,
14.11–0.2, 15.80–0.2, 15.99–0.2, 17.26–0.2, 19.53–0.2, 20.08–0.2, 21.06–0.2,
21.4810.2, 21.92–0.2, 22.85–0.2, 23.91–0.2, 25.28–0.2, 26.06–0.2, 27.34–0.2,
27.51–0.2, and 29.6710.2.
10

10. The crystalline salt form of Claim 9 wherein the powder x-ray diffraction
pattern comprises two or more diffraction peaks at 20 values selected from 10.52–0.2, 13.85–0.2, 15.80–0.2, 17,26–0.2, and 21.06–0.2.

15 11. The crystalline salt form ofClaim 8, wherein the crystalline salt forril is
characterized by a powder x-ray diffraction pattern in which the peak positions are substantially in accordance with the peak positions ofthe pattern shown in Figure 4.

12. The crystalline salt form ofClaim 8, wherein the crystalline salt form is
20 characterized by a differential scanning calorimetry trace substantially in accordance with
that shown in Figure 5.

13. A pharmaceutical composition comprising a pharmaceutically acceptable
carrier and a crystalline salt form of any one of Claims 1 to 12.
25

14. A method oftreating 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 crystalline salt form of any one of Claims I to
30 12.

15. A method oftreating a disorder ofreduced motility ofthe gastrointestinal
tract in a mammal, the method comprising administering to the mammal, a therapeutically
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effective amount of a pharmaceutical composition comprising a pharmaceutically-
acceptable carrier and a crystalline salt form of any one of Claims ito 12.

16. The method of Claim 15, wherein the disorder ofreduced motility is
5 selected from chronic constipation, constipation-predominant irritable bowel syndrome,

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diabetic and idiopathic gastroparesis, and functional dyspepsia.

17. A crystalline crystalline salt form as claimed in any one ofClaims 1 to 12
for use in therapy.

18 Use of a crystalline salt form of any one ofClaims Ito 12 for manufacture
of a medicament.

19. The use ofClaim 18 wherein the medicament is for the treatment of a
15 medical condition in a mammal associated with 5-HT4 receptor activity.

20. The use ofClaim 19 wherein the medical condition is a disorder of
reduced motility ofthe gastrointestinal tract.

20 21. The use ofClaim 19 wherein the medical condition is selected from
chronic constipation, constipation-predominant irritable bowel syndrome, diabetic and idiopathic gastroparesis, and functional dyspepsia.

22. A process for preparing a crystalline hydrochloride salt of 1 -isopropyl-2-
25 oxo- 1,2-dihydroquinoline-3-carboxylic acid {(1S,3R,5R)-8-[(R)-2-hydroxy-3-
(methanesulfonyl-methyl-aniirio)propylj-8-azabicyclo[3.2. 1]oct-3-yl} amide, the process comprising:
(a) contacting 1 -isopropyl-2-oxo- 1,2-dihydroquinoline-3-carboxylic acid
{(1 S,3R,5R)-8-[(R)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-
30 azabicyclo{3.2. 1oct-3-yl} arnide with hydrochloric acid to form a reaction mixture; and
(b) isolating the crystalline hydrochloride salt from the reaction mixture.

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23. A process for preparing a crystalline hydrate ofthe hydrochloride salt of
1 -isopropyl-2-oxo- 1,2-dihydroquinoline-3-carboxylic acid {(1S,3R,5R)-8-[(R)-2-hydroxy-3-methanesulfonyl-methyl-amino)propy1]-8-azabicyclo[3.2.1]oct-3-yl} amide, the process comprising:
(a) dissolving 1 -isopropy1-2-oxo- 1,2-dihydroquinoline-3-carboxylic acid
{(1 S,3R,5R)-8-{(R)-2-hydroxy3-(rnethanesulfonyl-methyl-amino)propy1].S-azahicyclo[3.2.1] oct-3-yl} amide hydrochloride in water at a concentration greater than about 50 milligrams per milliliter to form a suspension; and
(b) isolating the crystalline hydrate from the suspension.

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0
5 10 15 20 25 30 35

20 (degrees)

Figure 1

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Temperature (°C)

Figure 2

250 300

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cJ)
0.500

C-)
- 0.500

- 1.500

- 2.500

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4000

- . 3000

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0

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- - Desorption . Initial Drying

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20 4b 1) 8’ 10

Relative Humidity (%)

Figure 3

5 10 15 20

20 (degrees)

Figure 4

25 30 35

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- 2.5

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Exo Up

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3/3

50 100 150 200

Temperature (°C)

Figure 5

- 2.500 I

Relative Humidity (%)

Figure 6

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105

100

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90 I
85

- + 80
300

FORMA CRISTALINA DE UN COMPUESTO DE QUINOLINONA-CARBOXAMIDA ANTECEDENTES DE LA INVENCION
Campo de la invenciOn
La invenciOri est6 dirigida a formas de sales cristalinas de un compuesto de quinolinona-carboxamida que son de utilidad coma agonistas del receptor 5-NT4. La nvención tambiØn tiene como objetos las composiciones farmacØuticas quo compreriden dichos compuestos cristalinos, mØtodos para usar dichos compuestos para tratar afecciories mOdicas mediadas par la actividad del receptor 5-HT4 y procesos ütiles para preparar dichos compuestos.
Estado do la tØcnica
En la Solicitud de Patente Provisoria de EE.UU. N°; 60/560.076, presentada el 7 de
abril, 2004, y en la Solicitud de Patente do EE.UU. N°: 11/100.113, presentada el 6 de abril, 2005, ambas cedidas al mismo cesionarlo, se describen nuevos compuestos do quinolinona-carboxamida que so espera sean de utilidad para el tratamiento de trastornos de motilidad reducida del tracto gastrointestinal. En particular, el compuesto {(1 S,3R,5R)- 8-[(R)-2-hidroxi-3-(metansulfonimetiI-amino)propil]-8-azabiciclo[3.2. 1 joct-3-il}amida del Æcido 1-isopropi1-2-oxo-1,2-dihidroquinolin-3-carboxilico se describe especificamente en estas solicitudes por demostrar actividad agonista 5-HT4.
La estructura qu Imica de Ia {(1 S,3R,5R)-8-[(R)-2-hidroxi -3-(metansulfonhl-metil-amino)propil]-8-azabiciclo[3.2.l]oct-3-iI}amida del âcido 1-isopropil-2-oxo-1 2-dihidroquinalin-3-carboxiuco estØ representada en Ia siguiente fOrmula I:
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11 0NN-S-
OH H3

aN 0

Para utilizar eficazmente este compuesto como un agente terapØutico, seria deseable contar con una forma saUna en estado sólido que fuera fÆcil de elaborar y que presente una estabilidad quimica y fisica aceptable. Por ejemplo, seria muy deseable contar con una forma salina que fuera tØrmicamente estable, par ejemplo a temperaturas que exceden los 200 °C aproximadamente y que no sea higroscOpica ni delicuescente, facilitando de esa manera el procesamiento y almacenamiento del material. En general,

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so prefieren los sólidos cristalinos frente a las formas amorfas, pars aumentar la pureza y estabilidad del producto elaborado.
No se han descrito previamente formas do sales cristalinas del compuesto de Is formula I. Por consiguiente, persiste Is riecesidad de una forma sauna cristalina estable del compuesto de fOrmula t quo no sea higroscOpica o delicuescente y que exhiba una estabilidad tØrmica favorable.
RESUMEN DE LA INVENCION

La presente invención provee una sat clorhidrato cristalina de Is {(1S,3R,5R)-84(R) 2-hidroxi-3-(metansulfonhl-metilamino)propilJ-8-azabiciclo[3,2,1]oct-3-iI}amida del Æcido 1-isopropil-2-oxo-1,2-dihidroquinolin-3-carboxilico o un solvato do Is misma. En un aspecto, Is forma de sal cristalina de Is irivención es una sal clorhidrato cristalina del compuesto de fOrmula I. En otro aspecto, Is forma de sal cristalina de Is invención Os un hidrato cristalino de Is sal clorhidrato del compuesto do fOrmula I.
Sorpresivamente, so ha encontrado que Is sal clorhidrato cristalina de la invenciOn
es tØrmicamente estable a temperaturas mayores que 200 °C aproximadamente y quo exhibe un cambio de peso menor quo un 02% aproximadamente cuando es expuesto a un rango do valores de humedad relativa entre aproximadamente 2% y aproximadamente 90% a temperatura ambiente. Aiin mØs, ni Is sal clorhidrato cristalina de Is invericiOn ni el hidrato do Is misma es delicuescente cuando es expuesta a una humedad relativa de hasta 90% a temperatura ambiente.
Entre otros usos, se espera que las formas de sales cristalinas do Is invenciOn sean
de utilidad pars preparar composiciones farmacØuticas para el tratamiento de trastornos de motilidad reducida del tracto gastrointestinal. La preserute invenciOn provee una sal clorhidrato cristalina do Is {(1 S,3R,5R)-8-[(R)-2-hidroxi-3-(metans ulfonil-metil-amino)propiul-8-azabiciclo[3,2. 1]oct-3-il}amida del Æcido 1-isopropil-2-oxo-1 2-dihidroquinolin-3-carboxIlico o un solvato de Is misma,
La invenci6n tambiOn provee un mOtodo do tratamiento do una enfermedad o afecciOn asociada con la actividad del receptor 5-HT4, por ejemplo un trastorno de motilidad reducida del tracto gastrointestinal, donde dicho mØtodo comprende administrar al mamifero, una cantidad eficaz pars uso terapOutico de una sal clorhidrato cristalina do Is {(1 S,3R,5R)-8-[(R)-2-hidroxi-3-(metansulfonil-metil-amino)propil]-8-azabiciclo[3,2. 1 joct-3-il}amida del 6cido 1-isopropil-2-oxo-1,2-dihidroquinolin-3-carboxilico o un solvato do Is misma.

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En otro aspecto del mØtodo, la invención provee un proceso para preparar una sal clorhidrato cristalina de la invención, donde dicho proceso comprende hacer reaccionr {(1 S,3R,5R)-8-[(R)-2-hidroxi-3-(metansulfonhl-metU-amino)propil]-8-azabiCiClO[3,2. 1]oct-3-il}amida del àcido 1 -isopropil-2-oxo-1,2-dihidroquinolin3-carboxilico con Æcido clorhidrico para formar una mezela de reacciOn y aislar la sal clorhidrato cristalina de dicha mezcla de

reacción

La invenciOn tambiØn provee una sal clorhidrato cristalina de la invención descrita
en a presente para su uso en terapias o como un medicamento, asi como el uso de una sal clorhidrato cristalina de la invonción en la elaboración de un medicamento, en especial a elaboración de un medicamento para el tratamiento de un trastorno de motilidad reducida del tracto gastrointestinal en un mamifero.
DESCRIPCION BREVE DE LAS FIGURAS

Diversos aspectos de la presente invención se ilustran con referencia a los dibujos adjuntos.
En la Figura 1 se muestra un patron de difracciOn por rayos X de polvo (PXRD) de
una Sal clorhidrato cristalina de la {(1S,3R,5R)-8[(R)-2-hidroxi-3-(metansuIfonimetiI-
amino)propil}-8-azabiciclo[3.2.1]oct-3-il}amida del Æcido 1isopropil-2-oxo-1 2-
dihidroquinolin-3-carboxilico de la invención.
En la Figura 2 se muestra un patrOn de difracciOn por rayos X de polvo (PXRD) de
una sal clorhidrato cristalina de la {(1S,3R,5R)-8-[(R)-2-hidroxi-3-(metansuIfonimetil-
amino)propil]-8-azabiciclo[3.2. 1]oct-3-il}amida del àcido 1 -isopropil-2-oxo-1 2-
dihidroquinolin-3-carboxilico de la invenciOn.
En la Figura 3 se muestra un grØfico de absorción dinÆmica de humedad (DMS)
para la sal clorhidrato cristalina de {(1S,3R,5R)-8-[(R)-2-hidroxi-3-(metansulfonU-metil-
amino)propil]-8-azabiciclo[3,2,1]oct-3-iI}amida del Æcido 1-isopropil-2-oxo-1 2-
dihidroquinolin-3-carboxilico de la invenciOn,
S E n la Figura 4 so muestra un grØfico de absorciOn dinØmica de humedad (DM5) para la sal clorhidrato cristalina de {(iS,3R,5R)-8-((R)-2-hidroxi-3-(metansulfonimet-
amino)propil]-8-azabiciclo[3,2. 1]oct-3-il}amida del Æcido 1 -isopropil-2-oxo-1 2-
dihidroquinolin-3-carboxilico de la invenciOn.
En la Figura 5 se muestra un gràfico do absorciOn dinmica de humedad (DM5)
para la sal clorhidrato cristalina de ((1S,3R,5R)-8-[(R)-2-hidroxi-3-(metansulfonil-metil-

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amfro)propil-8-azabiciclo[3 2.1]oct3-U}amida del Æcido 1 -isopropi -2-oxo-1 2-dihidroquinolin-3-carboxIlico de la invención,
En la Figure 6 Se muestra un grfico de absorción dinÆmica de humedad (DMS)
pare la sal clorhidrato cristaline de {(iS,3R, 5R)-8-[(R)-2-hidroxi-3-(metansulfonil-metil-amino)propil]-8-azabiciclo[3,2.1]oct-3-il}amida del àcido 1-isopropil-2-oxo-1,2-dihidroquinoliri-3-carboxIlico de la invención.
DESCRIPCION DETALLADA DE LA INVENCION

En la Figura 6 se muestra un grÆfico de absorciOn dinªmica de humedad (DM8)
para la sal clorhidreto cristalina de {(1S,3R,5R)-8-{(R)-2-hidroxi-3-(metansulfonil-metil-amino)propil]8-azabiciclo[32, 1]oct-3-il}amida del Æcido 1 -isopropil-2-oxo-1 2-dihidroquinolin-3-carboxilico de la invención.
Definiciones

Al describir los compuestos, composiciones y mOtodos de Ia invericiOn, los siguientes tOrminos tienert los siguientes significados, salvo que se indique 10 contrario. El tØrmino "caritidad eficaz para use terapØutico" hace referenda a una cantidad suficiente pare efectuar el tratamiento cuando se la administra a un paciente que necesita el tratamiento.

El tØrmino "tratamiento" como se lo utiliza en Ia presente significa el tratamiento de
una enfermedad, trastorno o efecciOn clinica en un paciente, come por ejemplo un mamifero (particularmente un humario) que incluye:
(a) prevenir que ocurra la enfermedad, trastorno o afección clinica, es decir,
tratamiento profilªctico de un paciente:
(b) mejorar la enfermedad, trastorno o afecciOn clinica, es decir, eliminar o causar
una regresión de la enfermedad, trastorno o afecciOri clinica en un paciente;
(c) suprimir la enfermedad, trastorno o afecciOn clinica, es decir, demorar o detener
el desarrollo de la enfermedad, trastorno o afecciOri clinica en un paciente; o
(d) aliviar los smntomas de la enfermedad, trastorno o afecciOn clinica en un
paciente.
El tØrmino ’solvato" significa un complejo o agregado formado por una o mªs molecules de un solute, es decir un compuesto de la invención o una sal aceptable para uso farmacØutico del mismo, y una o mÆs molØculas deun solverite, Dichos solvatos son tipicamente sôlidos cristalinos con una relaciOn molar de soluto y solvente sustancialmente fa. Algunos solventes representativos son a modo de ejemplo, agua, metanol, etanol,

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sopropanol, ªcido acØtico, y similares. Cuando el solvente es agua, el solvato formado se denomina especificamente hidrato,
El tØrmino sal clorhidrato cristalina" tal como se utiliza en la presente se refiere a un sólido cristalino que no incluye una fracciOn molar sustancialmente fija de molØculas de solvente en la red cristalina, es decir una que no es un solvato. Los solvatos, o especificamente los hidratos, de la invenciOn se identifican explicitamente.
Se debe tener en cuenta que, tal como se usan en la memoria descripUva y en las reivindicaciones adjuntas, las formes en singular ’un", ’una", "la" y ’el’ incluyen las referencias en plural, a menos que el contenido claramerite indique lo contrarlo.
El tØrmino "grupo protector de amino" significa un grupo protector adecuado para prevenir reacciones indeseadas en un nitrógeno de amino. Algunos grupos protectores de amino representativos incluyen, en un sentido no limitativo, formilo; grupos acilo, par ejemplo grupos alcanoilo, como por ejemplo acetilo; grupos alcoxicarbonilo, como par ejemplo ter-butoxicarbonito (Boc); grupos arilmetoxicarbonilo, coma por ejemplo benciloxicarbonilo (Cbz) y 9-fluoreriilmetoxicarbonilo (Fmoc); grupos arilmetilo, como por ejemplo bencilo (Bn), tritilo (Tr) y 1,1-di-(4’-metoxifenll)metilo; grupos sililo, coma par ejemplo trimetilsililo (TMS) y terbutildimetilsiliIo (TBDMS); y similares.
Acjonte activo
El agente activo en las formas salinas de la presente, es decir el compuesto de fOrmula I, se denomina {(15,3R,5R)8-((R)-2-hidroxi-3-(metansulfonimeth-amino)propil]-8 azabicictoç3,2,i]oct-3-iI}amida del Øcido 1-isopropil2-oxo-1,2-dihidroquinolin-3’carboxilico segün el software disporilble comerciatmente AutoNom (MDL Information Systems, GmbH, Frankfurt, Atemania). La denominaciOn (1S,3R,5R) describe (a orientaciOn relativa de los enlaces asociados con el sistema de anitlos biciclicos. Como alternativa, el compuesto se denomina N-[(a.endo)-8-[(R)-2-hidroxi-3-(metansuIfonimetfl-amino)propiI]-8-azabiciclo[3,2. 1 }oct-3-yl]-1 -(1 -metiletil)-2-oxo-1,2-dihidro-3-quinolinecarboxamida. Formasde las sales de la invencn

En un aspecto, la invención provee un clorhidrato cristalino de (a {(1S,3R,5R)-8-((R)- 2-hidroxi-3-(metansulfonil-metil-amino)propilj-8-azabiciclof3,2. 1 oct-3-il}amida del äcido 1-isopropit-2-oxo-1,2-dihidroquinotin-3-carboxilico.
La sal clorhidrato cristalina de la invenciOn contiene tipicamente entre aproximadamente 0,8 y aproximadamente 1,2 equivalentes molares do Æcido ctorhidrico por equivalente molar del compuesto de fOrmula I, incluyendo entre aproximadamente 0,9 y

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aproximadamente 1 1 equivalentes molares de ÆcidoclorhIdrico por equivalente molar del compuesto do formula I.
La relaciÆn molar do Æcido clorhidrico y agente activo puede determinarse fcilmente con los mOtodos disponibles para los especialistas en el arte. Por ejemplo, dichas relaciones molares pueden determinarse fÆcilmente portitulaciOn con una soluciOn estÆndar de nitrato de plata. Como alternativa, se pueden usar mØtodos de anÆlisis elemental, 1H NMR y cromatografla iÆnicapara determinar la relación molar.
En un aspecto, la sal olorhidrato cristalina de la presente invenciOn se caracteriza
por un patron de difracciOri par rayos X de polvo (PXRD) que tiene dos a mÆs picos do difracción a valores 2 seleccionados entre 4,41–0,2, 8,82–0,2, 9,08–0,2, 11,21–0,2, 14,40–0,2, 16,42–0,2, 17,35–0,2, 17,61–0,2, 18,14–0,2, 19,04–0,2, 19,95–0,2, 20,20–0,2, 21,23–0,2, 22,13–0,2, 22,48–0,2, 22,83–0,2, 24,16–0,2, 25,37–0,2, 25,56–0,2, 26,22–0,2, 27,33–0,2, 29,0810,2 y 29,61–0,2. En particular, en este aspecto, la forma cristalina se caracteriza por un patron de difracciOn par rayos X de polvo quo tiene dos o mÆs picos de difracción a valores 20 seleccionados entre 14,40–0,2, 1 7,35–0,2, 17,61–0,2, 19,04–0,2, 2123–0,2 y 22,13–0,2.
Coma es de conocimiento en el campo de la difracciOn par rayos X de polvo, las posiciones de los picas de los espectros do FXRD son relativamente menos sensibles a los detalles experimentales, tales coma detalles de la preparación do la muestra y geometrIa del instrumento, que las alturas relativas do los picos. Asi, en un aspecto, la sal clorhidrato cristalina del compuesto de fOrmula I so caracteriza por un patrOn do difracciOn par rayos X do polvo en el cual las posicionos do los picos son sustancialmente acordes con las quo so muestran en la Figura 1.
La sal clorhidrato cristalina do la presente invenciOn tambiØn so caracteriza por su estabilidad tØrmica a temperaturas altas coma lo demuestra el grØfico de calorimetria diferencial por barrido (DSC) que muestra un pica en el flujo de calor endotØrmico en el rango entre aproximadamente 230 °C y aproximadamente 260 °C, como se ilustra en la Figura 2. AOn mÆs, el grÆfico del anØlisis tØrmicogravimØtrico (TGA) no muestra ningtn evento tØrmico significativo debajo do los 225 °C aproximadamente.
En aUn otro aspecto, Ia sal clorhidrato cristalina so caracteriza por su espectro do absoroiOn infrarrojo que muestra bandas de absorciOn significativos en 758, 783, 798, 802, 949, 981, 1149, 1158, 1217, 1332, 1377, 1453, 1467, 1487, 1525, 1566, 1575, 1615, 1672 y 3197 GM-1 aproximadamente.

