QUINOLINONE-CARBOXAMIDE COMPOUNDS AS 5-HT$_4$ RECEPTOR AGONISTS

BACKGROUND OF THE INVENTION

Field of the Invention

The invention is directed to quinolinone-carboxamide compounds which are useful as 5-HT$_4$ receptor agonists. The invention is also directed to pharmaceutical compositions comprising such compounds, methods of using such compounds for treating or preventing medical conditions mediated by 5-HT$_4$ receptor activity, and processes and intermediates useful for preparing such compounds.

State of the Art

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

In particular, characterization of 5-HT$_4$ receptors and identification of pharmaceutical agents that interact with them has been the focus of significant recent activity. (See, for example, the review by Langlois and Fischmeister, *J. Med. Chem.* **2003**, *46*, 319-344.) 5-HT$_4$ receptor agonists are useful for the treatment of disorders of reduced motility of the gastrointestinal tract. Such disorders include irritable bowel syndrome (IBS), chronic constipation, functional dyspepsia, delayed gastric emptying, gastroesophageal reflux disease (GERD), gastroparesis, post-operative ileus, intestinal

pseudo-obstruction, and drug-induced delayed transit. In addition, it has been suggested that some 5-HT_4 receptor agonist compounds may be used in the treatment of central nervous system disorders including cognitive disorders, behavioral disorders, mood disorders, and disorders of control of autonomic function.

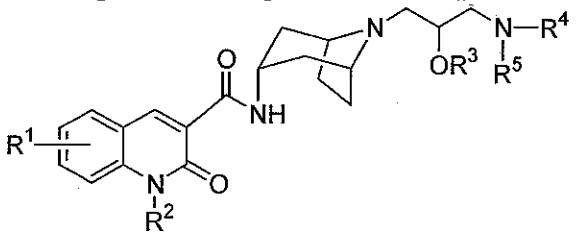
Despite the broad utility of pharmaceutical agents modulating 5-HT_4 receptor activity, few 5-HT_4 receptor agonist compounds are in clinical use at present. One agent, cisapride, that was utilized extensively for treatment of motility disorders of the gastrointestinal tract was withdrawn from the market, reportedly due to cardiac side effects. Late stage clinical trials of another agent, prucalopride, have been suspended.

Accordingly, there is a need for new 5-HT_4 receptor agonists that achieve their desired effects with minimal side effects. Preferred agents may possess, among other properties, improved selectivity, potency, pharmacokinetic properties, and/or duration of action.

SUMMARY OF THE INVENTION

The disclosure provides novel compounds that possess 5-HT_4 receptor agonist activity. Among other properties, compounds of the disclosure have been found to be potent and selective 5–HT_4 receptor agonists. In addition, compounds of the disclosure have been found to exhibit favorable pharmacokinetic properties which are predictive of good bioavailability upon oral administration.

According to a first aspect, there is provided a compound of formula (I):



(I)

wherein:

R^1 is hydrogen, halo, hydroxy, C_{1-4}alkyl, or C_{1-4}alkoxy;

R^2 is C_{3-4}alkyl, or C_{3-6}cycloalkyl;

R^3 is hydrogen or C_{1-3}alkyl;

R^4 is —$S(O)_2R^6$ or —$C(O)R^7$;

R^5 is hydrogen, C_{1-3}alkyl, C_{2-3}alkyl substituted with –OH or C_{1-3}alkoxy, or —CH_2—pyridyl;

R^6 is C_{1-3}alkyl;

or, R^5 and R^6 taken together form C_{3-4}alkylenyl; and

R^7 is hydrogen, C_{1-3}alkyl, or pyridyl;

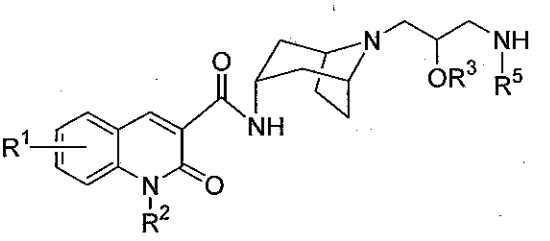
or a pharmaceutically-acceptable salt or solvate or stereoisomer thereof.