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So ha demostrado que a sal clorhidrato cristalina del compuesto do fOrmula I tiene
un perfil reversible do absorciOnldosorción con un nivel excepcionalmente bajo de higroscopicidad (es decir, menos que aproximadamente 0,2% de aumento de peso en el rango do humedad entre 2% do humedad relativa y 90% do humedad relativa a temperatura ambiente) come so muestra en la Figure 3.
AdemÆs, se ha encontrado quo la sal clorhidrato cristalina del compuesto do fOrmula I es estable frente a la exposiciOn a una temperatura y humedad elevadad por un periodo prolongado. Per ejemplo, despuOs del almacenamiento durante 24 semanas a 40
° C y 75% de humedad relativa, el anÆlisispor HPLC no mostrO degradaciOn quimica y no
hubo cambios detectables en los resultados do DSC, TGA o PXRD,
En otro aspecto, la invenciOn provee una sal clorhidrato cristalina hidrato del
compuesto do fOrmula I.
En un aspecto, la sal clorhidrato cristalina hidrato de la presente invenciOn se
. caracteriza por un patron de difracciOn por rayos X do polvo (PXRD) quo tiene dos o mOs
picos de difracciOn a valores 20 seleccionados entre 5,30–0,2, 7,43–0,2, 872–0,2,
10,52–0,2, 13,85–02, 14,11–0,2, 15,80–0,2, 15,99–0,2, 17,26–0,2, 19,53–0,2,
20,08–0,2, 21,06–0,2, 21,48–0,2, 21,92–0,2, 22,85*0,2, 23,91–0,2, 25,28–0,2,
26,06–0,2, 27,34–0,2, 2751–0,2 y 29,67–0,2. En particular, en este aspecto, ia forma
cristalina so caracteriza per un patrOn de difracciOn por rayos X de polvo que tiene dos o
mÆs picos do difracciOn a valores 20 seleccionados entre 10,52–0,2, 1385–0,2, 15,80–0,2, 17,26–0,2 y 2106*0,2.
En otro aspecto, la sal clorhidrato cristalina hidrato del compuesto de fOrmula I se
caracteriza per un patrOn do difracciOn P01 rayos X do polvo en el cual las posiciones pico
son sustancialmente acordes con las quo se muestran en la Figura 4.
La sal clorhidrato cristalina hidrato de la presente invenciôn tambiØn se caracteriza
per su grÆfico do calorimetria diferencial per barrido (DSC) quo presenta un pico
1191 sustancial en el flujo de calor endotØrmico identificado con la fusiOn del cristal en el range
entre aproximadamente 225 °C y aproximadamente 250 °C, con endotermias generales o
dObiles monores ternperaturas come se ilustra on la Figura 5. AOn mÆs, el grÆficodel
anÆlisis tOrmico gravimOtrico (TGA) muestra que a temperature de degradaciOn so
encuentra por encima do 250 °C aproximadamente.
Se ha demostrado quo la sal clorhidrato cristalina hidrato del compuesto de formula
I tiene un perfil reversible de absorciOn/desorciOn a temperatura ambiente en todo el range

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entre aproximadamente 2% aproximadamente 90% de humedad relativa, como se ilustra en la Figura 6. El hidrato cristalino exhibe menos quo un 025% aproximadamente de aumento de peso entre aproximadamente 40% aproximadamente 75% de humedad relativa.

Estas propiedades de las formas salinas do esta invención se ilustran con mÆs detalles mÆs adelanteen los ejemplos.
Procedimientos de sintesis

El agente activo, {(I S3R, 5R)-8-[(R)-2-hidroxi3-(metansulfonil-metil-amino)propil]-8-azabiciclo[3,2, 1]oct-3-il}amida del Æcido 1 -isopropil-2-oxo-1,2dihidroquinolin-3 carboxilico, se puede preparar a partir de materiales iniciales fÆcilmento disponibles usando los procedimientos que so describen ms adelante en los ejemplos o usando los procedimientos que se describen en las Solicitudes de Patonte de EE.UU. asignados a un cesioriario comUn enumerados en la secciOn do Antecedentes de esta solicitud.
Para preparar la sal clorhidrato cristalina do la invenciÆn, la {(1S,3R,5R)-8-[(R)-2-hidroxi-3-(metansulfonil-metilamino)propiI]-8-azabicicIo[3,2, I]oct-3-U}amida del ªcido 1-isopropil-2-oxo-1,2-dihidroquinoliri-3-carboxilico se hace reaccionar tipicamente con entre aproximadamente 1 aproximadamente 1,5 equivalentes molares, incluyendo entre aproximadamente 1 aproximadamente 1,2 equivalentes molares, de àcido clorhidrico concentrado. En general, esta reacción tiene lugar en un diluyente inerte a una temperature quo varfa en un rango entre aproximadamente 20 °C aproximadamente 80 ° C. Los diluyentes inertes adecuados para esta reacciOn incluyen, en un sentido no limitativo, etanol, metanol, isopropanol, acetato de etilo, acetonitrilo, tolueno, tetrahidrofurano combinaciones de Los mismos.

Una vez completada la reacciOn, la sal cristalina de la invención so aisla de la mezcla do reacciOn mediante cualguier medio convencional, tal como precipitación, conceritración, centrifugaciOn semejantes.
El hidrato cristalino se puede preparar disolviendo la sal clorhidrato de la
{(I S,SR,5R)-8-[(R)-2-hidroxi-3-(metansulfonhl-metil-amino)propil]-8-azabiciclo[3,2, 1 }oct-3-il}amida del Æcido 1-isopropii-2-oxo1,2-dihidroquinolin-3-carboxilico en agua a una concentraciOn superior a 50 mg/ml aproximadamente, quo provee una suspension a partir de la cual se puede aislar el hidrato cristalino resultante de manera convencional.

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Comosiciones farmacØuticas
Las formas do sat clorhidrato cristalina de la invención Se administrari tipicamente a
un paciente en la forma de una composiciOn farmacØutica. Dichas composiciones farmacØuticas pueden administrarse al paciente a travØs do cualquier ruta de administraciOn aceptable, incluyendo, sin limitaciones, modos de administración oral, rectal, vaginal, nasal, por inhalación, tOpica (incluyendo transdOrmica) y parenteral.
Por consiguiente, en uno do los aspectos de las composiciones, la invenciOn estÆ dirigida a una composición farmacØutica que comprende un vehIculo o excipiente aceptable para uso farmacØutico y una cantidad eficaz para Lso terapØutico de la sal
clorhidrato cristalina de tin compuesto de fOrmula I. Opcionalmente, dichas composiciones
farmacOuticas pueden contoner otros agentes torapØuticos y/o do formulación si se
deseara.

Las composiciones farmacØuticas do la invenciOn contienen tfpicamente contain
una cantidad ef:caz para uso terapØutico de la sal cristalina de la presente invención. STipicamente, tales composiciones farmacOuticas contienen entre aproximadamente 0,1 y aproximadamente 95% en peso del agente activo, incluyendo entre aproximadamente 1 y
aproximadamente 70% en peso; tat como entre aproximadamente 5 y aproximadamente
60% en peso del agente activo.
Puede usarse cualquier vehiculo o excipiente convencionat en las composiciones
farmacØuticas de la irivenciÆn. La selecci6n do un vehiculo o excipiente particular, o do
combinaciones do vehiculos o excipientes, dependerÆdel modo do administración usado para tratar un paciente, o del tipo do condiciOn mØdica o estado do enfermedad particular. Rospecto do esto, la preparaciOn do una composiciOn farmacØutica adecuada para un modo de aciministraciOn particular estÆ dentro del alcance do aquellos entrenados en la tOcnica farmacØutica. AdemØs, los ingredientes para dichas composiciones estÆn disponibles comercialmente, por ejomplo, en Sigma, P.O. Box 14506, St. Louis, MO 63178. A mode de ilustraciOn adicional, so describen tØcnicas convencioriales de

formulaciOn en Remington: The Science and Practice of Pharmacy, 20a edición, Lippincott Williams & White, Baltimore, Manly (2000); y H.C. Ansel et at., Pharmaceutical Dosage Forms and Drug Delivery Systems, 7a ediciOn, Lippincott Williams & White, Baltimore, Manly (1999).
Los ejemplos representativos de materiales quo pueden servir come veh(culos aceptables para el uso farmacOutico incluyen, sin limitaciones, los siguientes: (1) azücares,

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tales como lactosa, glucosa y sacarosa; (2) almidones, tales como almidôn de maiz y almidón de papa; (3) celulosa y sus derivados, tales como carboximetilcelutosa de sodio, etil celulosa y acetato de celulosa; (4) tragacanto on polvo; (5) malta; (6) gelatina; (7) talco;
(8) excipientes, tales como manteca do cacao y ceras para supositorios; (9) aceites, tales como aceite de mani, aceite do semilla de algodOn, aceite de cârtamo, aceite de sØsamo, aceite de oliva, aceite de maiz y aceite de soja; (10) glicoles, tales como propilenglicol;
(11) polioles, tales como glicerina, sorbitol, manitol y polietilenglicol; (12) Østeres, tales como oleato de etflo y Iaurato de etilo; (13) agar; (14) agentes amortiguadores, tales como
hidróxido do magneslo e hidrOxido de aluminio; (15) àcido alginico; (16) agua libre de
pirógenos; (17) solución sauna isotónica; (18) solución de Ringer; (19) alcohol etilico; (20)
soluciones amortiguadoras de fosfato; y (21) otras sustancias no tóxicas compatibles
empleadas en composiciones farmacØuticas.
Las composiciones farmacØuticas de Ia irivenciOn so preparan tipicamente
. mezclancto o combiriando de forma exhaustiva e intima un compuesto do la invenciOn con un vehiculo aceptable para el uso farmacØutico y uno o mÆs ingredientes opcionales. Si
resulta necesario o se to desea, puede luego moldearse la mezcla uniforme combinada
resultarite, o puede cargârsela en tabletas, cÆpsulas, pildoras y semejantes usando
procedimientos y equipos convencionales.
Las composiciones farmacØuticas de la invención preferentemente se envasan en
formas de dosificación unitaria, El tØrmino "forma de dosificaciÆn unitaria" hace referenda a una unidad fisicamente discreta adecuada para admiriistrar una dosis a un
paciente, es decir, quo cada unidad contiene una cantidad predeterminada do agente
active calculada para producir et efecto terapØutico deseado, ya sea solo o en
combinaciOn con una o mØs unidades adicioriales. For ejemplo, dichas formas do
dosificación unitarias pueden ser cÆpsulas, tabletas, pildoras, etcetera.
En una realizaciôn preferida, las composiciones farmacOuticas de la invenciôn son Sapropiadas para una administraciOn oral. Las composiciones farmacØuticas apropiadas para administración oral pueden tomar la forma de cÆpsulas, tabletas, pildoras, pastitlas,
sachØts, grageas, polvos, grÆnulos; o coma una soluciôn o una suspensiOn en un liquido
acuoso o no acuoso; o coma emulsiones Iiquidas de aceite en agua a agua en aceite; a
como elixires o jarabes; y semejantes; donde cada uno contiene una caritidad
predeterminada de un compuesto de la presente invención como ingrediente active.

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Cuando se desee una administración oral en una forma de dosificación sólida (es
decir, como cÆpsulas,tabletas, pildoras y semejantes), las composiciones farmacØuticas de la irvenciOn comprenderÆntipicamente un compuesto de la presente invenciôn, como ingredierite activo, y uno o mÆsvehiculos aceptables para el uso farmacØutico, tales como citrato de sodio o fosfato dicàlcico. Opcionalmente a coma alternativa, dichas formas de dosificaciOn sólidas pueden comprender tambiØri: (1) rellenos 0 extensores, tales coma almidones, lactosa, sacarosa, glucosa, manitol y/o Æcido silicico; (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, almidÆn de papa o tapioca, kido alginico, detecminados silicatos,
y/o carbonato de sodlo; (5) agentes Clue demoran la solubilizaciOn, tales como parafina; (6)
aceleradores de Ia absorción, tales como compuestos de amonio cuaternario; (7) agentes
humectantes, tales como alcohol cetilico y/o monoestearato de glicerol; (8) absorbentes,
. tales como caolin y/o arcilla bontonita; (9) lubricantes, tales coma talco, estearato de calcio, estearato de magnesia, polietilenglicoles sOlidos, lauril sulfato de sodio y/o mezclas
de los mismos; (10) agentes colorantes; y (11) agentes amortiguadores.
TambiØn puede haber agentes de liberaciön, agentes humectantes, agentes de
recubrimiento, edulcorantes, agentes saborizantes y perfumantes, conservarites y
antioxidantes presentes en las composiciones farmacØuticas de esta invención. Los
ejemplos de antioxidantes aceptables para el uso farmacØutico incluyen: (1) antioxidantes solubles en agua, tales como Æcido ascôrbico, clorhidrato de cisteina, bisulfato do sodio, metabisulfato de sodio, sulfito de sodio y semejantes; (2) antioxidantes solubles en aceite, tales como patmitato de ascorbilo, hidroxianisol butilado (BHA), hidroxitolueno butilado (BHT), lecitina, galato de propilo, alfa-tocoferol y semejantes; y (3) agentes quelantes de metales, tales como Æcidocitrico, àcido eti lend iaminotetraacØtico (EDTA), sorbitol, 6cido tartØrico, Æcido fosfórico y semejantes. Los agentes de recubrimiento para tabletas, cpsuIas, pildoras y semejantes incluyen aquellos usados para recubrimientos entØricos, tales como ftalato de acetato do celulosa (CAP), ftalato do acetato de polivinilo (PVAP), ftalato de hidroxipropilmetilcelulosa, copolimeros de ester de Æcido metacrilico,trimetilato de acetato de celulosa (CAT), carboximetil etilcelulosa (CMEC), succinato do acetato de hidroxipropilmetilcelulosa (HPMCAS), y semejantes.
Si se to desea, las composiciones farmacØuticas do la prosento invenciOn tambiØn pueden formularse para obtener la liberaciOn lenta a controlada del ingrediente activo

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usando, a modo de ejemplo, hidroxipropil metil celulosa en proporciones variables; u otras matrices de polimeros, liposomas y/o microesferas.
AdemÆs, las composiciones farmacØuticas de Ia presente invención pueden contener opcionalmente agentes opacantes y so pueden formular de modo quo liberen el ingrediente activo solamente, o con preferencia, en una determinada porciôn del tracto gastrointestinal, opcionalmente de una manera demorada. Los ejemplos do composiciones do embebido quo se pueden usar incluyen sustancias polimØricas y ceras. El ingrediente activo tambiØn puede estar en una forma micro-encapsulada, si resulta
apropiado, con uno o mÆsde los excipientes descriptos previamente.
Las formas do dosificaciOn lIquidas para administración oral incluyen, a modo do
ilustraciÆn, emulsiones, microemulsiones, soluciones, suspensiones, jarabes y elixires
aceptables para el uso farmacØutico. Dichas formas de dosificación liquidas comprenden
tipicamente el ingrediente activo y un diluyente inorte, tal como, por ejemplo, aqua u otros
. solventes, agentes solubilizantes y emulsionantos, tales como alcohol etIlico, alcohol isopropilico, carbonato de etilo, acetato do etilo, alcohol bencilico, benzoato de bencilo,
propilenglicol, 1,3-butilenglicol, aceites (por ejemplo, aceite de semilla de algodOn, mani,
maiz, germen, oliva, castor y sØsamo), glicerol, alcohol tetrahidrofurilico, polietilenglicolos y
Østeres do Æcidos grasos de sorbitn, y mezclas do los misnios. Las suspensiones,
ademÆs del ingrediente activo, pueden contener agentes de suspension, tales como, por
ejemplo, alcoholes isoestearilicos etoxilados, polioxietilen sorbitol y Østeros do sorbitÆn, celulosa microcristalina, metahidróxido de aluminio, bentonita, agar-agar y tragacanto, y
mezclas do los mismos.

Como alternativa, las composiciones farmacØuticas do la invenciOn se pueden
formular para una administraciOn por inhalaciOn. Las composiciones farmacØuticas
adecuadas para administraciOn por inhalaciOn tomarªn tipicamente la forma do un aerosol
0 Ufl polvo. Dichas composiciones se administran generalmente usando dispositivos do Sadministraci6n bien conocidos, tales como un nebulizador do inhalaciOn, un inhalador do dosis medidas, un inhalador de polvo seco o un dispositivo de administraciOn similar.
Cuando so las administra por inhalación usando un recipiente presurizado, las
composiciones farmacØuticas do la invenciOn comprenden tIpicamente el ingrediente
activo y un propelente apropiado, tal como diclorodifluorometano, triclorofluorometano,
diclorotetrafluoroetano, diOxido de carbono u otro gas apropiado.