In a second aspect, there is provided a pharmaceutical composition comprising a therapeutically effective amount of the compound as defined above and a pharmaceutically acceptable carrier.

In a third aspect, there is provided a compound as defined above for use in therapy.

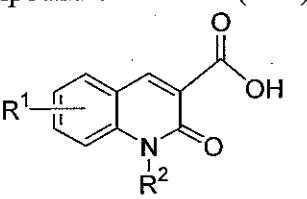
In a fourth aspect, there is provided the use of a compound as defined above for manufacture of a medicament for the treatment of a disorder of reduced motility of the gastrointestinal tract in a mammal.

In a fifth aspect, there is provided a process for preparing a compound as defined above, the process comprising: (a) reacting a compound of formula (III):



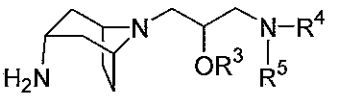
(III)

with compound of the formula L—R^4 wherein L is a leaving group, or L—R^4 represents HO-C(O)R^7; or (b) reacting a compound of formula (VIII):



(VIII)

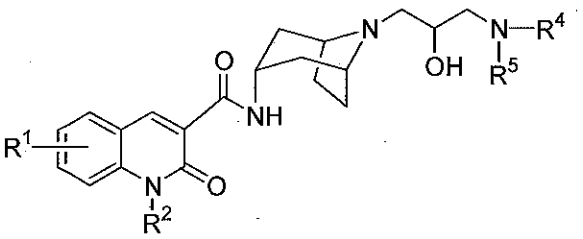
with a compound of formula (IX):



(IX)

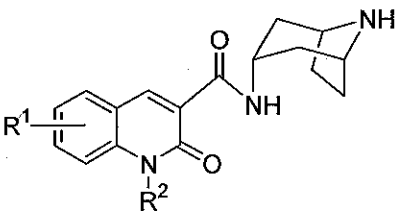
to provide a compound of formula (I), or a pharmaceutically-acceptable salt or solvate or stereoisomer thereof.

In a sixth aspect, there is provided a process for preparing a compound of formula (I'),



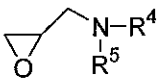
(I')

wherein R^1, R^2, R^4, and R^5 are defined above; or a pharmaceutically-acceptable salt or solvate or stereoisomer thereof, the process comprising reacting a compound of formula (IV):



(IV)

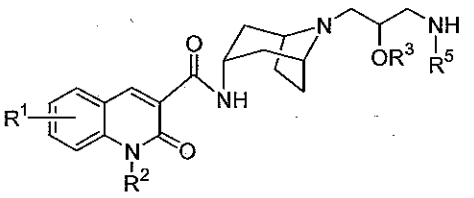
or a salt thereof with a compound of formula (XI):



(XI)

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

In a seventh aspect, there is provided a compound of formula (III):



(III)

wherein:

R^1 is hydrogen, halo, hydroxy, C_{1-4}alkyl, or C_{1-4}alkoxy;

R^2 is C_{3-4}alkyl, or C_{3-6}cycloalkyl;

R^3 is hydrogen or C_{1-3}alkyl;

R^4 is —$S(O)_2R^6$ or —$C(O)R^7$; and

R^5 is hydrogen, C_{1-3}alkyl, C_{2-3}alkyl substituted with –OH or C_{1-3}alkoxy, or —CH_2—pyridyl; or a salt or stereoisomer or protected derivative thereof.

In an eighth aspect, there is provided an in vitro method of studying a biological system or sample comprising a 5-HT_4 receptor, the method comprising: (a) contacting the biological system or sample with a compound as defined above; and (b) determining the effect caused by the compound on the biological system or sample.

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

The disclosure also provides a method of treating a disease or condition associated with 5-HT_4 receptor activity, e.g. a disorder of reduced motility of the gastrointestinal tract, the method comprising administering to the mammal, a therapeutically effective amount of a compound of the disclosure.

Further, the disclosure provides a method of treating a disease or condition associated with 5-HT_4 receptor activity in a mammal, the method comprising administering to the mammal, a therapeutically effective amount of a pharmaceutical composition of the disclosure.