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AdemÆs, la composición farmacŁuticapuede tomar la forma de una cÆpsula a un cartucho (por ejemplo, de gelatina) que comprende un compuesto de la invención y un polvo apropiado para usar en un irihalador de polvo. Las bases de polvo apropiadas incluyen, a modo de ejemplo, lactosa a almidôn.
Los compuestos de la invención tambiØn pueden administrarse par via transdØrmica, usando sistemas de administraciOri transdØrmica y excipientas conocidos. Por ejemplo, puede mezclarse un compuesto de la invención con potenciadores de la permeabilidad, tales coma propilenglicol, monolaurato de polietilenglicol, azacicloaIcan2-onas y semejantes, e incorporarse en un parche o un sistema de administración similar. Si se desea, en dichas composiciones transdermicas se pueden utilizar excipientes adicionales que incluyen agentes gelatinizantes, emulsionantes y soluciones amortiguadoras de pH.
Las siguientes formulaciones ilustran composiciones farmacØuticas representativas
de la presente invención:

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jmnlo de Formulación A
So prepararon cªpsulas de gelatina dura para administraciôn oral de la manera siguiente:
Ingredientes Cantidad
Sal de la invenciOn 50 mg
Lactosa (secada por aspersiOn) 200 mg
Estearato de magnesio 10 mg
Procedimiento renresentativo: Los ingredientes se mezclan exhaustivamente y luego so cargan en una capsula do gelatina dura (260 mg de composiciOn por cÆpsula).
Ejemplo de FormulaciOn B
Se prepararon cÆpsulas de gelatina dura para administraciOn oral de la manera siguiente:
Ingredientes Cantidad
Sal de la invenciOn 20 mg
Almidón 89 mg
Celulosa microcristalina 89 mg
Estearato do magneslo 2 mg
Procedimiento representativo: Los ingredientes se mezclan exhaustivamente luego Se hacen pasar por un tamiz do malla N° 45 do EE.UU. se cargan en una cÆpsula de gelatina dura (200 mg de composiciOn por cÆpsula).
Ejemplo de FormulaciOn C
Se preparan cÆpsulas paraadministraciOn oral de la siguiente forma:
Ingredientes Cantidad
Sal de la invenci6n 10 mg
Monooleato de polioxietilen sorbitano 50 mg
Polvo de almidOn 250 mg
Procedimiento representativo: Los ingredientes se mezclan exhaustivamente luego so cargan en una cÆpsula de gelatina(310 mg de composiciOn porcÆpsula).
templo do FormulaciOn D
Se preparan tabletas para administraciôn oral como se indica a continuaciOn: Ingredientes Cantidad
Sal de la invenciOn 5 mg
AlmidOn 50 mg

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Celulosa microcristaUna 35 mg
Polivinilpirrolidona (10% en peso en 4 mg
agua)
Carboximetil almiclOn do sodia 4,5 mg
Estearato de magnesia 0,5 mg
Talco 1 mg
Procedimiento rresontativo: Se hce pasar el ingrodiente activo, el almidOn la celulosa a travØs de un tamiz do malla N° 45 EE.UU. se mezcla exhaustivamente. La solución de polivinilpirroUdona se mezola con los polvos resultantes esta mezcla Se pasa luego a travØs de un tamiz de malla N° 14 EE.UU.. Los grØnulos asi producidos so secan a 50-60 OC so pasan a travØs de un tamiz de malla N° 18 EE.UU.. Luego se agrega carboximotil almidOn sOdico, estearato do magnesia talco (tamizados previamente con una malla N° 60) a los grØnulos. DespuØs do mezclar, se comprime la mezcla en una mÆquinatableteadora para obtener una tableta con un peso de 100 mg.
Ejemplo de Formulación E
So preparan tabletas para administración oral coma se indica a continuación: Ingredientes Cantidad
Sal do la invenciÆn 25 mg
Celulosa microcristalina 400 mg
DiÆxido dosiliclo ahumado 10 mg
Acido esteârico 5 mg
Procedimionto renrosontativo: Los ingrediontos so mezclan exhaustivamente luego se comprimen para formar comprimidos (440 mg do composiciOn par comprimido). Ejemalo do Formulación F
Se preparan tabletas monorranuradas para administraciOn oral do la siguiente forma:

Ingrodientes Cantidad
Sal do la invención 15 mg
AlmidOn do maiz 50 mg
Croscarmelosa sÆdica 25 mg
Lactosa 120 mg
Estearato do magnesio 5 mg

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Procedimiento representativo: Los ingredierites se mezclan exhaustivamente y so comprimeri para formar un comprimido monorranurado (215 mg de composición por comprimido).
Elemolo de FormulaciOn G

Se preparó una suspensiOn para administraciôn oral de la siguiente manera:
Ingredientes Cantidad
Sal do la invención 0,1 g
Acido fumÆrico 0,5 g
Cloruro de sodio 2,0 g
Metil parabeno 0,159
Propil parabeno 0,05 g
AzUcar granulado 25,5 9
Sorbitol (70% solución) 12,85 g
Veegum k (Vyerbilt Co,) 1,0 g
Saborizante 0,035 ml
Colorantes 0,5 mg
Agua destilada c.s.p. lOOmI
Procedimiento representativo: Los ingredientes se mezolaron para formar una suspensiOn que contenia 10 mg do ingrediente activo por lOmi do suspensiOn.
Ejemplo de FormulaciOn H
Se preparO un polvo seco para administrar per inhalaciOn mediante el siguiente procedimiento representative.
Ingredientes Cantidad
Sal de la invenciOn 1,0 mg
Lactosa 25 mg
Procedimiento representative: El ingredierite activo se microniza y luego se combina con lactosa. Esta mezcla luego so carga en un cartucho de inhalaciOn do gelatina. Los contenidos del cartucho se administran usarido un inhalador para polvo.
Elemplo de tormulación I
Se preparO un polvo seco para administrar por inhalaciOn en un inhalador do dosis modida do la manera siguiente:
Procedimiento representativo: Se prepara una suspension que contiene 5% en peso do una sal de la invenciOn y 0,1% en peso do lecitina dispersarido 109 de compuesto activo,

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coma particulas micronizadas con un tamaæo promedio menorqua 10 pm, en una soluciOn formada a partir con 0,2 g de lecitina disueltos en 200 ml de agua desmineralizada. La suspension se secO par aspersiOn y el material que se obtuvo se micronizO para dar
particulas con un diÆmetro media menor de 1,51m. Las particulas se cargaron en
cartuchos con 1,1,1,2-tetrafluoroetano presurizado.
Eiemplo de formulaciOn J
Se preparO una formulaciOn inyectable de la siguiente manera:
Ingredientes Cantidad
Sal de la invenciOn 0,2 g
soluciOn amor-tiguadora de pH do 40 ml
acetato do sodio (0,4M)
HCI (0,5N) o NaOH (0,5N) c.s.p. pH 4
Agua (destilada, estØril) asp. 20m1
Procedimiento representativo: Se mezclaron los ingredientes anteriores se ajustO el pH a 4t 0,5 usando HCI 0,5N o NaOH 0,5N.
Eiemplo de formulaciOri K
Se preparan cªpsulas para admirristraciOn oral de la siguiente forma:
Ingredientes Cantidad
Sal de la invenciOn 4,05 mg Celulosa microcristalina (Avicel PH 103) 259,2 mg Estearato do magnesia 0,75 mg Procedimiento representativo: Los ingredientes so mezclan exhaustivamente luego se cargan en una cÆpsula degelatina (Tamaæo #1, Blanca, Opaca) (264 mgde composición por cOpsula).
Eiemplo de formulación L
Se preparan cÆpsulaspara administraciOn oral do Ia siguiente forma:
Ingredientes Cantidad
Sal de la invención 8,2 mg
Celulosa microcristaliria (Avicel PH 103) 139,05 mg
Estearato de magnesia 0,75 mg
Procedimiento representativo: Los ingredientes se mezclan exhaustivamente luego se cargan en una cÆpsulade gelatina (Tamaæo#1, Blanca, Opaca) (148 mg de composiciOn par cÆpsula).

ON

Utilidados

El campuesto do formula I, {(1 S,3R,5R)-8-[(R)2-hidroxi-3-(metansulfonil-metil-amino)propilj-8-azabiciclo[3,2, 1]oct-3-il}amida del Æcido 1 isopropil-2-oxo-1 2-dihidroquinolin-3-carboxilico, es un agonista del receptor 5-HT4 y por ella se espera quo as presentes formas de sales cristalinas del compuesto do fOrmula I sean do utifidad para tratar las afecciones mØdicas mediadas por receptores 5-HT4 o asociados con la actividad del receptor 5-HT4, es decir las afecciones mØdicas que mejorari con el tratamiento con un agonista del receptor 5-HT4. Dichas condiciones mØdicas incluyen, sin
Umitarse, s(ndrome del intestino irritable (IBS), constipación crOriica, dispepsia funcional,
retardo del vaciado gØstrico, enfermedad de reflujo gastroesofØgico (GERD),
gastroparesis, gastropatIa diabØtica e idiopOtica, flea postoperatorio, pseudoobstrucciOn
intestinal, y trOnsito con retardo inducido par drogas. AdemÆs,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 do Ænimo, y trastornos de control do la funciOn
autOnoma.

En particular, las formas salinas de la invenciOn aumentan la motilidad del tracto
gastrointestinal (Cl) y par Ia tanto so espera que sean Otiles para tratar trastornos del tracto
GI a causa de la motilidad reducida en mamiferos, incluyendo humanos. Dichos trastornos
do la motilidad GI incluyen, a modo de ilustraciOn, constipación crOnica, sIndrome del iritestino irritable con predominio de constipaciOn (C -IBS), gastroparesis diabØtica e idiopÆtica,y dispepsia funcional.
Por ella, en un aspecto la invenciOn provee un mØtodo para incrementar la motilidad
del tracto gastrointestinal en un mamifero, donde dicho mØtodo comprende la administraciOn a dicho mamIfero de una cantidad eficaz para usa terapØutico de una composiciOn farmacØutica que comprende un vehiculo aceptable para usa farmacØutico y una sal cristalina do la invenciOn.
Cuando so utilizan para tratar trastornos de motilidad reducida del tracto GI u otras condiciones mediadas par receptores 5-I-IT4, las formas salinas de la invenciOn se administrarØn tipicamente por via oral en una Cinica dasis diana a en varias dosis par dia, aunque se pueden utilizar atras formas de administración. La cantidad de agente activo quo se administra por dosis a la cantidad total quo se administra par dia serà determinada tipicamento par un medico, en base a las circunstancias relevantes, que incluyen la

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condiciOn quo se debe tratar, la ruta de admirstraciOn seleccianada, el compuesto quo se estÆ administrando y su actividad relativa, la odad, peso, y respuesta del paciente individual, la gravedad de los sintomas del paciente, etcetera.
Se espera quo la dosis apropiada para tratar trastarnos de motilidad reducida del tracto GI u otros trastornos mediados par receptores 5-HT4 vane dentro del rango entre aproximadamente 0,0007 y aproximadamente 20mg/kg/dia do agonte activo, incluyendo entre aproximadamente 0,0007 y aproximadamente lmg/kg/dia. Para un humano promedlo de 70 kg, la misma padnia sen do entre aproximadamente 0,05 y aproximadamente 70 mg par dia de agonte activa.
En un aspecto de b invenciôn, as formas salinas de la invenciÆn se utilizan para tratar la constipaciÆncrOnica. Cuando so utilizan para tratar Ia constipaciÆn crónica, las sales de la invenciôn so administrarÆntipicamente par via oral en una Onica dosis diana o en varias dosis par dia. So espera que la dosis para tratar la constipaciÆncrónica vane . dentro del rango entre aproximadamente 0,05 y aproximadamente 70mg par dia. En otro aspecto do la invención, las formas salinas do la invenci6n so utilizan para tratar el sIndrome del intestino irritable. Cuando so utiliza para tratar el sindrome del intestino irritable con predominia de constipación, las sales do la invenciOn so administrarªn tipicamente par via oral en una Onica dosis diana o en varias dosis por dia. So espera que la dosis para tratar el sindrome del intestino irritable con predominlo de canstipaciOn vane dentro del rango entre aproximadamente 0,05 y aproximadamente 70mg par dia.
En otro aspecto do la invención, las formas salinas de a invención so usan para tratar la gastroparesis diabØtica e idiopàtica. Cuando so utilizan para tratar gastroparesis diabØtica e idiapÆtica, la sales dola invención tipicamente se administran par via oral on una sola dosis diana a en mØltiples dosis par dia. Se espera quo Ia dosis para tratar Ia gastroparesis diabØtica vane dentro del rango entre aproximadamente 0,05 y aproximadamente 70mg par dia.
En a0n otro aspecto de la invenciôn, las formas salinas do la invenciön se utilizan
para tratar la dispepsia funcional. Cuando so utilizan para tratar la dispepsia funcional, los compuestos do la invención so administrarÆn tipicamente parvia oral en una Onica dosis diana a en varias dosis por dia. So espera quo la dosis para tratar la dispepsia funcional vane dentro del rango entre aproximadamente 0,05 y aproximadamente 70 mg par dia.

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La invenciôn tambiØn provee un mØtodo para tratar un mamifero con una
enfermedad o condiciOn asociadas con Ia actividad del receptor 5-HT4, donde dicho mØtodo comprende administrar al mamifero una cantidad efectiva para uso terapØutico de una forma saUna de la invenciOn o de una composición farmacØutica que comprende una forma sauna de la invenciÆn.

Como se describió previamente, las formas salinas de la invenciÆn sonagonistas
del receptor 5-HT4. Par ello, la invención provee ademÆs un mOtodo de agonizar un
receptor 5-HT4 en un mamIfero, donde dicho mØtodo comprende la administración de una forma sauna de la invenciOn a dicho mamifero,
Estas propiedades, asi come la utilidad de las formas de sal clorhidrato de la invención, se pueden demostrar utilizando diversos ensayos in vitro e in v/ye bien conocidos per los especialistas en el arte. Los ensayos representativos se describirØn con mayr detalle en los siguientes ejemplos,
EJEMPLOS

Los siguientes ejemplos sintØticos biológicos se ofrecen para ilustrar la invenciOn,
no deberi verse de ninguna manera come una limitaciOn del alcance de la invenciÆn. En los ejemplos a cantinuaciOn, las siguientes abreviaturas tienen los siguientes significados salvo que se indique lo contrario. Las abreviaturas que no se definen a continuación tienen sus significados aceptados generalmente.
Boc terbutoxicarbon! lo

(Boc)20 di-tert-butil dicarbonato
DCM Diclorometano
DMF N,N-dimetilformamida
DM80 dimotilsulfóxido
EtOAc acetate de etilo
mCPBA Æcido m-clorobenzoico
MeCN Acetonitrilo
MTBE Øter ter-butilmetilico;
PyBop hexafluorofosfato de benzotriazol-1 -iloxitripirrolidin-fosfonlo
Rf factor de retenciOn
RT temperatura ambiente,
TFA Acido trifluoroacØtico
THF tetrahidrofurano

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Los reactivos (incluyerido aminas secundarias) y solventes se adqufrieron do proveedores comerciales (Aldrich, Fluka, Sigma, etc.), y so utilizaron sin purificación adicional. Las reacciones se levaron a cabo baja atmOsfera do nitrOgeno, salvo quo se indique to contrario. El progreso do las mezclas do reacción fue monitoreado mediante cromatografia en capa delgada (TLC), cromatografia liquida do alta presióri anaUtica (HPLC-anal.) y espectrometria do masa, cuyos detalles so ofrecen mÆs adelante y por separado en ejemplos especificos do las reacciones. Las mezclas de reacciOn se sometieron a tratamiento posterior coma se describe especificamente en cada reacciOri; comUnmente so purificaron por extracciOn y otros mtodos de purificaciOn tat coma cristalizaciOn dependiente de temperatura y solvente, y precipitaciOn. Adems, las mezclas do reacción se purificaron par rutina por HPLC preparativa: La caracterización de los productos do reacción so llevó a cabo por rutina par espectrometria do masa y NMR. Para la medición de NMR, las muestras se disolvieron en solvente deuterado (CD30D, CDCI3, o DMSO-d5), y los espectros 1H-NMR se adquirieron con un iristrumento Varian Gemini 2000 (300 MHz) bajo condiciones de observaciOn estªndar, La identificaciOn par espectrometria de masa de los campuestos se efectuô mediante un mOtodo de ionización con olectroatomizaciOn (ESMS) con un instrumento do Applied Biosystems (Foster City, CA) modelo API 150 EX a un instrumento Agitent (Palo Alto, CA) modelo 1100 LC/MSD. El contenido de agua so determin6 par titulación de Karl Fischer usando un coulómetro Brinkmann (Westbury, NY) Metrohm Karl Fischer Modelo 813. Preparacón 1: ter-butilØster de Æcido(IS,3R,5R)-3.amino-8-azabiciclo[3.2.ljoctari-

8-carboxifico

a. Preparación de 8-bencil-8-azabiciclof3.2.1 loctan-3-ona
So agregO ªcido clorhidrico coricentrado (30 ml) a una soluciOn heterogØnea do 2,5-dimetoxitetrahidrofurario (82,2 g, 0,622 mat) en ague (170 ml) baja agitaciOn. En un recipiente separado enfriado a 0°C (baæo do hielo), (92 ml) se agregó Æcido clorhidnico concentrado lentamente a una solución do bencilamina (100 g, 0,933 mot) en agua (350 ml). La sotución de 2,5-dimetoxitetrahidrofurano se agitó durarite aproximadamento 20 mini, so diluyó con agua (250 ml), y luego se agregó la solución do bencilamina, y a continuaciOn se agregO una solucion do Æcido1,3-acetoniadicarboxitico (100 g, 0,684 mol) en agua (400 ml) y luego fosfato Æoidode sodio (44 g, 0,31 mol) en agua (200 ml). El pH so ajustO de pH 1 hasta pH - 4,5 utilizando NaOH 40%. La solución turbia color amarillo pÆlido resultanteso agitó durante la noche. La solución luego se acidificO a pH 3 desde

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pH 7,5 utilizando ªcido clorhidrico 50%, se calentO a 85°C y se agitô durante 2 horas. La solución se enfrió a temperatura ambierite, se basificó a pH 12 utilizando NaOH 40%, y se extrajo con diclorometano (3 x 500 ml). Las fases orgÆnicas combinadasso lavaron con salmuera, so secO (MgSO4), so filtrO y so concentró bajo presiOn reducida para producir el intermediario del titulo crudo como un aceite viscoso color marrOn.
A una solución del intermediario crudo en metanol (1000 ml) se agrego dicarbonato
de di-ter-butilo (74,6 g, 0,342 mol) a 0 00. La solución se dejó calentar hasta temperatura ambiente y se agitó durante la noche. El metanol so eliminO bajo presión reducida y el
aceite resultante se disolvió en diclorometano (1000 ml). El intermediario so extrajo con
H3PO4 1 M (1000 ml) y se lavó con diclorometano (3 x 250 ml). La fase acuosa so basificó
a pH 12 utilizando NaOH acuoso, y se extrajo con diclorometano (3 x 500 ml). Las fases
orgÆnicas combinadasso secaron (MgSO4), so filtrO y se concentrô bajo presiOn reducida
para producir el intermediario del titulo como un aceite viscoso color marrOn claro.
. 1H-NMR (CDCI3) (ppm) 7,5-7,2 (m, 5H, C6H5), 3.7 (s, 2H, CH2Ph), 3,45 (ancho s, 2H, CH-NBn), 2.7-2,6 (dd, 2H, CH200), 2,2-2,1 (dd, 2H, CH200), 2,1-2,0 (m, 2H, CH2CH2), 1,6 (m,
2H, CH2CH2). (m/z): [M+H} calculado para C141-117N0 216,14; encontrado, 216,0.
b. Prpraciôn do tor-butilØster de àcido 3-oxo-8-azabiciclol3.2.1]octan-8-carboxitico
A una solución do 8-bencil-8-azabiciclo[3.2.1]octan-3-ona (75 g, 0,348 mol) en
EtOAc (300 ml) se agregó una solución do dicarbonato de di-ter-butilo (83,6 g, 0,383 mol,
1,1 eq) en EtOAc (300 ml), Se agregO la solución resultante y solvente de enjuague (100 ml EtOAc) a un recipiente do hidrogenaciÆn Parrdo IL quo contenIa 23 g de hidrÆxido de paladio (Pd 20 % en peso, base soca, sobre carbon, -50% de humedad de agua; por ejemplo catalizador do Pearlman) bajo una corriente de nitrOgeno. El recipiente de reacciOn so desgasificó (alternando vaclo y N2 cinco veces) y se presurizO a 60 psi de gas H2. La soluciOn de reacciôn so agitó durante dos dias y se recargO con H- segOn fuera necesario para mantener la presiOn de I-t a 60 psi hasta resultar completa la reacciôn segün to indicado por la cromatografia en capa delgada sobre silice. La solución negra luego se filtró a travØs do una almohadilla de Celitefi y so concentrO bajo prestOn reducida para dar el intermediario del titulo en forma cuantitativa como un aceite viscoso color amarillo anaranjado. Se utilizO en el paso siguiente sin tratamionto adicional. 1H NMR (CDCI3) ?(ppm) 4,5 (ancho, 2H, CH-NBoc), 2.7 (ancho, 2H, CH200), 2,4-2,3 (dd, 2H, CH2CH2), 2,1 (ancho m, 2H, CH2CO), 1.7-1,6 (dd, 2H, CH2CH2), 1,5 (s, 9H, (CH3)3C000N)).

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C. PreparaciÆn de ter-butilŁster de Æcido (1 S,3R,5R_)-3-amino-8-azabiciclol3.2. I loctan-8-carboxilico

A una so(ución del producto del paso anterior (75,4 g, 0,335 mol) en metanol (1 L)
se agregó formato de amonlo (422,5 g, 6,7 mol), agua (115 ml) y 65 g de paladio sobre carbOn activado (10% en base seca, -50% hümedo con agua; tipo Degussa E101NEJW) bajo una corriente de N2 bajo agitaciôn per medlo de un agitador mecânico. DespuØs de 24 y 48 horas, se agregaron mÆs porcionesde formato de amoriio (132g, 2,1 mol) cada vez. Una vez terminado el progreso de la reacciOn, segOn el monitoreo per HPLC anailtica, se agregÆ Celite (>500g) y Ia suspensiOn espesa resultarìte se filtrO y luego el sOlido recogido se enjuagO con metanol (-500 ml). Los filtrados se combinaron y se concentrô bajo presiOn reducida hasta ser eliminado todo el metanol. La solucióri turbia bifÆsicaluego se diluyO con àcido fosfOrico 1 M hasta un volumen final de -1,5 a 2,0 L a pH 2 y se lava con diclorometano (3 x 700 ml). La fase acuosa se basificó a pH 12 utilizando NaOH aq. 40%, y so extrajo con diclorometano (3 x 700 ml). Las fases orgÆnicas combinadas se secaron sobre MgSO4, se flitraron y se concentraron por evaporaciOn rotativa, y luego alto vacio dando 52 g (70%) del intermediario del titulo, de nombre tradicional N-Boc-endo-3-aminotropano, come un sOlido color blanco hacia amarillo pÆlido. LaproporciOn de isOmeros de endo a exoamina del producto resultó >99 segun el anÆlisisper 1H-NMR (>96% do pureza per HPLC analItica). 1H NMR (CDCI3) 6 (ppm) 4,2-4,0 (ancho d, 2H, CHNaoc), 3,25 (t, 1H, CHNH2), 2,1-2,05 (m, 4H), 1,9 (m, 2H), 1,4 (s, 9H, (CH3)3000N), 1,2-1,1 (ancho, 2H). tn/z): [M+H] calculado para C121-122N202 227,18;
encontrado, 227,2. HPLC anatitica (rnØtodo isocrtico; 2:98 (A:6) a 90:10 (A:B) a Ia largo
de 5 tiempo de retenciOn = 2,14 mm,
Preparación 2: âcido I -isopropil-2-oxo-I,2-dihidroquinolin-3-carboxiliCo
En primer lugar se agregO acetona (228,2 ml, 3,11 mol) a una suspensiOn agitada
de 2-aminofenilmetanol (255,2 g, 2,07 mol) y ªcido acØtico (3,56 ml, 62 mmol) en agua (2
S L)a temperatura ambiente. Despu6s de 4 In, la suspensiOn se enfriO a 0 °C y se agitO durante otras 2,5 h y luego so filtrO. El sOlido se recogiO y se IavO can agua y el sólido
hUmedo se enfriO y se secÆ per tiofilización para dar 2,2,-dimetil-1,4-dihidro-2H-
benzo[1,3]oxazir,a (332,2 g, 98 %) coma un sOIido blancuzco. 1H NMR (CDCI3; 300MHz):
1,48 (s, 6H, C(CH)2), 4,00 (bs, 1H, NH), 4,86 (s, 2H, C), 6,66 (d, 1H, ArH), 6,81 (t, 1H,
An -I), 6,96 (d, 1H, AM), 7,10 (t, 1H, Art-I).

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Una solucion de 2,2,-dimetil-1,4-dihidro-2H-benzo[1,3]oxazina (125 g, 0,77 mol) en
THF (1 L) so ftrO a travØs de on embudo de centefleo y luego so agregO por goteo a travOs do on embudo de adiciOn, a lo largo do on periodo do 2,5 h, a una so!uciOn agitada
de LiAIH4 1,0 M en THF (800 ml) a 0 °C. La reacciOn so detuvo por adiciÆn lenta en
porciones de Na7SO410H20 (110 g), a In largo do on periodo do 1,5 h, a 0°C. La mezcla do reacciOn se agitO durante la noche, Se filtrO y las sales sOlidas se lavaron ciudadosamerite con THF. El filtrado se concentrÆ bajo presión reducida pam dar 2-isopropilaminofenilmetanol (120 g, 95 %) como on aceite color amarillo. 1H NMR (CDC(,;
300MHz): 1,24 (d, 6H, CH(CH3)2), 3,15 (bs, IH, OH), 3,61 (sept, 1H, CH(CH:)2),4,57 (s,
2H, CH2), 6,59 (t, IH, An -I), 6,65 (d, I H, ArH), 6,99 (d, 1H, An -I), 7,15 (t, 1H, ArH).
So agregó diOxido de manganeso (85 % 182,6 g, 1,79 mol) a una soluciOn agitada
de 2-isopropilaminofenilmetanol (118 g, 071 mol) en tolueno (800 ml) y la mezcla do
reacciOn so calentO a 117°C durante 4 h. La mezcla de reacción se dejO enfriar hasta
. temperatura ambiente durante la noche y luego se filtró a travØs do una almohadilla do Celite quo se eluyO con tolueno. El filtrado se concentró bajo presiOn reducida para dar 2-isopropilaminobenzaldehido (105 g, 90 %) como on aceite anaranjado, H NMR (CDCI3;
300MHz): 1,28 (d, 6H, CH(CH3)2), 3,76 (sept, IH, CH(CH)7), 6,65(t, 1H, ArH), 6,69 (d, 1H,
ArH), 7,37 (d, IH, ArH), 7,44 (t, 1H, An -f), 9,79 (s, 1H, CHO).
Se agregó 2,2-dimetil-[1,3]ciioxan-4,6-diona, do nombre tradicional Æcido de
Meldrum, (166,9 g, 1,16mol) a una solución agitada de 2-isopropilaminobenzaldehido (105 g, 064 mol), àcido acØtico (73,6 ml, 1,29 mol) y etilenediamina (43,0 ml, 0,64 mol) en metanol (1 L) a 0 °C. La mezcla do reacciÆn so agitó durante 1 h a 0 °C y luego a temperatura ambiente durante la noche. La suspensiOn resultante so filtró yel sOlido so lavó con metarnol y so recogio pare dar el intermediarlo del titulo, Æcido 1-isopropil-2-oxo-1,2-dihidroquinoiin-3-carboxilico (146 g, 98 %) como urn sOlido blancuzco.1H NMR (CDCIj; 300MHz): 1,72 (d, 6H, CH(CH3)), 5,50 (bs, IH, CH(CH3)2), 7,44 (t, IH, ArH), 7,75-7,77 (m, 2H, ArH), 7,82 (d, 1H, AM), 8,89 (s, 1H, CH).
Ejemplo 1: Sintesis do âcido 1-isopropiI-2-oxo-1,2dihidroquinoIin-3-carboxilico ((1 S,3R,5R)-8-[(R)-2.hidroxi-3-(metansuIfoniI-metiI-amino)propiIJ-8-aza-bIciclo[3.2.l]oct-3-iI}amlda
a. Preoaración do ter-butilØster do Æcido (1S,3R,5R)-3-F1-isornropil-2-oxo-1,2-
dihidropuinolin.3-carbonil)aminol-8-azabiciclo[3.2.1]octarn-8-carboxilko

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En un recipiente de 3 L, ªcido 1-isoprop-2-oxo-1,2-dihidroquinofln-3-carboxIlico (112,4 g, 0,486 mol, 1,1 eq) se suspendió en tolueno (1 L). La mezcla se calentó a 85 °C y so agregO cloruro de tionilo (86,74 g, 0,729 mol) por goteo a Jo largo de 70 La mezcla se calentO a 95 °C durante 15 h con agitaciOn y luego se dejó enfriar hasta temperatura ambiente.
En un recipiente separado do 12 L, se suspendió ter-butilØster de Æcido
(1 S3R,5R)-3-amino-8-azabiciclo[3.2, 1 loctan-8-carboxilico (100,0 g, 0,442 mol, I eq) en tolueno (1 L) y Se agregó NaOH 3 M (4 eq). La mezcla se agitô a temperatura ambiente durante 10 min y luego se enfrió hasta aproximadamente 5 °C, La soluciOn de cloruro do Æcido se agregO lentamente con agitaciOn a 10 largo do 40 min manteniendo la temperatura interna par debajo de 10 °C. La mezcla so agitO a 3-5 °C durante 30 min y se deja separar las fases durante la noche. La fase en tolueno (-2,5 L) so recogió, se concentrO hasta aproximadamente la mitad (-1,2 L) por evaporaciôn rotativa, y se utilizÆ . diroctamente en el prÆximo paso.
b. Preparación de {(1S,3R,5R)-8-azabiciclof3.2.1]oct-3-il}amida de Æcido 1-isopropil-2-oxo-1,2-dihidroguinolin-3-carboxilico
A la soluciOn en tolueno preparada en el paso anterior (-1,2 L) se agregó Æcido trifluoroacØtico (200 ml) a Jo largo do 20 min a 20 °C con agitaciôn. La mezcla so agitO a 20 °C durante 2 h, So agregO agua (1,55 L) y la mezcla so agito durante 30 min a 20 C. Despues de 30 mm, la mezcla se soparó en tres fases. La faso inferior (-350 ml), un aceite viscose color marrón, contenia el intermediario crudo.
A un recipiente de 12 L cargado con MTBE (2,8 L), se agregó el aceite marrOn
crudo a Jo largo de 1 a 1-2 °C con agitaciOn. La suspensiOn so agitO a a misma temperatura durante 1 h y luego se filtrO. El filtrado se lavO con MTBE (2 x 300 ml) y so secó bajo vaclo a temperatura ambiente durante 4 dias para dar Ia sal trifluoroacetato del intermediario del titulo (163,3 g) como un polvo color amarillo pÆlido.
C. PreparaciOn de N-metil-N-[(S)-2-oxiran-2-ilmetillmetansutfonamida
Un recipiente de 12 L se cargo con agua (1 L) y a continuaciOn se agregO NaOH
(50 % en agua, 146,81 g, 1,835 mol). El vaso con NaOH se lavO con agua (2 x500 ml) y los lavados se agregaron al recipiente. La mezcla se agitó a temperatura ambiente durante 10 min y Se enfriO a 8 00. Se agregO (N-metil)metansulfonamida (200,2 g, 1,835 mol) an agua (500 ml) a lo largo de 5 mm. La mezcla so agitO durante I h a 4 00 y so agregO (S)-2-clorometiloxirano (339,6 g, 3,67 mol). La mezcla so agitO durante 20 h a 3-4

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° C. Se agregO diclorometano (2 L) y la mezcla se agitO durante 30 min a 5-10 °C. Las dos
fases se dejaron separar a Ia largo de 10 min y se recogieron. La fase orgÆnica (-2,5L) volvió a agregarse al recipiente de 12 L y so lavó con H3PO4 1 M (800 ml) y salmuera (800 ml). El diclorometano so eliminó par evaporaciOn rotativa. Al producto crudo, so agregó tolueno (400 ml) y se eliminO por evaporaciOn rotativa. Luego do tres ciclos adicionales del proceso con tolueno, se obtuvo el intermediarlo del titulo (228,2 g) que so utilizO sin purificación adicional en el próximo paso.
d. Sintesis do {(i S,3R,5R)-8-1(R)-2-hidroxi-3-(rnetansulfonimetiI-amino)proill-8-aza-biciclol3.2. 1 loct-3-il}amida de Æcido 1-isopropil-2-oxo-1,2-dihidroguinolin-3-carboxIlico En un recipiente de 3 L, so suspendió trifluoroacetato de {(1S,3R,5R)-8 azabiciclo[3.2. 1]oct-3-il}amida de ªcido 1 -isopropil-2-oxo-1,2-dihidroquinolin-3-carboxilico (105,0 g, 0,232 mol) en etanol absoluto (400 ml). A esta suspensiÆn,so agregO NaOH (50 % en agua, 0,243 mol. 1,05 eq) disuelta en etanol absoluto (100 ml) a temperatura ambiente. El vaso que contenia el NaOH se lavO con etanol (2 x 50 ml) i los lavados se agregaron a Ia mezcla de reacciÆn. DespuØsde 30 min de agitación, se agregó una solución de N-metil-N-[(S)-2-axiran-2-ilmetil]metansulfonamida (62,0 g, 1,5 eq) en etanol absoluto (100 ml). La mezcla so somotió a reflujo durante 2 h, so enfrió a temperatura ambiente y se agregaron ntcleos de cristal del compuesto del titulo. DespuØs de aproximadamente 5 min do agitaciOn se formO un sOlido blanco. La mezcla se enfrió a 3-5
° C y se agitó durante 2 h. El sOlido blanco so filtró y la almohadUla htimeda se lavO con
etanol absoluto frio (3 x 50 ml), El sólido so secó baja vacio a 30 °C durante 60 h para dar
el compuesto del titulo (93,8 g, contenido de agua segin el mØtodo do Karl Fischer 2,03
%). 1H NMR (CDCI3) 5 ppm 10,52 (d, IH), 8,83 (s, IH), 7,75 (d, 2H), 7,64-7,60 (m, 2H),
7,28-7,26 m, 1H), 4,33-4,26 (m, 2H), 3,78-3,75 (m, 1H), 3,27-3,20 (m, 4H), 3,01 (s, 3H),
2,88 (s, 3H), 2,58-2,53 (m, 1 H), 2,30-1,81 (m, 11 H), 1,68 (d, 6H).
Los ntcleos de cristal so obtuvieron de una preparaciôn previa del compuesto del

40 titulo segOn el mØtodo de este ejemplo en menor escala, en Ia que la cristalizaciOn ocurriO
de manera espontØnea.
Ejemplo 2: Sintesis de sal clorhidrato cristalina de {(IS,3R,5R)-8-[(R)-2-hidroxi-3-(metansulfonfl-metil-amino)propil]-8-aza-biciclo[3.2.ljoct-3-U}amida de Æcido 1-isopropil-2-oxo-1,2-dihidroquinolin-3-carboxilico
En un recipiente do IL, {(1 S,3R 5R)-8-[(R)-2-hidroxi-3-(metansulfonil-metil-ammno)propil]-8-aza-biciclo[3.2.1]oct-3-iI}amida de Æcido 1-isopropil-2-oxo-1,2-

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dihidroquinolin3-carboxilico (34,7 g, 0,069 mol) so suspendiO en etanol absoluto (210 ml). Se agregó HCI concentrado (1,1 eq) a temperatura ambiente con agitación. La mezcla so agitó a reflujo durante 30 min y se enfriÆ atemperatura ambiente y so agito durante 2 h. El sólklo se filtró y la almohadilla hümeda se lavO con etanol absoluto frio (3 x 50 ml). El sôlido se secô bajo vacio a 30 °C durante 48 h para dar el compuesto del titulo (34,5 g, 93.7 % de rendimierito, coritenido de agua segün el mØtodo do Karl Fischer 0,13 %). Ejemplo 3: Sintesis de hidrato cristalino de la sal clorhidrato de {(IS,3R,5R)-8-[(R)- 2-hidroxi3.(motansuIfonhl-metil-amIno)propilJ-8-aza-biCiClO[3.2.1]OCt-3-il}arflida de
ÆcidoI -isopropil-2-oxo-I,2.dihidroquinolin-3-carboxilico
Se dlsolviô clorhidrato de {(1 S,3R,5R)-8-[(R)2-hidroxi-3-(metansulfonhl-metil-
amino)propil]-8-aza-biciclo[3.2. 1]oct-3-iI}amida de ªcido 1 -isopropil-2-oxo-1 2-dihidroqumnolin-3-carboxulico (139 mg, 0,28 mmol) en agua esterilizada para inyección
(2 ml). Al cabo do unas horas, Ia soluciÆnse tornô una suspension turbia. La suspensiOn
. se agitO y se dejO en reposo durante la noche a temperatura ambiente resultando en nfl precipitado blanco. El sOlido so recogiO P01 filtracióri y se secO Wrante 2 min en
condiciones ambiente (aproximadamente 40-50 % de humedad relativa) para dar el
compuesto del titulo (130 mg, 91 % do rendimiento)
Ejemplos 4-9: Propiedades de las Formas Salinas de la lnvenciön
Se analizaron muestras de la sal clorhidrato cristalina do {(1 S,3R,5R)-8-[(R)-2-hidroxi-3-(metansulfonil-metil-amino)propil}-8-aza-biciclo[3.2. ljoct-3-il}amida de àcido 1-isopropil-2-oxo-1,2-dihidroqumnolin-3-carboxIlico (compuesto de formula I), preparado como en el Ejemplo 2 y del hidrato cristalino de la sal clorhidrato del compuesto de formula I, preparado como en of Ejemplo 3, por difracciOn de rayos x on polvo (PXRD), calorimetria de escaneo diferencial (DSC), anÆlisistermogravimØtrico (TGA), espectroscopia infrarroja (IR) y anlisis elemental.
Ejemplo 4 Difracción por rayos X de polvo
Se obtuvieron patrones de DifracciOn por rayos X de polvo con un DifractOmetro de Rayos X Thermo ARL. Modelo X’TRA (Thermo ARL SA, Suiza), usando radiaciÆn de Cu Ka a 1,542 A (45 kV, 40 mA) con un detector Si(Li) de estado sólido. El anOlisis se efectuO tipicamente a una velocidad de anÆlisis de20/minuto, con un tamaæo depaso de 0,030 P01 punto en un rango de entre 2 y 3011 en dos Ængulostheta. Las muestras, tat como se las recibió o motidas en un polvo fino, envasaron cuidadosamente en un inserto comOn de pequeæovolumen, diseæado a medida para que se ajuste en el recipiente de

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carga de muestra superior del instrumento, para efectuar el anlisis, Se verificó semanalmente la calibración del instrumento hasta dentro do –0,020 del Ængulodos-theta por comparaciOn con un estàndar de un metal siliconado. Los patrones de PXRD representativos para las muestras de la sal clorhidrato cristalina y del hidrato de la sal clorhidrato de la irivenciOri se muestran en las Figuras 1 y 4, respectivamente.
Ejemplo 5 Anâlisis tØrmico
La calorimetrIa diferencial por barrido (DSC) se efectuó usando el módulo de TA Instruments Modelo Q-100. Los datos se recolectarori y analizaron usando el software de TA Instruments Thermal Advantage para Q SeriesTM. Se pesÆ conprecisiOn una muestra de aproximadamente 1-10 mg en una plancha de aluminlo con tapa. La muestra so evaluO usando una rampa de calentamierito lineal do 10 °C/min desde 5 °C hasta, tipicamente, 265 °C. La celda de DSC se purgO con nitrOgeno seco durarite el uso. Los grÆficosde DSC representativos para las muestras de la sal clorhidrato cristalina y de la sal clorhidrato cristalina hidrato del compuesto I se muestran en las Figuras 2 y 5, respectivamente.
El anÆlisis termogravimØtrico (TGA) so efectuO usando un mOdulo de TA Instruments Modelo 0.500. Los datos se recolectaron y analizaron usando el software de TA Instruments Thermal Advantage para Q Series T11. Se colocO una muestra que pesaba 1-5 mg aproximadamente en una bandeja de atuminio sobre un soporte de platirio y se barriO desde temperatura ambiente hasta 300 °C con una velocidad do calentamiento lineal de 10 ’C/min. Las cÆmaras de equilibrio y horno fueron purgadas con flujos de nitrOgeno durarite el uso. Las Figuras 2 y 5, respectivamente, representan un trazado TGA representativo para muestras de una sal clorhidrato cristalina y de una sal clorhidrato cristalina hidrato de compuesto I.
El trazado de DSC en la Figura 2 demuestra quo la sal clorhidrato de la presente invenciOn tiene una excelente estabilidad tØrmica con un mÆximo en el flujo de calor endotØrmico en el rango entre aproximadamente 230 ’C y aproximadamente 260"C y sin eventos tØrmicos significativos debajo de los 225 °C aproximadamente. La comparaciOn do los trazados de DSC y TGA indica que la sal clorhidrato de la presente invenciOni sufre simultØnemente fusiOn y descomposiciOri a temperaturas superiores a los 230 C aproximadamente.
El trazado de DSC en la Figura 5 para la forma hidrato de la presente exhibe un
pico sustaricial en el flujo de calor endotØrmico identificado por fusiOn del cristal en el

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rango entre aproximadamente 225 C y aproximadamente 250 °C y endotermias generates o dØbiles a temperaturas mÆs bajas. LacomparaciOn de los trazados do DSC y TGA indican que la descomposiciÆnde la forma cristalina hidrato no es significativa a la temperature de transición de fusiOn.
Ejemplo 6 EvaIuacôn de la absorciôn dinÆmicade humedad
La evaluación de absorciOn dinmica de la humedad (DMS) se efectuO a 25 C usando una microbalanza atmosfØrica VII, SCA-100 System (VTI Corp., Hialeah, FL 33016), Se usO un tamaæo de muestra de aproximadamente 5-10 mg y a humedad se
dofinió en el valor ambiente at comenzar et anªlisis. Un anØlisis tipico do DMS consistió
en tres barridos: humedad retativa (HR) del ambiente hasta 2%, entre 2% de HR y 90% do
HR, entre 90% de HR y 5% de HR a una velocidad de anÆlisis de 5% do HR/paso. La
masa se midió cada dos minutos y la RH se cambió por el valor siguiente (– 5% RH)
cuando la masa de la muestra era estable hasta dentro de 002% para 5 puntos
. consecutivos. Los grÆficos de DMS representativos para las muestras de una sal clorhidrato cristalina y de una sal clorhidrato cristalina hidrato del compuesto I se muestran
en las Figuras 3 y 6, respectivamerite.
La sal clorhidrato exhibe un perfil de absorciOn/desorciOn reversible con un cambio
de peso menor que un 0,2% sobre todo el rango de 2% a 90% de RH. La forma hidrato
exhibiO un perfil do absorciOn/desorciOn reversible con aproximadamente un 2,3% de
pOrdida de peso a medida quo la muestra so secaba desde el valor ambiente hasta 2% de RH que so volviÆa aumentar cuando la muestra era expuesta de 2% de RH a 40% de RH, La forma hidrato tenia menos que aproximadamente 0,25% do aumento de peso en el rango de 40 %RH a 75% RH,
Ejemplo 7 AnÆlisis infrarrojo
So determinO el espectro de absorciOn infrarroja (IR) para el rango do frecuencia do entre 4000 y 675 cm-’ usando un espectrOmetro Avatar 360 FT-R, equipado con un dispositivo de contenciOn de muestras Nicolet con reflexiOn total atenuada (ATR). El espectro de absorciOn IR representativo para una muestra de a sal clorhidrato cristalina de Ia invención tenia bandas de absorciOn significativas en 758–1, 783–1, 795–1, 802–1, 949–1, 981–1, 1149–1, 1158–1, 1217–1, 1332–1, 1377–1, 1453–1, 1467–1, 1467–1, 1525–1, 1566–1,1575–1,1615–1,1672–1 y3197–1 cm-’,