The compounds of the disclosure can also be used as research tools, i.e. to study biological systems or samples, or for studying the activity of other chemical compounds. Accordingly, in another of its method embodiments, the disclosure provides a method of using a compound of formula (I), or a pharmaceutically acceptable salt or solvate or stereoisomer thereof, as a research tool for

studying a biological system or sample or for discovering new 5-HT_4 receptor agonists, the method comprising contacting a biological system or sample with a compound of the disclosure and determining the effects caused by the compound on the biological system or sample.

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

The disclosure also provides a compound of the disclosure as described herein for use in medical therapy, as well as the use of a compound of the disclosure in the manufacture of a formulation or medicament for treating a disease or condition associated with 5-HT_4 receptor activity, e.g. a disorder of reduced motility of the gastrointestinal tract, in a mammal.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides novel quinolinone-carboxamide 5-HT_4 receptor agonists of formula (I), or pharmaceutically-acceptable salts or solvates or stereoisomers thereof. The following substituents and values are intended to provide representative examples of various aspects of this invention. These representative values are intended to further define such aspects and are not intended to exclude other values or limit the scope of the invention.

In a specific aspect of the invention, R^1 is hydrogen, halo, C_{1-4}alkyl, or C_{1-4}alkoxy.

In other specific aspects, R^1 is hydrogen, halo, or C_{1-4}alkyl; or R^1 is hydrogen or halo; or R^1 is fluoro; or R^1 is bromo.

In yet another specific aspect, R^1 is hydrogen.

In a specific aspect, R^2 is C_{3-4}alkyl or C_{3-6}cycloalkyl.

In another specific aspect, R^2 is C_{3-4}alkyl. Representative R^2 groups include *n*-propyl, isopropyl, *n*-butyl, *sec*-butyl, and *tert*-butyl.

In another specific aspect, R^2 is isopropyl.

In yet other specific aspects R^2 is C_{3-4}alkyl or C_{4-5}cycloalkyl; or R^2 is isopropyl or C_{4-5}cycloalkyl.

In a specific aspect, R^3 is hydrogen or C_{1-3}alkyl.

In other specific aspects, R^3 is hydrogen, or R^3 is methyl.

In a specific aspect, R^4 is $—S(O)_2R^6$ wherein R^6 is C_{1-3}alkyl.

In another specific aspect, R^4 is $—S(O)_2CH_3$.

In a specific aspect, R^4 is $—C(O)R^7$ wherein R^7 is hydrogen, C_{1-3}alkyl or pyridyl.

In other specific aspects, R^4 is $—C(O)R^7$ wherein R^7 is hydrogen or C_{1-3}alkyl; or R^4 is $—C(O)R^7$ wherein R^7 is hydrogen or methyl; or R^4 is $—C(O)R^7$ wherein R^7 is hydrogen; or R^4 is $—C(O)R^7$ wherein R^7 is methyl.

In yet another specific aspect, R^4 is $—C(O)R^7$ wherein R^7 is 3-pyridyl or 4-pyridyl.

In a specific aspect, R^5 is hydrogen; C_{1-3}alkyl; C_{2-3}alkyl substituted with –OH or C_{1-3}alkoxy; or $—CH_2—$pyridyl.

In other specific aspects R^5 is hydrogen, C_{1-3}alkyl, or $—CH_2—$pyridyl; or R^5 is hydrogen or C_{1-3}alkyl.

In yet other specific aspects, R^5 is $—CH_2—$3-pyridyl; or R^5 is hydrogen or methyl; or R^5 is hydrogen; or R^5 is methyl.

In yet other specific aspects, R^5 and R^6 taken together form —$(CH_2)_3$— or —$(CH_2)_4$; or R^5 and R^6 taken together form —$(CH_2)_3$—.

In one aspect, the invention provides a compound of formula (I) wherein R^3 is hydrogen.

In another aspect, the invention provides a compound of formula (I) wherein R^4 is —$S(O)_2R^6$.

In another aspect, the invention provides a compound of formula (I) wherein R^4 is —$C(O)R^7$

The invention further provides a compound of formula (I) wherein R^1 is hydrogen or halo; R^2 is isopropyl or C_{4-5}cycloalkyl; and R^3, R^4, R^5, R^6, and R^7 are defined as in formula (I).