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Ejemplo 8 Evaluación de la estabilidad en estado sólido
Las muestras de la sal clorhidrato de la invenciOn so guardaron en multiples viales
do vidrio abiertos a 40 °C y 75% de RH. A intervalos especificos, se retiraba 01 contenido do un vial represeritativo y se lo analizaba por DSC, TGA, PXRD y por HPLC para determinar su pureza quimica. DespuØs de 24 semanas do almacenamiento, no hab[a cambios detectables en los termogramas de D$C o TGA ni en el patrOn do PXRD. La pureza quimica de la muestra guardada era del 99,6%.
Ejemplo 9 Determinaciôn de la relación molar de los contralones
La relación molar do contraiones para Æcido clorhidrico (HA) con respecto a

{(1 S,3R,5R)8-[(R)-2-hidroxi.3-(metansuIfonimetiamino)propil]-8-aza-biciclo[3,2, 1]oct3-
il}amida del Æcido 1-isopropil-2-oxo-1,2-dihidroquinolin-3-carboxilico (compuesto de
fOrmula I) se calculó de acuerdo con la siguiente fOrmula:
RelaciOn de contralones (WHA/MWHA) / (W1/MW1)
. donde WHA es el peso porcentual do HCI en la muestra, MWkjA es el peso molecular de HCI, MW el el peso molecular del compuesto do fOrmula I (504,6 amu) y W es el peso
porcentual del compuesto do fOrmula I en la muestra, calculado do acuerdo con la fOrmula:
WI = 100WHXWH20--WR8

donde WH20 es el peso porcentual del contenido do agua, WRS es el peso porcentual del
solvente residual, suponiendo que el compuesto I no tieno impurezas.
La relaciOn molar del Æcido clorhidrico con respecto al compuesto I para una muestra do una sal clorhidrato cristalina de la invenciOn se calculO como do 0,94:1 usando el peso porcentual de HCI (WHA) de 6,3% y los valores do WH20 0,26 % y WRS = 0,47%. El contenido do HCI se determinO por titulaciOn con una soluciÆn estÆndarde nitrato do plata, el contenido de agua WH20 so doterminO por titulación coulomØtrica do Karl Fisher y el contenido de solvente residual WRS so determinó por cromatografla gaseosa.
Ejemplo Comparativo 1: Sintesis de sal de Æcido citrico de {(IS,3R,5R)-8-[(R)-2-hidroxi-3-(metansulfonil-metil.amino)propil]-8-aza-biciclo[3.2.1]oct-3-il}amida de Æcido1-isopropil-2-oxo-1,2-dihidroquinolin-3-carboxIlico
Se suspendiO {(1 S,3R,5R)-8[(R)-2-hidroxi-3-(motansulfonft-metil-amino)propil]-8-aza-biciclo[3.2.l]oct-3-il}amida do äcido 1 -isopropil-2-oxo1,2-dihidroquinolin-3-carboxilico (0,1 g, 0,2 mmol) en etanol (1 ml). A esta suspensiOn se agregO una soluciOn IM do Æcido citrico en etanol (0,072 ml, 0,072 mmol, 0,33 eq). La mezcla so sonicO brevemente hasta resultar transparente, so tapO, y luego so dejO en reposo durante la nocho. La tapa luego

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se quito y la mezcla se dejó evaporar bajo condiciones ambiente hasta observarse sOlidos. La mezcla luego volvió a taparse y se dejO en reposo durante 72 h. El sólido resultante so filtró y se lavO con etanol frio pam dar el compuesto del titulo como un sOUdo (74,3 mg).
Ejemplo Comparativo 2: SIntesis de sales Æcidas de{(1S,3R,5R)-8-((R)-2-hidroxi-3-(metansuIfon1l-metil-amino)prop1l]-8-aza-bRiCIO[3.2.1]oct-3-iI}amida de Æcido 1-isopropil-2-oxo-1,2-dihidroquinolin-3-carboxIlico
Siguiendo el procedimiento del Ejemplo Comparativo 1, se prepararon las siguientes sales Æcidas de {(1 S,3R,5R)-8-[(R)-2-hidroxi-3-(metansulfonimetil-amino)propil]-8-aza-bicicIo[3.2.1]oct-3il}amida de Æcido 1-isopropil-2-oxo1,2-dihidroquinolin3-carboxilico en forma sOlida utilizando los equivalentes indicados de Æcido (peso del producto en segundo parØntesis): adipico (0,5 eq) (48,5 mg); fosfOrico (0,5 eq) (86,6 mg); sulfOrico (0,5 eq) (27,0 mg); tartØrico (0,5 eq) (66,3 mg); mÆlico (0,5 eq) (25,3 . mg): y bromhIdrico (1 eq) (62,9 mg). Ejemplo Comparativo 3: Sintesis do sal de ªcido metansulfônico de {(IS,3R,5R)-8-[(R)2-hidroxi-3-(motansuIfoniI.metiI-amino)propiI]-8-aza-biCiCIO(3.2.1]Oct-3-iI}amida do ªcido 1 -isopropil-2-oxo-1,2-dihidroquinolin-3-carboxilico
A una soluciÆnde ((1 S,3R,5R)-8-[(R)-2-hidroxi-3-(metansulfonil-metil-amino)propil]- 8-azabiciclo[3.2. 1]oct-3-il}amida de 6cido 1 -isopropil2-oxo-1,2-dihidroquinolin-3-carboxilico (0,1 g, 0,2 mmol) en acetoriitrilo/agua 50 % (1 ml) se agregO una solución 1M de äcido metansulfónico en etanol (0,2 ml, 0,2 mmol, 1 eq). La mezcla luego se congelÆy se liofilizO a sequedad durante la noche. El sólido resultante so disolvió en isopropanol (1 ml) con calentamiento suave y so deja enfriar. El sólido resultante se recogiO por filtración y se lavô con isopropanol frio pam dar el compuesto del tItulo como un sólido (90 mg). Ejemplo Comparativo 4: Sintesis do sales âcidas do {(IS,3R,5R)-8-[(R)-2-hidroxi-3-(metansuIfonil-metil-amino)propiI]-8-aza-biciclo3.2.1]oct-3-iI}amida do Æcido 1-isopropil-2-oxo-1,2-dihidroquinolin-3-carboxilico
Siguienido el procedimiento del Ejemplo Comparativo 3, se prepararon las siguientes sales ªcidas de {(1 S,3R,5R)8[(R)-2-hidroxi-3-(metansulfonimetil-amino)propil]-8-aza-biciclo[3.2. 1]oct-3-il}amida de Æcido 1 -isopropil-2-oxo-1 2-dihidroquinolin-3-carboxIlico en forma sOlida utilizando los equivalentes indicados do ªcido (peso del producto en segundo parØntesis): fumÆnico (1 eq) (107,2 mg); benzoico (1 eq) (105,2 mg); y (R)-mandØlico (1 eq) (96,1 mg).

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Ejemplo comparativo 5: Propiedades de los compuestos de los ejemplos comparativos 14.
Las sates cristalirias do ªcidos do los compuesto do formula I de los Ejemplos comparativos 1-4 fueron analizados par PXRD, DSC y TGA. En todos los casos, se observO una pØrdida do peso par TGA a temperaturas inferiores a los 100 °C aproximadamente, muy probablemente debido a la pØrdida do solverite. La o las temperaturas a las cuales so observó flujo de calor endotØrmico par DSC, excepto las caracteristicas de temperatura baja atribuidas a la pØrdida do solvente, junta con la confirmaciOn do la cristaliriidad par PXRD se resumen en la siguiente Tabla I, donde la estoquiometra estØ indicada par el nümero do equivalentes Æcido usados parapreparar la

sat Æcida.

Tabla I: Comparación de sales
Acido Equivalentes
de Æcido

Endotermia DSC PXRD

CItrico 033 -125 °C, -160-180
OC

cristalino

Adipico 0,5 -150 °C cristalino
FosfOrico 0,5 -215-220°C cristalino
Sulfürico 0,5 -150 °C, -190-205
° C, -240 °C

cristalino

TartØrico 0,5 -140 °C, -185 °C cristalino
Mälico 0,5 -110 °C, -’185 °C cristalino
Bromhfdrico 1 -155 °C, -210°C cristalino
MetansuifOnico 1 -175 °C, -235 °C" cristalino
FumØrico 1 -125°C cristalino
Benzoico 1 <150 °C cristalino
(R)-miØtico 1 -120 °C cristalino
* mOttiples polimorfos, so observO exotormia
comienzo do fusiOn y descomposiciOn
Ensayo 1: Ensayo de union a radioligando en receptores 5-HT4() humanos
a. PreparaciOn de membranas con 5-HT4(C)
Se cultivaron cØlutas HEK-293 (riæOnembrionarlo humano) transfectadas en forma estable con ADNc del receptor 5-HT4> humano (Bmax = aproximadamente 6,0 pmol/mg do proteina, determinado usando el ensayo de uniOn a radioligando en membrana con [3H]-GR1 13808) en frascos T-225 con media do Eagle modificado par Dulbecco (DMEM), quo contenia 4500 mg/I de D-glucosa y clorhidrato do piridoxiria (GIBCO-lnvitrogen Corp., Carlsbad CA: N° Cat 11965) suplemeritado can 10% de suero fetal bovino (FBS) (GIBCO-

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Invitrogen Corp.: NO Cat 10437), L-glutamina 2 mM y (100 unidades) penicilina (100 pg) estreptomicina/mI (GIBCO-lnvitrogen Corp.: NO Cat 15140) en una incubadora hUmeda con 5% de CO2 a 37°C. Se cultivaron las cØlus bajo una presión do selección continua agregando 800 l.JgImI de geneticina (GIBCO-lnvitrogen Corp.: NO Cat 10131) al medlo. So cultivaron las cØlulas hasta atcanzar aproximadamente 60-80% de confluencia
(< 35 de pasajes de subcultivo). 20-22 horas antes de la cosecha, so lavaron las cØlulas dos veces y so les agregÆ DMEM libre do suero. Todos los pasos do fabricación se realizaron en hielo. Se levant6 la monocapa de cØlulas por agitacion mocªnica y so tltulÆ con una pipeta de 25 ml. Se recolectaron las cØlulas por centrifugaciÆna 1000 rpm (5 minutes).
Para la preparación de membrana, so suspendieron los precipitados celulares en Øcido 4-(2-hidroxietil)-1-piperazinetansulf6nico 50 mM (HEPES), pH 7,4 (amortiguador de preparaciOn de membrana) (40 ml/rendimiento celular total de 30-40 frascos T225) y se homogenizó usando un destructor do tejidos politron (con parÆmetros de 19, 2 x 10 sogundos) en hielo. Los homogenates resultantes so centrifugaron a 1200 0 per 5 minutes a 4°C, Se descartO el precipitado y se centrifugó el sobrenadante a 40000 G (20 minutos). So lavO al precipitado una vez resuspendiØndo!o con amortiguador do preparaciOn de membranas y centrifugÆndolo a 40000 G (20 minutos). Se suspendiO nuevamente el precipitado final en HEPES 50 mM, pH 7,4 (amortiguador do ensayo) (equivalerite a I frasco T225 /1 ml). Se determinO la concentraciOn de protoinas en la suspension do membrana empleando el mØtodo de Bradford (Bradford, 1976). Se almacenaron las membranas en alicuotas a -80°C.
b. Ensayos de union a radioligandos
So realizaron ensayos do union a radioligandos en placas do ensayo do polipropileno do 96 cavidades profundas de 1,1 ml (Axygen), en un volumen de ensayo total do 400 p1, que contenian 2 pg do proteinas de membrana en HEPES 50 mM, pH 7,4, con 0,025% de albUmina do suero bovino (BSA). Se realizaron los estudios de saturaciOn para determinar los valores de Kd del radioligando usando (3H]-GR1 13808 (Ame(sham Inc., Bucks, Reino Unido: NO Cat TRK944; actividad especifica do aproximadamente 82 Ci/mmol) a 8-12 conceritraciones diferentes, que variaron entre 0,001 nM y 5,0 eM. Se realizaron los ensayos de desplazamiento para determinar los valores de pKj de los compuestos con [3H]-GR113808 0,15 nM y once concentraciones diferentes del compuesto, quo variaron entre 10 pM y -100 pM.

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Los compuestos de prueba se recibieron coma soluciones madre 10 mM en DMSO, y se los diiuyó haste 4001M en HEPES 50 mM, pH 7,4, a 25°C, quo contenia 0,1% de BSA, y luego se reahzaron diluciones senates (1:5) en el mismo amortiguador. Se determinO la union no especifica en presencia de GR113808 1 iM sin marcar. Se incubaron los ensayos por 60 minutos a temperatura ambiente, y luego so terminaron as reacciones de uniOn realizando una filtraciOn rÆpida en placas de fibre do vidrio do 96 cavidades GFIB (Packard BioScience Co., Meriden, CT), empapadas previamente on 0,3% de polietilenimina. So lavaron las placas do filtro ties veces con amartiguador do
filtracjsn (HEPES 50 mM helado, pH 7,4) para eliminar la radiactividad no unida, Se
secarori las placas, so agregaron 35 p1 de fluido de centelleo liquido Microscint-20
(Packard BioScience Co., Meriden, CT) a cede cavidad y so reaiizO el conteo do las
places en un contador de centelleo liquido Packard Topcount (Packard BioScience Co.,
Meriden, CT).
. Los datos do uniOn fueron analizados mediante un antisis do regresiOn no lineal con el paquote do software GraphPad Prism (GraphPad Software, inc., Sari Diego, CA)
usando el modelo do 3 perªmetros para competencia de un sitio. Se ajustO el BOTTOM
(minimo de la curve) do acuerdo con el valor de la uniOn no especifica, detorminado en
presencia de GR1 13808 1 iM. Se calcularon los valores do K pare los compuestos do
prueba, en Prism, para los valores de IC50 do mayor ajuste, y el valor do Kd del
radioligando, usando la ecuaciOn do Cheng-Prusoff (Cheng y Prusoff, Biochemical Pharmacology, 1973, 22, 3099-108): K = lC0f(1 + [L}/Kd), donde [L] = concentraciOn
(’HI-GR113808. Se expresan los resultados como el logaritmo decimal negativo de los
valores de K, pK,
Los compuestos de prueba con mayores valores do pKi en este ensayo presentan
una mayor afinidad do uniOn per el receptor 5-HI4. El compuesto de fOrmula 1 tenia un
valor de pKj mayor que aproximadamente 7,5 en este ensayo. SEnsayo 2: Ensayo do union a radioligando en receptores 5-HTA humanos: determinación do la solectividad por subtipos del receptor
a. PreparaciOn do membranes con 5.HT3A
Se obtuvieron cØlulas HEK-293 (riæOn ombrionario humano)transfectadas en forma
estable con ADNC del receptor 5-HT-3A humano del Dr. Michael Bruess (Universidad do
Bonn, RDA) (Bmax = aproximadamente 9,0 pmotfmg de proteiria, determinado usando el
ensayo do uniOn a radioligando en membrane con rHJ-GR65630) en frascos T225 o

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fÆbricas de cØlulas con medio de Eagle modificado por Dulbecco (DMEM) al 50% (GIBCO-lrivitrogen 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 Nra SH30070,03) y (50 unidades) penicilina (50 pg) estreptomicina/mi (GIBCO-lnvitrogen Corp.: N° Cat 15140) en una incubadora hümeda con 5% de CO2 a 37°C.
Se cultivaron las cØlulas hasta alcanzar aproximadamente 70-80% de confluencia
(< 35 de pasajes de subcultivo). Todos los pasos de fabricación se realizaron en hielo. Para cosechar las cØlulas, se aspiraron los medios y se lavaran las cØlulas con soluciOri sauna amortiguada con fosfato de Dulbecco, libre de Ca24

y Mg2 (dPBS). Se levantó la
monocapa de cØlulas aplicando una agitaciÆn mecÆnicasuave. Se recolectaron las cØlulas por centrifugaciOn a 1000 rpm (5 minutos). Los pasos subsiguientes de la preparación de membrana se realizaron de acuerdo con el protocolo descripto previamente para las membranas que expresaban receptores 5-HT4(1,).
b. Ensayas de union a radioligandos
Se realizaron ensayos do uniOn a radioligandos en placas de ensayo de polipropileno do 96 cavidades, en un volumen de ensayo total de 200 ii, quo contenian 1,5-2 ig do proteinas de membrana en HEPES 50 mM, pH 7,4, con 0,025% de amortiguador de ensayo do BSA. Se realizaron los estudios de saturaciOn para determinar los valores do K del radioligando usando rH)-GR65630 (Perkin Elmer Life Sciences Inc., Boston, MA: N° Cat NET1011, actividad especIfica do aproximadamente 85 Ci/mmol) a doce concentraciones diferentes, que variaron entre 0,005 nM y 20 nM. Se realizaron los ensayos de dosplazamiento para determinar los valores de pKi do los compuestos con [3H-GR65630 0,50 nM y once concentraciones diferentes del compuesto, que variaron entre 10 pM y 100 IN. Los campuestos se recibieron como soluciones madre 10 mM en DM80 (vØase la sección 3,1), y se los diluyo hasta 400 IM en HEPES 50 mM, pH 7,4, a 25°C, que contenIa 0,1% de BSA, y luego so realizaron diluciones seriales (1:5) en el mismo amortiguador. Se determinó la union no especifica on presencia do MDL72222 10 iJM sin marcar. Se incubaron los ensayos por 60 minutos a temperatura ambiente, y luego se terminaron las reacciones de uniOn realizando una filtración rØpida en placas de fibra de vjdrio do 96 cavidades GFIB (Packard BioScience Co., Meriden, CT), empapadas previamente en 0,3% de polietilenimina. Se lavarori las placas do filtro tres veces con amortiguador do filtracisn (HEPES 50 mM helado, pH 7,4) para eliminar Ia radiactividad

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no unida. Se secaron las placas, se agregaron 35 II de fluido de centelleo liquido Microscint-20 (Packard BioScience Co., Meriden, CT) a cada cavidad y se realizó el conteo de las placas on un contador de centelleo liquido Packard Topcount (Packard BioScience Co., Meriden, CT).
So analizaron los datos de uniOn empleando el procedimlento de regresiOn no lineal descripto previamente para deterrninar valores de K. Se ajustO el BOTTOM (minimo do la curva) de acuerdo con el valor de a union no especifica, determinado en presencia de MDL72222 10 iJM. Se definiO la cantidad [LI en la ecuación de Cheng-Prusoff como la
concentraciOn de [3H]-GR65630.
Se calculó la selectividad de subtipos del receptor 5-HT4 per subtipos del receptor
5-HT3 como la relaciOn K(5-HT3A)/K(5-HT4(C)). El compuesto de formula I presentaba una
selectividad par el subtipo del receptor 5-HT4/5-HT3 mayor que aproximadamente 1000 en
este ensayo.
, Ensayo 3: Ensayo de acumulación de AMPc en cØlulas completas en places instantÆnoas,con cØlulas HEK-293 quo expresan receptores 5-HT4()
En este ensayo so determinó la potencia funcional de un compuesto de prueba
midiendo la cantidad de AMP cIclico producido cuando se pusieron en contacto cØlulas
HEK.293, quo expresaban receptores 5-HT4, con distintas concentraciones de compuesto
de prueba.
a. Cultivo de las cØlulas

Se prepararon cØlulas HEK-293 (riæOn embrionario humario) transfectadas en forma estable con ADNc clonado del receptor 5HT4(;) humano, que expresaban el receptor a dos derisidades diferentes: (1) una densidad de aproximadamente 0,5-0,6 pmol/mg de proteina, determinada usando un ensayo do union a radioligando [3H-GR113808 en membrane, y (2) una densidad de aproximadamente 6,0 pmol/mg de proteina. Se cultivaron las cØlulas en frascos T-225 con medlo de Eagle modificado per Dulbecco (DMEM), que contenia 4500 mg/I do D-glucosa y clorhidrato de piridoxina (GIBCO-lnvitrogen Corp.. Carlsbad CA: N° Cat 11965) suplementado con 10% de suero fetal bovino (FBS) (GIBCO-lnvitrogen Corp.: N° Cat 10437), L-glutamina 2 mM y (100 unidades) penicilina (100 pg) estreptomicinalml (GIBCO-Invitrogen Corp.: NO Cat 15140) en una incubadora hOmeda con 5% de CO2 a 37°C. Se cultivarori las cØlulas bajo una presiOn de selecciOn continua agregando geneticina (800 pg/mI: GIBCO-lnvitrogen Corp.: NO Cat 10131) al media.