In yet another aspect, the invention provides a compound of formula (I) wherein:

R^1 is hydrogen;

R^2 is C_{3-4}alkyl or C_{4-5}cycloalkyl;

R^3 is hydrogen;

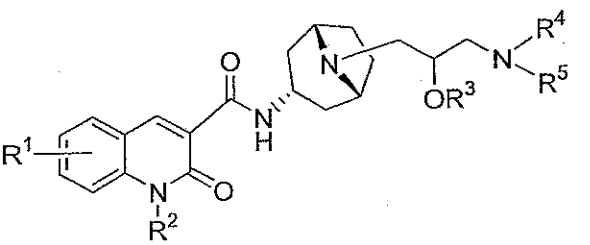
R^4 is —$S(O)_2R^6$ or —$C(O)R^7$;

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

R^6 is C_{1-3}alkyl; and

R^7 is hydrogen or C_{1-3}alkyl.

In yet another aspect, the invention provides a group of compounds of formula (II):



(II)

wherein R^1 is hydrogen, R^2 is isopropyl, and R^3, R^4, R^5, and R^6, or R^3, R^4, R^5, and R^7 take the values shown in Table I and Table II, respectively.

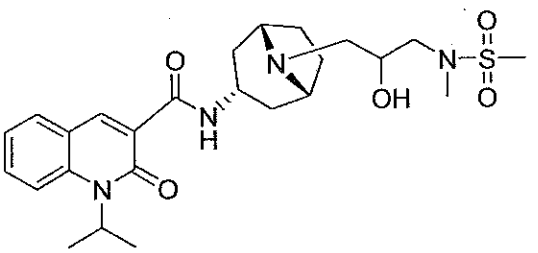
Table I

$R^4 = —S(O)_2R^6$			
Example No.	R^3	R^5	R^6
1	H	CH_3	CH_3
2	CH_3	CH_3	CH_3
3	CH_3	—CH_2-3-pyridyl	CH_3
4	H	H	CH_3
5	H	—CH_2-3-pyridyl	CH_3
6	H	CH_3	CH_3
7	H	CH_3	CH_3
21	H	C_2H_5	CH_3
22	H	H	CH_3
23	H	—$(CH_2)_3$—	

Table II

$R^4 = —C(O)R^7$			
Example No.	R^3	R^5	R^7
8	H	CH_3	4-pyridyl
9	H	H	4-pyridyl
10	CH_3	CH_3	CH_3
11	CH_3	CH_3	4-pyridyl
12	CH_3	—CH_2-3-pyridyl	CH_3
13	H	H	CH_3
14	H	CH_3	CH_3
15	H	CH_3	H
16	H	H	H
17	H	CH_3	CH_3
18	H	CH_3	H

The chemical naming conventions used herein are illustrated for the compound of Example 1:



which is designated 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide, according to the AutoNom software, provided by MDL Information Systems, GmbH (Frankfurt, Germany). The designation (1*S*,3*R*,5*R*) describes the relative orientation of the bonds associated with the bicyclic ring system that are depicted as solid and dashed wedges. The compound is alternatively denoted as *N*-[(3-endo)-8-[2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl]-1-(1-methylethyl)-2-oxo-1,2-dihydro-3-quinolinecarboxamide. In all of the compounds of the invention depicted above, the quinolinone-carboxamide is endo to the azabicyclooctane group.

Particular mention may be made of the following compounds

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(methanesulfonylamino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*S*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(acetyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(formyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-3-(acetyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-3-(formyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide; and

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonylamino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide.

As exemplified by particular compounds listed above, the compounds of the invention may contain a chiral center, specifically, at the carbon atom in formulas (I) or (II) bearing the substituent —OR^3. Accordingly, the invention includes racemic mixtures, pure stereoisomers, and stereoisomer-enriched mixtures of such isomers, unless otherwise

indicated. When a particular stereoisomer is shown, it will be understood by those skilled in the art, that minor amounts of other stereoisomers may be present in the compositions of the invention unless otherwise indicated, provided that any utility of the composition as a whole is not eliminated by the presence of such other isomers.

Definitions

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

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

The term "alkylenyl" means a divalent saturated hydrocarbon group which may be linear or branched or combinations thereof. Unless otherwise defined, such alkylenyl groups typically contain from 1 to 10 carbon atoms. Representative alkylenyl groups include, by way of example, methylene, ethylene, *n*-propylene, *n*-butylene, propane –1,2-diyl (1-methylethylene), 2-methylpropane-1,2-diyl (1,1-dimethylethylene) and the like.

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

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

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

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

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

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

(a) preventing the disease, disorder, or medical condition from occurring, i.e., prophylactic treatment of a patient;

(b) ameliorating the disease, disorder, or medical condition, i.e., eliminating or causing regression of the disease, disorder, or medical condition in a patient;

(c) suppressing the disease, disorder, or medical condition, i.e., slowing or arresting the development of the disease, disorder, or medical condition in a patient; or

(d) alleviating the symptoms of the disease, disorder, or medical condition in a patient.

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

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

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

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

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

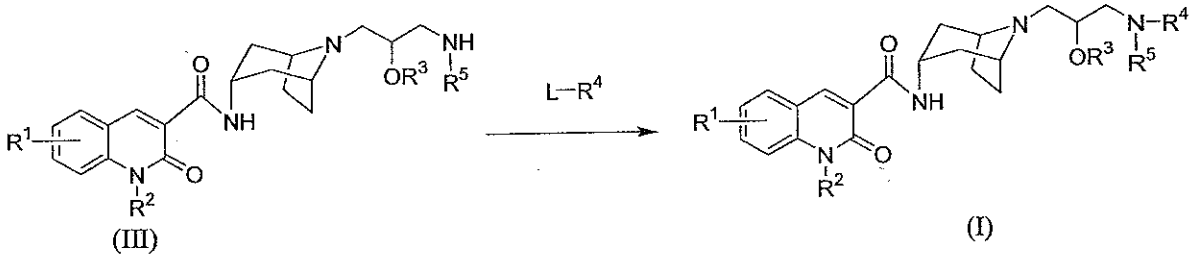
General Synthetic Procedures

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

Additionally, as will be apparent to those skilled in the art, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. The choice of a suitable protecting group for a particular functional group, as well as suitable conditions for protection and deprotection, are well known in the art. For example, numerous protecting groups, and their introduction and removal, are described in T. W. Greene and G. M. Wuts, *Protecting Groups in Organic Synthesis*, Third Edition, Wiley, New York, 1999, and references cited therein.

In one method of synthesis, compounds of formula (I) are prepared as illustrated in Scheme A. (The substituents and variables shown in the following schemes have the definitions provided above unless otherwise indicated).

Scheme A



(III) (I)

In Scheme A, L represents a leaving group such as chloro, bromo, iodo, or ethoxy, or the reagent L—R^4 is the carboxylic acid HO-C(O)R^7, i.e. L formally represents hydroxy.

Optimal reaction conditions for the reaction of Scheme A may vary depending on the chemical properties of the reagent L—R^4, as is well known to those skilled in the art.

For example, when L is a halo leaving group, such as chloro, the reaction is typically conducted by contacting intermediate (III) with between about 1 and about 4 equivalents of a compound of formula L—R^4 in an inert diluent, such as dichloromethane, in the presence of an excess of base, for example between about 3 and about 6 equivalents, of base, such as *N,N*-diisopropylethylamine or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Suitable inert diluents also include *N,N*-dimethylformamide, trichloromethane, 1,1,2,2-tetrachloroethane, tetrahydrofuran, and the like. The reaction is typically conducted at a temperature in the range of about -100 °C to about 30 °C for about a quarter hour to about 2 hours, or until the reaction is substantially complete. Exemplary reagents L—R^4 in which L is chloro include methanesulfonylchloride and acetylchloride.

When the reagent L—R^4 is a carboxylic acid, Scheme A represents an amide coupling reaction which is typically conducted by contacting intermediate (III) with between about 1 and about 4 equivalents of a compound of a carboxylic acid L—R^4 in an inert diluent, for example, *N,N*-dimethylformamide, in the presence of a coupling agent such as benzotriazol-1-yloxytripyrrolidino-phosphonium hexafluorophosphate (PyBop). The reaction is typically conducted at ambient temperature, for about a quarter hour to about 2 hours, or until the reaction is substantially complete. Suitable alternative coupling agents include 1,3 dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC), and PyBop combined with 1-hydroxy-7-azabenzotriazole (HOAt).