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b. Preparación de las cØlulas
So cultivaron las cØlulas hasta alcanzar aproximadamente 60-80% do confluencia. Entre veinte y veintidOs horas antes del ensayo, so lavaron las cOlulas dos veces y se les agregÆ DMEM libre de suero, quo contenia 4500 mg/1 de D-glucosa (GIBCO-Invitrogen Corp.: NO Cat 11965). Para cosechar las cOlulas, se aspiraron los medics y se agregaron 10 ml de Versene (GIBCO-lnvitrogen Corp.: NO cat. 15040) a cada frasco T-225. Las cØlulas se incubaron durante 5 min a RT y luego so desprendieron del frasco par agitación mecÆnica. Se transfirió la suspension celulr a un tubo do centrifuga quo contenia un
volumen igual de dPBS tibia (370C), y se centrifugo par 5 minutos a 1000 rpm. Se
descartÆ el sobrenadante y se suspendiO nuevamente el precipitado en amortiguador do
estimulaciOn entibiado con anterioridad (37°C) (equivalente a 10 ml par cada 2-3 frascos
T-225). Se registrO este momenta y so lo anotó coma cero. Se coritaron las cØlulas con un
contador Coulter (conteo superior a 8 pm, el rendimiento por frasco fue de 1-2 x iOT

. cØlulas/frasco). Se suspendieron nuevamente las cØlulas a una concentración do 5 x 105

cØlulas/mI, en amortiguador do estimulación ontibiado con anterioridad (37°C) (tal coma so
obtiene en el conjunto de elementos de placas instantªrieas), y se preincubO a 37°C par 10
minutos.

Se realizaron ensayos do AMPc on tin formato de radioinmunoensayo, usO el
sistema de ensayo de activaciOn do adenilil ciclasa en placas instantØneas con 1251-AMPc
(SMP004B, Perkin Elmer Life Sciences Inc., Boston, MA), do acuerdo con las instrucciones del fabricante.

Las cØlulas se cultivaron y so las preparO coma Se describió previamente. Las concentraciones finales do las cØlulas en el ensayo fueron de 25 x 103 cOlulas/cavidad, y el volumen do ensayo final fuo do 100 ii. Los compuestos do pruoba se recibieron coma soluciones madre 10 mM en DMSO, y so los diluyO hasta 400 1M en HEPES 50 mM, pH 7,4, a 25°C, que contenia 0,1% de BSA, y luego so realizaron diluciones seriales (1:5) en 01 mismo amortiguador. Se realizaron ensayos do acumulaciOn de AMP ciclico con 11 concentraciones diferentes do compuesto, que variaron entre 10 pM y 100 iM (concentraciones finales do ensayo). Se incluyO una curva do coricentración do 5-HT-respuesta (entre 10 pM y 100 IM) en cada placa. Se iricubaron las cØlulas, con agitaciOn, a 37°C par 15 minutos, y se terminO la reacciÆn agregando 100 l.jl do amortiguador de detecciOn helado (coma so obtiene en el conjunto de elementos de placas instantÆneas)a cada cavidad. Se sellaron las placas y se las incubô a 4°C durante la noche. Se cuantificó

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la radiactividad unida por espectroscopia do proximidad do centelleo usando el dispositivo Topcount (Packard BioScience Co., Meriden, CT).
Se extrapolo la cantidad do AMPc producido por ml do reacciOn a partir de la curva
de referencia do AMPc, do acuerdo con las instrucciones proporcionadas en el manual de usuarlo del fabricante. So analizaron los datos realizarido anÆlisis de regresiOn no lineal con el conjurito de software GraphPad Prism, usarido el modelo do respuesta a Ia dosis sigmoidal do 3 parÆmetros(pendiente restririgida a la unidad). So informan los datos do potencia como valores de pEC50, el logaritmo decimal negativo del valor do EC), donde
EC5o es la coricentraciOn efectiva para obtener un 50% do respuesta maxima.
Los compuestos do prueba con un mayor valor do pEC50 en este ensayo tienen una
potencia de agonismo mayor sobre el receptor 5-HT4. El compuesto de formula I evaluado
en este este ensayo en la linea celular (1) con una densidad de aproximadamente
05-0,6 pmollmg de proteina, tenia un valor pEC.5o mayor que aproximadamente 7,5.
. Ensayo 4: Ensayo do pinza do tensiOn en vivo do la inhibición do Ia corriente de iones do potasio en cØlulas completas que expresan el canal do potasio cardiaco hERG
Se obtuvieron cØlulas CHO-K1 transformadas en forma estable con ADNc de hERG
do Gail Robertson, en Ia Llniversidad de Wisconsin. Se mantuvieron las cØlulas en
almaceriamiento criogOnico hasta utilizarlas. Se expyieron las cØlulas y so las sometiO a un
pasaje en medlo Eagle modificado por Dulbecco/F 12, suplementado con 10% de suero
fetal bovino y 200 ig/ml do geneticina. So sembraron las cØlulas en cubreobjetos de vidrio recubiertos con poll-D-lisina (100 pg/mI), en placas de 35 mm2 (que contenian 2 ml de medio), a una densidad que permitió seleccionar las cØlulas aisladas para realizar estudios de pinza de tensiOn. So mantuvieron las placas en un ambiente humidificado con 5% de CO2 a 37°C.

Se preparO solución extracelular al menos cada 7 dias y so la almacenO a 4°C miontras no so la utilizaba. La solución extracelular contenIa (mM): NaCl (137), KCI (4), CaCl2 (1,8), MgCl2 (1), glucosa (10), àcido 4-(2-hidroxietil)-1-piporazinetansulf6nico (HEPES) (10), pH 7,4, con NaOH. La soluciOn extracelular, en ausencia o presencia de compuesto do prueba, so colocO en recipientes do reserva, desde los quo so la hizo fluir hacia la camara do registro a aproximadamente 0,5 mI/minuto. Se preparO la soluciOn intracelular, se la separó en alicuotas y so la almaconO a -20°C hasta el die en quo se la usO. La soluciOn intracelular contenia (mM): KCI (130), M9CL (1), sal de Æcido etilenglicol-bis(beta-aminoetil Øter) N,N,N’,Ntetra acØtico (EGTA) (5), MgATP (5), Æcido 4-(2-

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hidroxiet)-1-piperazinetansulf6mco (HEPES) (10), pH 7,2, con KOH. Todos los experimontos se realizaron a temperatura ambiente (20-221C).
So transfiiieron los cubreobjetos donde se habian sembrado las cØlulas a una cØmara de registro, y se los perfuridió en forma continua. Se formaron sellos Gigaohm entre las cØlulas y los electrodas do patch. Una voz obtenido un patch estable, se comenzÆ el registrO en el modo de pinza de tensiÆn,con un potencial do retención inicial de -80 mV. DespuØs de obtener una corriente de cØlulas complotas estable, so expusioron las cØlulas al compuesto de prueba. El protocolo do pinza do tension fue: un paso con un potencial de retenciOn de entre -80 mV y +20 mV por 4,8 segundos, repolarización a -50 mV par 5 segundos y luego retorno al potencial de retenciOn original (-80 mV). Se puso en prÆctica este protocolo do tensiOn cada 15 segundos (0,067 Hz). So determinaron las amplitudes de las corrientes pico durante la fase de repolarizaciön usando el software pClamp. Se perfundieron los compuestos de prueba, a una concentración do 3 pM, sobre las cØlulas durante 5 minutos, a lo que siguio un periodo do lavado do 5 minutos en ausencia do compuesto. Finalmente, se agregó un control positivo (cisapride 20 nM) a Ia porciOn perfuncilda para determinar la función de las cØlulas. El paso de camblo entre -80 mV y +20 mV activa el canal hERG, (a quo da coma resultado una corriente hacia el exterior. El paso do retorno a -50 mV da coma resultado una corriente hacia el exterior, a medida que el canal so recupera de la inactivaciOn y so desactiva.
So determinaron las amplitudes de las corrientes pica durante la fase do repolarizaciOn usando el software pCLAMP. So exportaron los datos do los articulos control y do prueba a Originfi (OriginLab Corp., Northampton MA), donde se normalizaron a amplitudes do las corrientes individuales respecto do (a amplitud do (a corriente inicial en ausencia do compuesto. So calcularon los promedios y los errores estOndar para cada condiciOn, y so los representO versus el transcurso del tiempo on el oxperimento.
So realizaron comparacionos entre las inhibiciones observadas de la corriente de
K despuØs do una exposiciOn de cinco minutos al art(culo de prueba a al vohiculo control (usualmente DM80 al 0,3%). Se realizaron comparacianos estadisticas entre grupos experimantales usando una prueba de t indopendierite para dos poblaciones (Microcal Origin v. 6,0). Las diferencias so consideraron significativas para p < 0,05.
A menor porcentaje de inhibiciOn de la corriente iOnica on esto ensayo, monor es el potencial de los compuestos do prueba do cambiar el patrÆn dorepolarizaciOn cardiaca cuando so los usa como agentes terapØuticos. El compuesto do fOrmula I se evaluó en

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este ensayo a una concentraciôn de 3 tM y presentaba una inhibición do la corriente del ion de potasio menor qua un 15% aproximadamente.
Ensayo 5: Modelo de biodisponibilidad oral on vitro: ensayo de permeabilidad

Caco-2

Se realizó el ensayo de permeabilidad Caco-2 para modelar la capacidad do los compuestos do prueba de pasar a travØs del intestino y alcanzar el torrento sanguineo despuOs de una administración oral. Se determinó la volocidad a la qua los compuestos de prueba an solución atravesaban una monocapa celular diseæada para imitar la uniOn estrecha entre las monocapas del intestino humano.
Se obtuvieron cØlulas Caco-2 (adenocarcinoma de colon humano) on la ATCC (ColocciOn Americana do Tipos de Cultivo; Rockville, MD). Para el estudlo de permeabilidad, se sembraron las cØlulas a una densidad de 63000 c6lulas/cm2 an filtros de policarbonato con trans-cavidades humedecidas previamente (Costar; Cambridge, MA). So formO una monocapa do cØlulas despuOs do 21 dias do cultivo. DespuØs do realizar el cultivo de cOlulas an la placa con trans-cavidades, se retirO Ia membrana quo contonia la monocapa celular de la placa con trans-cavidades y se la insertó an la cØmara de difusión (Costar; Cambridge, MA). La càmara do difusión so insertO an el bloque de calentamiento qua estaba equipado con agua circulanto externa, regulada con termostato a 37 ’C para el control de la temperatura. El colector de aire administraba 95% do 02/5% de CO2 a carla mitad do una cÆmara de difusión, y creaba un patron de flujo laminar a travØs do la monocapa celular, lo quo resultó ofectivo para reducir la capa limitrofe indeseada.

Se realizó el ostudlo de permeabilidad con concentraciones de compuesto de prueba de 100 iiM, y con 14C-manitol para monitorear la integridad de la monocapa. Todos los experimentos se condujeron a 37 °C durante 60 mm. Se tomaron muestras a los 0, 30 y 60 min de ambos lados donante y receptor de Ia cÆmara. Se analizaron las muestras por HPLC o conteo do centelleo liquido para determinar las concentraciones de compuesto y manitol. Se calculO el coeficiente do permeabilidad (Kr)) en cm/segundo.
En este ensayo, so considera qua un valor de K mayor qua aproximadamente 10 x
10’ cm/segundo indica una biodisponibilidad favorable. El compuesto do fOrmula I tue evaluado an este ensayo y exhibia un roIor do Kp mayor que aproximadamente 20 x 10 cm/seg.

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Ensayo 6: Estudlo farmacocinØtico en ratas
Se prepararon formulaciones en soluciÆnacuosa de los compuestos de pruebas en
cido lÆctico 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 par dosificaciOn intravenosa (IV), a una dosis de 2,5 mg/kg o par administración oral (P0), a una dosis de 5 mg/kg. El volumen de dosificación fue de 1 ml/kg para la administraciôn IV y 2 ml/kg para la administraciOn P0. Se extrajeron muestras de sangre en serie de los animales antes de la dosificación, y 2 (IV
solamonte), 5, 15 y 30 minutos, y 1, 2, 4, 8 y 24 horas despuØs de la dosificación, Se
determinaron [as concentraciones de compuestos de prueba en plasma sangufneo par
media de un anÆlisisde cromatografia IIquida-ospectrometrIa de masa (LC-MS/MS) (MDS
SCIEX, API 4000, Applied Biosystems, Foster City, CA), con un limite de cuantificación
inferior de 1 rig/ml.
. Se evaluaron los parÆmetrosfarrnacocinØticos convencionales realizando anIisis no compartimentados (modelo 201 para la dosificación IV y modelo 200 para la
dosificaciOn P0), usando WinNonlin (version 4,0,1, Pharsight, Mountain View, CA). El
mÆximo en la curva de concentraciOn de compuesto de prueba en plasma sanguineo
versus tiempo se denota C,. Se calculO el area bajo la curva de concentraciOn versus
tiempo desde el momento de la dosificación hasta la ültima concontración mensurable
(AUC(0-t)) empleando la regla trapezoidal lineal. Se calculó la biodisponibilidad oral (F(%)), es decir, la relaciOn entre AUC(0-t) para una administraciOn P0 y AUC(0-t) para
una administraciOn IV, normalizada de acuerdo con la dosis, como se indica a
continuaciOn:

F(%) = AUCPOfAUCIV x Dosisv/Dosisp0 X 100% Se espera que los compuestos de prueba con mayores valores de los parÆmetros
AUC(0-t) y F(%) en este ensayo presenten una mayor biodisponibilidad cuando se Slosadministren par via oral. El compuesto de fOrmula I tenla un valor de 0,16 pg/mI, un valor de AUC(0-t) de 0,46 pghr/ml y una biodisponibilidad oral (F(%)) en el modelo de
ratas del 19% aproximadamente.
Si bien se ha descripto la presente invenci6n con referenda a realizaciories
especificas, los especialistas en la tØcnica deberÆn comprenderque pueden efectuarse
diversos cambios y pueden sustituirse elementos equivalentes, sin apartarse del espiritu
verdadero y el alcance de la invenciOn. AdemOs, pueden efectuarse muchas

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modifioacones para adaptar una situacón particular, un material, una composiciOn reacionada, un proceso, uno o ms pasos de un proceso al objetivo, el espiritu y el alcance de la presente invend6n. 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 documentaciôn se incorporan por completo a mode de referenda en la presente documentaciOn, en la misma extension en que se incorpora cada uno individualmente a modo de referenda.

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REIVINDICACIONES

1. Una forma sauna cristalina que es la sal clorhidrato de {(1 S,3R,5R)-8[(R)-2-hidroxi-
3-(metansulfonU-metll-amino)propil]-8-aZabiciClO[3.2. 1]oct3-l}amida de Ocido 1 -isopropi-2-oxo-1,2-dihidroquinolin-3-carboxilico a un solvato del mismo.
2. La forma sauna cristalina de la reivindicaciÆn 1 donde la forma salina cristalina es
uria sat clorhidrato cristalina.
3. La forma sauna cristalina de la reivindicaciOn 2, donde la forma sauna cristalina se
caracteriza par an patron do difracción do rayos x on polvo con dos a mÆs picas do
difracción a valores do 20 seteccionados entre 441–0,2, 8,82–0,2, 9,08–0,2, 11,21–0,2,
14,40–0,2, 16,42–0,2, 17,35–0,2, 17,61–0,2, 18,1410,2, 19,04–0,2, 19,95–0,2,
20,20–02, 21,23–0,2, 22,13–0,2, 2248–0,2, 22,83–0,2, 24,16–0,2, 25,37–0,2,
25,56–0,2, 26,22–0,2, 27,33–0,2, 29,08–0,2, y 29,61–0,2.
4. La forma salina cristalina do la reivindicaciOn 3 donde el patron do difracciOn de
. rayos x compreride dos o ms picas de difracciOn a valores de 20 soleccionados entre 14,40–0,2, 17,35–0,2, 17,61–0,2, 19,04–0,2, 21,23–0,2, y 22,13–0,2,
5. La forma salina cristalina de la reivindicaciOn 2, donde a forma salina cristalina so
caracteriza par an patron de difracción de rayos x en polvo en donde las posiciones do los
picos estÆn sustancialmente do acuerdo con las posiciones do los picas del patron
mostrado en Ia Figura 1.
6. La forma salina cristalina do la reivindicaciOn 2, donde la forma salina cristalina so
caracteriza par un trazada de calorimetria do escaneo diferencial quo muestra an mOximo de flujo de calor endotOrmico a una temperatura mayor quo aproximadamente 230 °C.
7, La forma salina cristalina do la reivindicaciOn 2, donde la forma salina cristalina so
caracteriza par an trazado de calorimetria do escaneo diferencial sustancialmente do acuerdo con el mostrado en la Figura 2.
8. La forma salina cristalina do la reivindicaciOn 1 donde la forma salina cristalina es
un hidrato.
9. La forma sauna cristalina de la reivindicaciOn 8 donde la forma salina cristalina so
caracteriza par un patrOn do difracciOn de rayos x en polvo con dos a mÆs picos de difracciOn a valores de 20 soleccionados entre 5,30–0,2, 7,43–0,2, 8,72–0,2, 10,52–0,2, 13,85–0,2, 14,11–0,2, 15,80–0,2, 15,99–0,2, 17,26–0,2, 19,53–0,2, 20,08–0,2, 21,06–0,2, 21,48–0,2, 21,92–0,2, 22,85–0,2, 23,91–0,2, 25,28–0,2, 26,06–0,2, 27,34–0,2, 27,51–0,2, y29,67–O,2.

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10. La forma saline cristalina de la reivindicación 9 donde el patron de difracciOn de
rayos x comprende dos o mOs picos de difracciOn a valores de 28 seleccionados entre 1052–0,2, 1 385–0,2, 15,80–0,2, 17,26–0,2, y 2106–0,2.
11. La forma sauna cristalina do la reivindicación 8, donde la forma sauna cristalina se
caracteriza per un patron de difracciOn de rayos x en polvo en donde las posiciones de los picos estÆn sustancialmente do acuerdo con las posiciones de los picos del patron mostrado en la Figure 4,
12. La forma sauna cristalina de la reivindicaciOn 8, donde Ia forma sauna cristalina so
caracteriza por un trazado de calorimetria do escaneo diferencial sustancialmerite de acuerdo con el mostrado en la Figure 5.
13. Una composición farmacØutica que comprende un vehiculo aceptable para uso
farmacØutico una forma sauna cristalina de cualquiera de las reivindicaciones 1 a 12.
14, Un mOtodo para tratar un mamifero qua sufra un estado clinico asociado con Ia
actividad del receptor 5-HT4, que comprende administrar al mamifero, una cantidad eficaz come terapia de una composición farmacØutica que comprende un vehIculo aceptable para uso farmacØutico una forma sauna cristalina de cualquiera de las reivindicaciones 1 a 12.

15. Un mØtodo para tratar un trastorno de motilidad reducida del tracto gastrointestinal
en un mamifero, que comprende administrar al mamifero una cantidad eficaz come terapia do una composición farmacØutica quo comprende un vehiculo aceptable para uso farmacØutico una forma salina cristalina de cualquiera de las reivindicaciones I a 12.
16, El mØtodo de la reivindicaciOn 15, donde el trastorno do motilidad reducida so
selecciona entre constipación crónica, sindrome do intestino irritable con constipaciOn predominante, gastroparesia diabØtica e idiopØtica, dispepsia funcional.
17. Una forma saUna cristalina de acuerdo con cualquiera de las reivindicaciones 1 a
12 que Os para su uso en terapia.
18. El uso de una forma sauna cristalina de cualquiera de las reivindicaciones 1 a 12
que es para la fabricación de un medicamento.
19. El uso de la reivindicaciOri 18 donde el medicamento es para el tratamiento do un
estado clinico en.in mamifero asociado con la actividad del receptor 5-HT4.
20. El use de la reivindicaciOn 19 donde el estado dinico es un trastorno do motilidad
reducida del tracto gastrointestinal.

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21. El uso de la reivindioación 19 donde el estado clinico se selecciona entre
constipaciOn crOnica, sindrome de iritestino irritable con constipaciOn predominante, gastroparesia diabØtica e idiopØtica, y dispepsia funcional.
22. Un proceso para preparar una sal clorhidrato cristalina de {(1S,3R,5R)-8-[(R)-2-
hidroxi-3-(metansulfonil-metil-ami no) prop! l]-8-azabiciclo[3.2.1]oct-3-il}amida de Æcido 1-isopropil-2-oxo-1,2-dihidroquinolin-3-carboxilico, que comprende:
(a) poner en contacto {(1 S,3R,5R)-8-[(R)-2-hidroxi-3-(metansulfonimetiI-amino)propil]-8-azabiciclo[32. 1]oct-3-U}amida de àcido 1 -isopropil-2-oxo-1 2-dihidroquinoUn-3-carboxilico con Æcidoclorhidrico para formar una mezcla de reacción; y (b) aislar la sal clorhidrato cristalina de la mezcla de reacción.
23. Un proceso para preparar un hidrato cristalino de Ia sal clorhidrato de {(1 S,3R,5R)-
8-[(R)-2-hidroxi-3-(metansulfonil-metil-amino)propil]-8-azabiciclo[3.2.1]oct-3-ilamida de Æoido1 -isopropil-2-oxo-1,2-dihidroquinolin-3-carboxilico, que comprende:
(a) disolver clorhidrato de {(1 S,3R,5R)-8-[(R)-2-hidroxi -3-(metansulfonU-metil-amino)propil]-8-azabiciclo[3,2, 1]oct-3-iI}amida de âcido 1 -isopropil-2-oxo-1 2-dihidroquinolin-3-carboxIlico en agua a una concentraciôn mayor qua aproximadamente 50 miligramos per mililitro para formar una suspension; y
(b) aislar el hidrato cristalino de Ia suspensiOn.

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FORMA CRISTALINA DE UN COMPUESTO DE QUINOLINONA-CARBOXAMIDA RESUMEN

Una sal clorhidrato cristahna de ((1 S,3R5R)-84(R)-2-hidroxi-3-(metansulfon-metil-anhino)propHl-8-azabiciclo(3.2.11oct-3-il}amida de àcido 1-isopropU2-oxo1,2-dihidroquinolin-3carboxflico o un solvato del mismo, Composiciones farmacØuticas que comprenden dichas formas salinas cristalinas, mØtodos para utilizar dichas formas salinas cristalinas para tratar enfermedades asociadas con la actividad del receptor 5-HT4, y procesos ütiles para preparar dichas formas salinas cristalinas.

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Temperatura (°C)

Figura 2

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Humedad relativa (%)

Figura 3

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5 10 15 20 25 30 35

20 (Grados)

Figura 4

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- 2.5

- 3,0 4
0

Exo Up

50 100 150

Temperatura (°C)

Figura 5

200 250

105

95 89

85

- + 80
300

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2.500

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-2.500

Humedad relativa (%)

Figura 6

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PATENT COOPERATION TRES1Y

From the INTERNATIONAL SEARCHING AUTHORITY

I To:
THERAVNCE, IIC.
Attn. Hagnth, Jef4rey A
50. Gateway 9oulevaxd
LOLICh San Francisco, CA 94080
ATS-QtZS ’ARIQE

Applicant’s or agent’s file reference
P-211-PCT

International application No.
PCT/tJS2006/01278

Applicant

THERAVAI’TC, INC.

NOV 07 2006

ThRAVANCE, INC.
TMALIDEPARTMENT

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

(POT Rule 44.1)

I Date of mailing

(day/month/year) 07/11/2006

FOR FURTHER ACTION See paragraphs 1 and 4 below

International filing date

(day/month/year) 05/04/2006

1. The applicant Is hereby notified that thU international search report and the written opinion of the International Searching
Authority have been established and are transmitted herewith.
Piling of amendments and statement under Article 19:
The applicant Is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):
When? The time limit for filing such amendments is normally two months from the date of transmittal of the
International Search Report
Whore? Directly to the International Bureau of WIPO, 34 chemin des Colombettes
1211 Geneva 20, Switzerland, Fascimlie No.: (41-22) 338.32.70
For more detailed Instructions, sea the notes on the accompanying sheet,

2, D 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 (art) additional fee(s) under Rule 40.2, the applicant is notified that:

E 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 see dOGision Is made.

4, Reminders
Shortly after the expiration of 18 months trom the priority dote, 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 $0bIs.1 and S0b1s.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 applicait 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 tiled within 19
months.

See the Annex to Form POT/18/301 and, for details about the applicable time ilrnits, Office by Office, see the PCTApp!!canrf.s Ga’lde, Volume II, National Chapters and the WIPO Internet site.

NL-2280 HV Rijswijk Angela Lopez Navarro

Name and mailing address of the international Searching Authority Authorized officer

A))European

Patent Office, P.B. 81IS Patentiaari 2

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Form PCT/iSA/220 (October 2005) I+tZht?t! (See notes on accompanying,sheet)

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NOTES TO FORM PCT/15A1220

These Notesare Intended to give the basic Instructions concerning the filing Of amendments under article 19. ThS
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 PCTApPlicertf’s Guide, a publication of WIPO,

In these Notes, ’Article’,"Rule", and Section refer to the provisions of the POT, the POT Regulations and the POT 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 bO 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 at the claims under Article IS 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 PGlAppllcenr’s Guide, Volume hA, Annexes SI and 52)

The attention of the applicant Is drawn to the tact 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 PC7Appl/cet’s ufde, 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 134 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 18 months from the priority data, 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 lbS completion of the technical preparations for international publication (Rule 48.1).

Where not to file the amendments?

The amendments may only be flied 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 beenlls flied, see below.

(’tow? 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 or the claims which, on account of an amendment or amendments, differs from the sheet originally filed.
At the claims appearing on a replacement sheet roust 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 mustlmay 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 1907).

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.

Notes to Form PCT/ISA/220 (first sheet).(dotober 2005)

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U PATENT COOPERATION TREQ(

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 48 and 44)

Applicant’s or agent’s file reference FOR FURTHER see FormPT1lSA/22O ACTION as well as, where applicable, Item 5 below.
international applicatlon No. International filing date (day/montWyear) (Earliest) Priority Date (dsy/monthfyear)

?CT/U82006/012978 05/04/2006 L 06/04/200 Applicant

THRAVATCL 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 3 sheets.
It is also accompanied by a copy of each prior art document cited In this report.

1. Bests 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 flied

a translation of the international application into which Is the language or 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 (Bee Box No. Ii)

3. Unity of invention Is lacking (see Box No lii)

4. With regard to the title,
the text is approved as submitted by the applicant

0 the text has been established by this Authority to read as follows:

5. With regard to the abstract,

1-1 the text is approved as submitted by the applicant
LI the text has been established, according to lluIa 38.2(b), by this Authority as it appears in Box No. IV. The applicant
may, within one month from the date or mailing of this international search report, submit comments to this Authority

S. 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
11 as selected by this Authority, because this figure better characterizes the Invention
b. 0 none of the figures is to be published with the abstract
L.

Form PCTIi$N210 (first sheet) (April 2005)

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3

I IUPIRL RI-.rl (International application No
PCT/US2006/0 12978
A. CLASSt1CATICN OF SUBJECT MATTER

INV. C07D451/04 A61K31/40 A61F1/00

According to international Patent Classification ([PC) or to both national ciasslfIctiort and IPG
B. PIP-LOS SEARCHED
Minimum documentation searched (classification system fotoWed 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 database consulted during the International search (name of data base and. where practical, search terms user

EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category Citation of document, with Incitcatlon. Where appropriate, orthe relevant passages Relevant to claim No,

WO 2005/3.00350 A (THERAVANCE INC [US]; 1-23
MARQUESS DANIEL [US); EATHEREE PAUL R
[US]; TURNE) 27 October 2005 (2005-10-27)
cited in the application
example 25

A EP 0 564 650 Al. (KYOWA HAKKO KOYO KK 1-23
[JP]) 13 October 1993 (1993-10-13) example 6, reference example 10

IIFuflherdocumals are listed in the continuation of Box C. L?jSee patent fejnhiy annex.
* Spaciai categories of cited documents:

T later document published after the international hung date or priority date and not In conflict with the application but A citeit document defining the general State of the art which Is fl5t to understand the principle or theory underlylrrg hIts considered to be of particular relevance invention
E1 earlier document tot published on or slierthe International ’XI document of particular relevance; the claimed Invention filing date cannot be considered novel or cannot be Gonetciered to

’L document which may throw doubts on priority, claim(s)or Involvean Inventive step when the document Is taken alone which is chart to establish tea pubiication dale 01 another Y document of particular relevance; ins claimed invention citation or Otiwrspecloi reason (as specified) cannot be considered to involve an inventive step when the

0 document referring to an oral disclosure, use exhibition or document Is combined with one or more other such docu-
other means meats, such combination being obvious to a person s)dlteci

IPI document published prior to the International filing date but In the art.

later than the priority date SIstered & document member chile same patent family
Pale of the actual completion of the international search Date of mailing of the international search report

25 October 2006 07/11/2006

Name and mailing address of the ISN Authorized officer
European Patent Office, P.S. 6815 Pstentiasn 2

NL- 2280 NV Rl)swljk Tel. (+31-70) 840-2040, Tx. 31 651 epo ni
Fax: (+31-70) 340-3016 Schmid, Arnold

FoOn PUTfl5N210 iss*snri Chest) (Apri12005)

Farm FT/SN210(patent family annex) (ApU 2O)

/ 07

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I

0 PATENT COOPERATION OEATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: PCT

WRITTEN OPINION OF THE

see form PCTASA20 INTERNATIONAL SEARCHING AUTHORITY

(POT Rule 43b1s.1)

Date of mailing
(daynnonthV.sr) see form PCTA210 (second sheet)

Applicants or agents file reference FOR FURTHER ACTION
see form PCT/ISA1220 See paragraph 2 below

International application No, International filing date (day4nont4esr) JPriority date (oy/miJ’eai)

PCTiUS2008O1 2978 L05042006 f 06.04.2005
International Patent Classification (IPC) or both national ciassication and (PC
(NV. C07D451iO4 A611<31/40A61P1O0

Applicant
THERAVANCE, INC.

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

Box No. I Basis of the opinion
Box No. II Priority
C3 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 4bis.1 (a)(i) with regard to novelty, Inventive stop or Industrial
applicability; citations and explanations supporting such statement
19 Box No. VI Certain documents cited
Box No. VII Certain defeats 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 (PEA and the chosen IPEA has notifed the

International Bureau under Rule 66.lbis(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]PEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCTI1S20 or before the expiration of 22 months from the priority date,
whichever expires later.

For further options, see Form PCTSA/220.

S. For further details, see notes to Form PCTI1SA/20

Name and mailing address of the ISA: Date of completion of Authorized Officer
this opinion

EuropeanP see form D-80298 Munich Schmid, Arnold t Tel +49 69 2399 -0 Tx: 523656 epmu d

Fax: +49892399- 4485 Telephone No, +4989 2399-8591

Form (PGTASA,37) (Cover Sheet) (A01 2005)

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WRITTEN OPINION OF THE International application No. INTERNATIONAL SEARCHING AUTHORITY PCTflJS2006AJ12978

Box No. I Basis of the opinion
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
0 a translation of the International application into which Is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).
2. 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:
0 a sequence listing
0 table(s) related to the sequence listing
b. format of material:
O on paper
0 in electronic form
C. time of filing1urnish1ng:
0 contained in the International application as flied.
O tiled together with the International application In electronic form.
0 furnished subsequently to this Authority for the purposes of search.

3. 0 In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been tiled or furnished, the required statements that the Information in the subsequent or additional
copies Is Identical to that in the application as flied or does not go beyond the application as flied, as
appropriate, were furnished.
4. Additional comments:

Form PCTASA/237 (April 2005)

U

WRITTEN OPINION OF THE International application No. INTERNATIONAL SEARCHING AUTHORITY PCT1US2006i012978

Box No. V Reasoned statement under Rule 43b!s.1(a)(J) with regard to novelty, inventive step or
industrial applicability; citations and explanations supporting such statement
1. Statement

Novelty (N) Yes: Claims 1-23
No: Claims

Inventive step (IS) Yes: Claims 1-23
No: Claims

Industrial applicability (IA) Yes; Claims 1-23
No: Claims

2. Citations and explanations
see separate sheet

Box No. VI Certain documents cited

1 1. Certain published documents (Rules 43b1s.1 and 70.10)

and/or

2. Non-written disclosures (Rules 43b1s.1 and 70.9)
see, form 210

Box No. VII Certain defects In the international application
The following detects in the form or contents of the international application have been noted: see separate sheet

lomi PCTASN27 (April 2005)

WRITTEN OPINION OF THE International appflcatlon No. INTERNATIONAL SEARCHING

AUTHORITY (SEPARATE SHEET) PCT/US200W01 2978

Re item V

Reasoned statement with regard to noveIty, inventive stop or industrial applicability; citations and explanations supporting such statement

1) Reference is made to the following documents:

Dl: WO 20051100350 A (THERAVANCE INC [US]; MARQUESS DANIEL [US): FATHEREE PAUL A [US]; TURNE) 27 October 2005 (2005-10-27) cited in the application
D2: EP-A1-0 564 650 (KYOWA HAKKO KOGYO KK [JP]) 13 October 1993 (1993-10-13)

2) The present compound differs from the closest prior art represented by D2, example
6 and reference example 10 among others in the mathanesulfonyl-methyl-amino part. Moreover, no type of salt, especially no hydrochloride salt is mentioned.

Accordingly the present subject-matter Is clearly novel pursuant to Article 33(2) PCT,

3) A skilled person looking for 5HT receptor modulators would surely not try to modify
the known compounds of D2, which moreover concerns a problem different from the present one (5HT3 receptor antagonists), in the way as specifically claimed with a reasonable expectation to obtain compounds exhibiting the desired properties which
could be proved by the applicant on pages 35, 36, 38, 40 and 41.

Therefore, the present subject-matter also involves an inventive step with respect to
Article 33(3) POT.

Farm P0T115N237 (Sepra1e Sheet) (Shoot 1) (EPO-April 2005)

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WRITTEN OPINION OF THE International application No. INTERNATIONAL SEARCHING

AUTHORITY (SEPARATE SHEET) PCTIUS2006/012978

Re Item VI
Certain documents cited

Certain published documents
Application No Publication date
Patent No (day/mont fyear)

Filing date Priority date (valid claim)
(day/month/year) (day/month/yar)

WO-A-2005/100350 27/10/2005 06/04/2005 07/04/2004

WO-A-2005/100350 discloses in example 25 the present compounds as a hycirochiorid salt. However, nothing is mentioned as regards the fact that it may represent a crystalline form.

Reitem VII

Certain detects In the international application

1) D2 which represents a relevant prior art should be referred to in the description in accordance to Rule 5(1)(a)(U) PCT.

Form POT/1$N237 (Separate Sheet) (Sheet 2) (EPO-AprIl 2005)

P-211 -POT

PCT REQUEST J

C) 1/6 0

Original (for SUBMISSION)

0-4 Form PCTJ110I1 01 PCT Request

0-4-1 Prepared Using PCTSAFE [EASY mode]
Version 3.51.001.176 M/0P 20060101/0. 20.4rc.7

The undersigned requests that the
present International application be
processed according to the Patent
Cooperation Treaty
06 Receiving Office (specified by the
applicant) United States Vatent and Trademark

Office (tJSPTO) (RO/tlS)
0-7 Applicant’s or agents file reference

Title ofInvention CRYSTALLINE FORM OF A QUINOLINONE
CARBOXMIDE COPDXJIW
Ii Applicant
Il-i This person is

11-2 Applicant for all designated States except US
11-4 Name THERAVACE, INC.
((-5 Address 901 Gateway Boulevard
South San Francisco, California 94080 United States of America
11-6 State of nationality US
((-7 Slate of residence

((-8 Telephone No. (650) 808-6000
11-9 Facsimile No (650) 808-6078
Ill-i Applicant and/or inventor

((i-i-I This person is applicant and inventor
1(1-1-2 Applicant for US only
((1-1-4 Name (LAST, First) Paul R.
ill-i-S Address 921 Minnesota Street
San Francisco, California 94107 United States of America
((3-1-6 State of nationality
(il-I-i State of residence

P-211 -PCT

PCTREQLJEST C)

P/-i 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:
IV-1-1 Name (LAST, First)
IV-1-2 Address

IV-1-3 Telephone No.
IV-1-4 Facelmlie No.
IV-1-6 Agenfs registration No.
IV-2 Additional agent(s)
IV-2-1 Name (LAST, First)
P/-2-2 Address

IV-2-3 Telephone No.
IV-2-4 FacsTmile No.
IV-2-6 e-mail
IV-2-6 Agents registration No.
IV-3Additional agent(s)
lV--I Name (LAST, First)
IV-3-2 Address

IV-3-3 Telephone No.
IV-3-4 Facsimile No.
IV-3-5 0-mail
IV-3-6 Agents registration No.

316 0
Original (for SUBMISSION)

H2.GAL Ueffrey A.
L’HEIAV.ANCE, INC..
901 Gateway Boulevard
South San Francisco, California 94080 United States of America

(650) 808-6406
(650) 808-6078
35,175
agent
SAXON, Roberta F.
TRERAVANCE, INC.
901 Gateway Boulevard
South San Francisco, California 94080 United States of America
(650) 808-3764
(650) 808-6078

43,087

COIEN, Joyce G.
T1ERA.V.ANCE, INC.
901 Gateway Boulevard
South San Francisco, California 94080 United States of America
(650) 808-6144
(650) 808-6078

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P-211 -PCT

PCT REQUEST

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LI
Original (for SUBMISSION)

(I) ’ \S

W4 Additional agent(s) agent

W-4-1 Name (LAST, First) EBERLE, Shelley
IV-4-2 Address THERAvANCE, INC.
901 Gateway Boulevard
South San Francisco, California 94080 United States of America

IV-4-3 Telephone No. (650) 808-4010

IV-4-4 Facsimile No. (650) 808-6079
IV-4-5 e-mail

IV-4-6 Agent’s registration No. 31,411
V DESIGNATIONS

V-I The tiling of this request constitutes
under Rule 4.9(a)1 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.
V-3 Identification of parent application or
patent grant

V-3-I Designation US

V-3-1-1 Kind of parent application or grant continuation-in-part

V-3-1-2 Parent application or grant No. 60/668,780
V3-1-3 Parent application or grant date 06 April 2005 (06.04.2005)
VI-1 Priority claim of earlier national
application

VI-1-1 Filing date 06 April. 2005 (06.04.2005)
Vi-1-2 Number 60/668,780
Vi-1-3 Country

Vi-2 Priority document request

The receiving Office is requested to VI-1
prepare and transmit to the international
Bureau a certilled copy of the earlier
application(s) Identified above as
Item(s):

VII-1 international Searching Authority European Patent Office (EPO) (ISA/EP) Chosen

P-21 1 -POT

PCT REQUEST

516

Original (for SUBMISSION)

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VIII Declarations Number of declarations
VIII-1 Declaration as to the Identity of the
inventor

VIII-2 Declaration as to the applicants
entitlement, as at the international filing
date, to apply for and be granted a
patent
VIlI-3 Declaration as to the applicant’s
entitlement, as at the International filing
date, to claim the priority of the earlier
application
V111-4 Declaration of invontorthlp (only for the
purposes of the designation of the
United States of America)
Viii-5 Declaration as to non-prudIclal
disclosures or exceptions to lack of
novelty
IX Check list number of sheets electronic file(s) attached

tx-i Request (including declaration sheets) 6

IX-2 Description 41

IX-3 Claims 4

IX-4 Abstract 1

1*5 Drawings 3

IX-? TOTAL 55

Accompanying items paper document(s) attached electronic file(s) attached
IX-8 Fee calculation sheet

IX-17 POT-SAFE physical media

IX-18 other Transmittal Letter

to the US Receiving
Ofrice

IX-18 other Six General Power

of. Attorney
documents

IX-lB other Receipt Postcard
IX-I 9 Figure of the drawings which should
accompany the abstract

IX-20 Language of filing of the international English application
X-t Signature of applicant, agent or
common representative

X-1-1 Name (LAST. First) Roberta P.
X-1-2 Name of signatory
X-1-3 Capacity

P-211 -PCT

PCT REQUEST (_) 616

Original (for SUBMISSION)

C.