The amide coupling of intermediate (III) with the carboxylic acid L—R^4 alternatively can be performed by converting L—R^4 to an activated ester, such as an

N-hydroxy succinimide (NHS) ester or a *p*-nitrophenyl ester, or an acid imidazole, which is then reacted with intermediate (III).

Alternatively, when the reagent L—R^4 is a liquid, for example ethyl formate, the reaction can be performed by dissolving (III) in a large excess of the reagent L—R^4, and heating to a temperature of between about 50 °C and about 100 °C for about 12 to about 24 hours.

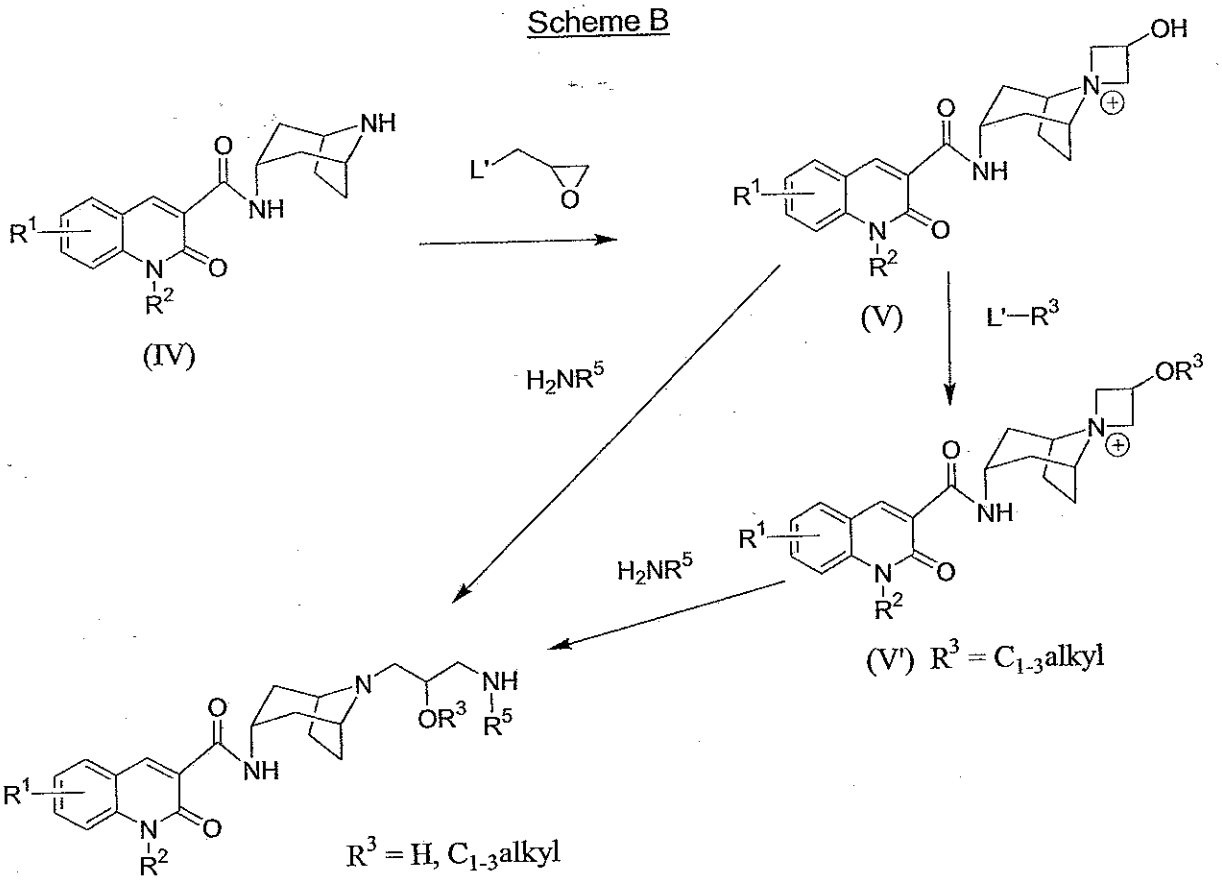
The product of formula (I) is isolated and purified by conventional procedures. For example, the product can be concentrated to dryness under reduced pressure, taken up in an aqueous weak acid solution and purified by HPLC chromatography.