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
1 0-3 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 I’CT Article 11(2)
10-5 International Searching Authority IsA/NP
10-6 Transmittal of search copy delayed
until search fee is paid

FOR INTERNATIONAL BUREAU USE ONLY

11-1 Date of receipt of the record copy by
the International Bureau

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NOVZ 7 2006

1 THERAVANCE, INC.
L LEGAL DEPARTMENT - From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE
OF A CHANGE

HAGENAHI Jeffrey A.
THRAVANCE, INC.
901 Gateway Boulevard
South San Francisco, California 94080

(?CT Rule 92bi’.1 and ETATS-LJNIS D’AMERIQUE
Administrative Instructions, Section 422)
Date of mailing (day/month/year)
14 November 2006 (14.11.2008)
Applicant’s or agent’s file reference

IMPORTANT NOTIFICATION

International application No. Intetnatfonal filing date (day/month/year)

PCT/US2006/012978 05 April 2006 (05.04.200)

1. The following indications appeared on record concerning:

the applicant the inventor 0 the agent Q the common representative
Name and Address State of Nationality State of Residence
TURNER, S. Derek US US 193b Warren Drive

Sin Francisco, California 94131 Telephone No.
United States of America
Facsimile No,

Teleprinter No

2. The international Bureau hereby notifies the applicant that the following change has been recorded concerning:

the person 0 the name 0 the address the nationality j the residence
Name and Address State of Nationality State of Residence
TURNER, S. Derek US US
6990 Falls Reach Drive #403
Fails Church, VA 22043

Telephone No.

United States of America
Facsimile No.

Teleprinter No.

3. Further observations if nit: cessaiy:

4. A copy of this notification has been sent to:
the receiving Office the designated Offices concerned
Z the International Searching Authority 0 the elected Offices concerned
the International Preliminary Examining Authority other:

The International Bureau of WIPO Authorized officer
34, chemin des Colombettes
1211 Geneva 20, Switzerland 2
Facsimile No. +4122 338 89 70
Facsimile Telephone

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L.

NOV 2 72006
From the INTERNATIONAL BUREAU THERAVANCE, INC.

LEGAL DEPARTMENT To:

NOTIFICATION OF THE HAGENAH, Jeffrey A.
OF A CHARGE THERAVANCE, INC.
901 Gateway Boulevard
South San Francisco, California 94080
(PCT Rule 92b1s.1 and ETATS-UNIS D’AMERIQUE
Administrative Instructions, Section 422)
Date of mailing (day/monthiyaar)

15 November 2006 (15.11.2006)

Applicant’s or agent8 file reference IMPORTANT NOTIFICATION

International application No. international filing date (day/rnonth.yea,)
PCT1U520061012978 05 April 2006 (05.04.2006)

1. The following indications appeared on record concerning:

the applicant the inventor 0 the agent Jj the common representative
Name and Address State of Nationality State of Residence
GQLIDBLIJM, Adam US US

505 Mollmo Drive Telephone No.
San Francisco, California 94127
United States of America
Facsimile No.

Teleprinter No,

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

D the person El the name CR the address 0 the nationality the residence
Name and Address State of Nationality State of Residence
GOLOBLUM, Adam U$ US

1320 Berkeley Way Telephone No.
Berkeley, CA 94702
United States of America
Facsimile No.

Teleprinter No..

3. Further observations, if necessary;

4. A copy of this notification has been sent to: the receiving Office the designated Offices concerned the International Searching Authority 0 the elected Offices concerned

the International Preliminary Examining Authority [] other:
The International Bureau of WIPO Authorized officer
34, chernin des Colombettes
1211 Geneva 20, Switzerland Konig Elisabeth
Facsimile No. +41 22 338 89 70
Facsimile No, +4122 338 82 70 Telephone No. +4122 338 87 48
torn1 1’tJI’fW/306 (October 2005) 1/C 1XLVC7YO

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NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions. Section 411)

Dale of mulling dczj,onth’’ec,r
14 June 2008 (14.06.2006)

AppUciit’t or agcnts file refcence
P-21 I -PCT

Uternatiojial application No.
PCT/US2006/012978

International pnblialion date (dly/11,on(h,)’eal’J
Not yet published

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From the INTERNATIONAL BUREAU

To

HA(3ENAH, Jeffrey A.
TI-IERAVANCE, INC.
901 Gateway Boulevard
South San Francisco, California 94080 ETATS.UN IS D’AMERIQUE

IMPORTANT NOTIFICATION

International filing date ck’/inonth/j’eai’)
05 April 2008 (05.04.2006)

Priority dale (dl’/mornh.’ew’)
06 April 2005 (06.04.2005)

THERAVANCE, INC. et al

1. By of this Form. which issued notification submission or transmittal of priority

2. ’JJ’applkcthle The letters "NR" appearing in the right-hand coluarn denote a priority document which, an the date of mailing of

priority document must he submitted by the applicant to the receiving Office or the International Bureau, but the applicant fails to

alter the time limit prescribed in Rule 17,1(a) or the request to prepare and transmit the priority document was submitted to the

compliance with Rule 17.1(a) or (h), the International Bureau will nevertheless transmit a copy of the document to the designated

TRADUCCION OFIC1AL No. 01/224 de un documento escrito en inglØs, el cuai para su identificaciOn se sella con el seflo del traductor al tiempo con la presente. Esta traducción ha sido preparada por Juan Pablo Piedrahita Aguilera, Traductor e IntØrprete Ofical debidamente reconocido de conformidad con el Certificado de Idoneidad Profesional No. 0045 expedido por la Universidad Nacional de Colombia.

(Se traducen un Certificado Notarial y Apostilla No. 125836 escritos en inglØs, adjuntos a un Pocler otorgado por la compaæIa THERAVANCEINC., con fecha Mayo 7, 2002, escrito en ingles y espaæol.)

CALIFORNIA RECONOCIMIENTO PARA TODOS LOS FINES
ESTADO DE CALIFORNIA

CONDADO de San Mateo) S.S.
En Mayo 7, 2002, ante ml, Joyce Cohen, Notario Püblico, (Nombre y Cargo del Funcionarlo (p.ej. "Jane Doe, Notarlo PUblico"), compareciO personalmente Jeffrey A. Hageriah, (Nombre(s) de el (los) firmante(s)),

r1 a quien(es) conozco personalmente
(en blancoij quien presentó pruebas basadas en evidencia suficiente quien es fs9n3 IaW persona$ cuyofs) nombref$ estâ suscrito(s)
en el instrumento adjunto y reconociO (eros) ante ml que
ØIfellos) suscribi6(eren3 el mismo en
autorizado(s) y que por su(s) firma(s)

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ll.dadjeo de
’r -;’ i.V

persona(s) o la entidad a nombre de quien lafe persona’s)
actüafn suscribiô(eron) el instrumento.
DA FE ml firma y sello oficial.
Firma: (Firmado) Joyce Cohen
Firma del Notarlo Püblico,
(Sello a Tinta: Joyce Cohen, ComisiOn # 1223928, NOTARIO PUBL1CO CALIFORNIA, Condado de San Mateo, ml comisiôn vence Junio 11, 2003).
Estampe el Sello Oficial Arriba

OPCIONAL

Aunque la informacion a continuación no so requiere por lay, puede sor valiosa Para quienes so basen en al clocumento y podrIan evitar qua fraudulentamente
so desprendan y so vuelvan a adjuntar otros documentos a este formulario. Descripción del Documento Adjunto
TItulo o Tipo de Documento:Poder Clarke modet,& Co Colombia
Fecha del Documento: Mayo 7, 2002 Nümero de PÆginas
Firmante(s) distintos a los arriba mencionados: (en blanco)
Facultad(es) Reivindicadas por el Firmante
CN Nombre del Firmante: J. Haqenah
4c

en blanco 1 INDJVIDUO
X FUNCIONARIO EMPRESARIAL-CARGO(S):
en blanco SOCtO [en blanco LIMITADO

en banco1 APODERADO

2

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1’ f.

2

en blanco SNDICO
Len bIanco CUSTOD10

en blanco OTRO:_. (EN BLANCO)

EL FIRMANTE REPRESENTA:
THERAVANCE INC.

(Sello Redondo en Relieve) Porte Superior del Pulgar Aqul

1997 AsociaciOn Naciorial de Notarios 9350 De Soto Ave, P.O. Box 2402, Chatsworth, CA 91313-2402 Prod. No. 5907 Pedido: Liamar a Linea Gratuita 1-800-876-6827.

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2,
3.
4.

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be (California
(Sello Pre-Impreso)
SECRETARIO DE ESTADO
APOSTILLA

(Convención de La Haya de Octubre 5 1961)
Pals: Estados Unidos de America
Este documerito pub//co
Ha s/do firmado por Joyce Cohen
Actuando en calidad de Notano PUblico, Estado de California
Lieva a/ se//olestampilla de Joyce Cohen, Notario PObiico, Estado de

California

En Sacramento, California

CERTIFICADO

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6. El 6 de Junio del 2002

7, Par el Sub-Secretario de Estado, Estado do California

8. No. 125836

9. S&lo/Estamplilla

(Se/la do Oblea del Estado do California, Se/Jo a tinta del Secretario do Estado) (So/la Redondo a Tinta del Secretano do Estado)
10. Firma (Ffrmado)
Bill Jones
Secretario de Estado
Por Firmado) CWasley 4
NP-24 A (Rev 1-96) asp 99 21639

ES TRADUCCION FIEL Y COMPLETA de la cual so deja copia en archivos

do esta oficina para su confrontaciOn y a Ia cual me remfto.

Bogota, Agosto 8 do 2002.

/ADUCCION11 OFCA N.

JUAN PABLO PEDRAHFAAGUILE.
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SECRETARY OF STATE

11 APOSTILLE
(Convention de La Haye du 5 octobre 1961)
1. Country: United States ofAmerica
This public document
2. has been signed by JOYCE COHEN
3. acting in the capacity of Notary Public, State of California
4. bears the seal/stamp of JOYCE COHEN, Notary Public, State of California
CERTIFIED
5. At Sacramento, California
6. the 6th day of June 2002
7. by Deputy Secretary of State, State of California
8. No. 125836

9. Seal/Stamp:

10. Signature

BILL JONES...

Secretary of$te,

BY (CJxCL
NP-24 A (Rev. 1-96 OSP 99 21629

CALIFORNIA ALL-PURPOSE ACKNOWLEDGMENT

State of California

County of..SCkY\

On fl before me, c- (tJfr3 lTf iLk Date Namaland Title of Officer)e,p,,jatte’boc, Notary Public)
personally appeared

1 JOYCE COHEN

amy

Notary

COMM.
Public-Californiaca

#1223926 m
SAN MATEO COUNTY

Place Notary Seal Above

personally known to me
Li proved to me on the basis of satisfactory evidence

to be the personçs4 whose name(g) is/afe subscribed to the within instrument and acknowledged to me that he/sba4hey executed the same in hi&4eitheir authorized capacity(is), and that by his/hcr/their signature(ron the instrument the person(s), or
the entity upon behalf of which the person(s)
acted, executed the instrument.

WITNESS my hand and official seal.

6,?nature of Notary Public

OPTIONAL
Though the information below is not required by law, it may prove valuable to persons relying on the document and could prevent fraudulent removal and reattachment of this form to another document.

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Signer(s) Other Than Named Above:

Capacity(ies) Claimed by Signer
Signer’s Name:..
Li Individual
Corporate Officer Title(s): --
Li Partner --.11 Limited LI General
Li Attorney in Fact
Trustee
Li Guardian or Conservator
Li Other:_

Signer Is Representing:

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Clarke, Modet & Co.
COLOMBIA

E-MAIL: clarkemodet@clarkcmodetcornco
SANTAFE DE BOGOTA, A.A. 92397

PODER

Yo (nosotros), abajo firmante(s)

an ml (nuestro) caracter de representante(s) legales de

una sociedad organizada y existente de acuerdo con las leyes de

yen actual cumplimiento do su objeto social, con domicilio an

por at presente instrumento confiero (conferimos) poder especial a favor de DILIA MARIA RODRIGUEZ DALEMAN, con CØdula do Ciudadonia No. 41.654.882 do Bogota, y tp. 20824 y CLAUDIA LUCIA CARO RAMIREL con CØdula de CiudadcrnIa No. 40.017.374 de Tunja, y tp. 36448, con domicilio en Santa Fe de Bogota, Colombia, para quo actuando en si.i orden en nombre del (de Ia) oforgante le (Ia) representen ante cualquier ertfidod, autoridad yb persona, especialmente ante las del orden Administrotivo, Contencioso Adminisfrativo, Judicial, Tribunales do Arbitramenfo y/o cuolquier afro en Ia referento a los siguientes usuntos:

1 I Solicitor. tramitor, obtener y/o registrar cualquiera do las modalidades do Propiedad Industrial, tales como: patentes do invenciOn modelos de ufilidad; diseOos industriales, variedades vegelales; marcas, lemas, nombres y enseæas comerciales y denominaciones do origen; asi coma sus renovuciones, prórrogas, fransferencias, cambios de nombre y/o domicilio, contratos y licencias.
2) Solicitor, tramitar, obtener y/o registrar derechos de autor, an cualquiera de sus presentaciones osi coma to protección do secretos industriales y know-how.
31 Solicitor, tramitar, obtener y/o registrar registros sanifarios, permisos y/o licencias de funcionamiento; cxi coma sus renovaciones a prörrogos, fransterencias, cambios de nombre y/o domicilio, contratos y licencias.
4) Ejercer acciones de oposición, observoción, reclamos, recorisideraci6n, reposiciOn, apelaciOn, cancelaciOn administrafiva o judicial, nulidad, profecciön at consumidor, concesiOn de licencias, protección do secretos industriales y demÆs acciones y recursos relocionados con derechos derivados de los asuntos arriba indicados y en contra do la competencia desleal, acciones derivadas do Ia obtención a explotaciOn de licencias, acciones de indemnización de perjuicios. Tambien podrâ(n) intervenir en juicios civiles, comercioles, penoles, contencioso-adrninistrativos, futelas y de policics; an acciones a juicios promovidos por el (Ia) mandante a en contra el (ella).
5) Registrar nombres de dominio ante Ia entidad competente.
6) En el ejercicio do este mandato los mandatarios arriba mencionados quedan focultados para: conciliar, cancelar, transigir, compromefer on órbifros, desisfir, recibir y tomar posesión de la cosa objeto de litigio y renunciar o desistir a derechos concedidos o on curso de concesiOn, admitir los hechos del proceso.
7) Ratificar o no la agenda ofic.iascs.
8) Asi mist-no faculto (facultamos) a los mencionados apoderados para recibir notiticaciones y designar apoderados judiciales o extrajudiciaoles, sustituir el presente poder y rcvocar las sustitucio-nes que hicieren.

Dade y firmado en

POWER OF ATTORNEY

I (we), the undersigned
THERAVANCE Inc

legal representative(s) of

an organization existing under the laws of the State of Delaware

and in present execution of its business activity, domiciled at 901 Gateway Boulevard, South San Francisco, California 94080, United States of America

do hereby grant POWER OF ATTORNEY to DILIA MARIA RODRiGUEZ D’ALEMAN, identification card No. 41,654-882 of Bogota, and tp- 20824 and CLAUDIA LUCIA CARO RAMIREZ, tp. 36448, identification card No. 40.017374 of Tunjo, residing in Santo Fe de Bogota, Colombia to act in the order of their appointment in behalf of grantor before any entity, authority and/or natural or juridical person, specially before those Administrative Administrative-Contentious, Judicial, arbitration courts, and/or any other, in all matters concerning the following:

To apply, process, obtain and/or register any of the modalities of Industrial Property, such as: Patents of Invention, Utility Models; Desings plant varieties; Marks Slogans, Trade Names and emblems and appellations of Origin; as well as their renewals, extensions, assignments, changes of name and/or domicile, agreements and licenses.

2) To apply, process obtain and/or register copyrights of any kind as well as protection of industrial secrets or know-how-

3) To apply, process, obtain and/or register sanitary registrations, operating authorizations and/or licenses; as well as their renewals or extentions, assignments, changes of name and/or domicile, agreements and licenses.
4) To bring actions of opposition, observations, claims, reconsideration, appeal or legal remedies, cancellations, nullity, protection to the consumer, granting of licenses, protection of trade secrets and other actions related with rights derived from the above- mentioned matters and against the unfair competition, actions resulting from the obtenfion or exploitation of licenses, recovering damages actions, They can also intervene or participate in Civil, Commercial, Criminal or Contentious-Administrative Trials; the actions for Writ mandamus, police actions: in actions, lawsuits or litigations instituted by the grantor or against him.
5) To register domain names before the competent entity.
6) To the effect of this mandate the above-named proxy are empowered to: conciliate, cancel, settle, submit to arbitration, withdraw and to receive and take possession of the litigation related objet and waive or renounce the rights granted or in process of granting, admit the facts of the
case

7) To ratify or not th afitaI9l
8) I (we) hereby ap)prAr*hthrjk Woi r3qjç ’kSh’#cyt itl-4’
powers to receiv ’nbfWti nsc fe rIfjtdialJ
and extrajudicial t 1ccy’ er oftdre

and to revoke sucsuitutioci, - U ()0.7
Granted and sign Siçh San cisco,. c1ffo4

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Assistant Secretary

Firma / s6uro THERAVANCE, INC.

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SUPLRINTE1NDENCA DE INDUSTRA Y COMERCIO

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Industra y Comercio
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REQUJSITOS MINIMOS PARA ADMISON A. TRAM.I.TE
PCT

PATENTE DE 1NVENCION MODELO DE UTILIDAD 3

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+ Datos de identiflcación del solidtanteo do La persona quo presont La soilcitud

+ D.escripciÆn doIa invenclOn {,y
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+ Comprobante d,,pagO do las tasas establecidas I.1

COMPLETA 1/, . 1NCOMPLETA 3

DISEO INDUSTRIAL 3

+ Thdicación de, qe, se socita:eL registro do Diselib Industrial [
4 Dt de identificación del solicitante o de Is persona qu prosentaJa:solicitud C 3

4, RepresentaciOn grªfica y fotogrfica cel D.iseo industrial a muestra del

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COMPLETA 3

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INCOMPLETA I.

+ Indicación do quo e soUcLta el registro do Un Esquerna do Trazadb .

+ Dates do Fden.tificaciÆ.ndel salicitante a do La persona quo’ presents is.solidtud [3
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COMPLETA f

Firma Responsable

Feha

INCOMPLETA j 3.

Carrera 13No, 27-00 PIso 5 7 y 10
Conrnuador:, 3 32 08 40
Fax: 350 522O

E-mail: InfosJoctov.cQ
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REFUBLICA DE COLOMBIA

SUPERXNTENDENCIA DE XNDUSTRXA Y COMERCIO

Bogota,
2020

Doctor (a)
RODRIGUEZ DALEMAN DILIA MARIA

Apoderado(a) y/o Representante de
THERAVANCE INC.

REFERENdA Radicaciôn No. Solicituci iriternacional
Fecha inicio fase nacional TrÆmite
Evento
Actuaci6n
Of icio No.

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Como resultado del examen de forma, realizado con fundamento en el artIculo 38 de
la Decision 486 de la ComisiOn de la Comurildad Andiria, articulo 27 del Tratado de
CooperaciOn en Materia de Fatentes (PCT), regla 51 bis del Reglainento y Circular
Unica de la Superintendencia de Industria y Comercio, se le comunica que:

1. La solicitud no contiene la copia del documento en que consta la cesi6n del
derecho a la patente del inventor al solicitante o a su causante. (Art. 26-k)
DecisiOn 486.

En cumplimierito de lo dispuesto per el artIculo 39 de la DecisiOn 486, se le
requiere para que dentro del tØrmino de dos meses siguientes a la fecha de
notificaciOn del presente of icio, complete los requisitos anteriormente enunciados.
En case de no dar cumplimiento al presente requerimiento, la solicitud se
considerarÆ abandonada y perderÆ su prelaciOn.

NotifIquese el present of icio conforme a lo dispuesto en el numeral 5.2, literal
e), del capItulo ntd1 titulo primero de la Circular Unica No.10 de 2001.

’ V

ALIX CARMENZACSPEDES DE VERGEL
Jefe de la dlv si6n de Nuevas Creaciones

El anter requerimiento fue notificado per fijaciOn en lista de
fecha
adpall