Alternatively, compounds of formula (I) can be prepared by *N*-alkylating a compound of formula (I) in which R^2 is hydrogen, which can be prepared according to Scheme A. The *N*-alkylation reaction is typically conducted by contacting a compound of formula (I) in which R^2 is hydrogen with between about 1 and about 4 equivalents of a compound of the formula L'—R^2 in which L' is a leaving group such as iodo or bromo. This reaction is typically conducted in a polar aprotic solvent such as dimethylformamide in the presence of between about 2 and about 4 equivalents of strong base, such as potassium *tert*-butoxide. Typically, the reaction is performed at a temperature of between about 60 °C and about 100 °C for between about 6 and about 24 hours, or until the reaction is substantially complete.

In yet another alternative, compounds of formula (I) in which R^1 is other than hydrogen are prepared by conventional processes from compounds of formula (I) in which R^1 is hydrogen.

Intermediates of formula (III) are prepared from readily available starting materials. For example, when the carbon bearing the substituent —OR^3 is not chiral, an intermediate of formula (III) is prepared by the procedure illustrated in Scheme B.

Scheme B



where L' independently represents a halo leaving group such as bromo, chloro, or iodo. A negatively-charged counterion is also present associated with the positively-charged intermediate (V) or (V').

First, an intermediate of formula (IV) is reacted with an oxirane compound, for example, 2-bromomethyloxirane (commonly, epibromohydrin) to form an azetidine salt of formula (V). This reaction is typically conducted by contacting (IV) with between about 2 and about 4 equivalents of 2-bromomethyloxirane in a polar diluent, such as ethanol. The reaction is typically conducted at ambient temperature for between about 24 and about 48 hours or until the reaction is substantially complete.

It will be understood that in the process of Scheme B and in other processes described below using intermediate (IV), intermediate (IV) can be supplied in the form of the freebase or in a salt form, with appropriate adjusment of reaction conditions, as necessary, as known to those skilled in the art.

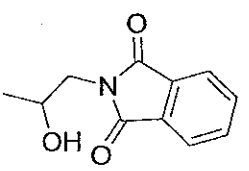
An intermediate of formula (V'), in which R^3 is C_{1-3}alkyl, can be prepared by contacting intermediate (V) with from slightly less than one equivalent to about one

equivalent of a compound of formula L'—R^3, where R^3 is C_{1-3}alkyl, in an inert diluent in the presence of between about 1 and about 3 equivalents of a strong base, such as potassium *tert*-butoxide or sodium hydride. The reaction is typically conducted at ambient temperature for between about a quarter hour to an hour, or until the reaction is substantially complete. Suitable inert diluents include dichloromethane, trichloromethane, 1,1,2,2-tetrachloroethane, and the like.

Next, the azetidine intermediate (V) or (V') is reacted with an amine of the formula H_2NR^5 to provide the intermediate (III). Typically, the azetidine intermediate is dissolved in an inert diluent, such as ethanol, and contacted with between about 1 and about 8 equivalents of the amine H_2NR^5. For example, when the amine H_2NR^5 is a volatile reagent, such as methylamine, preferably, between about 5 and about 7 equivalents of the amine are used. The reaction is typically conducted at a temperature of between about 50 °C and about 100 °C for between about 12 and about 24 hours or until the reaction is substantially complete.

An intermediate of formula (III) in which R^5 is hydrogen, can be prepared from the azetidine intermediate (V) or (V') using ammonium formate in place of ammonia, i.e. in place of the reagent H_2NR^5 indicated in Scheme B. Alternatively, to prepare intermediate (III) where R^5 is hydrogen, the azetidine ring of (V) or (V') can be opened by reaction with an azide, such as sodium azide, which is then followed by a reduction reaction to provide intermediate (III), or the ring can be opened by reaction with ammonium hydroxide.

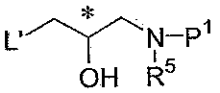
As described in detail in Example 4a, when R^3 and R^5 are hydrogen and the carbon bearing the substituent —OR^3 is not chiral, an intermediate of formula (III) can be prepared by reacting intermediate (IV) with an oxiranylmethyl compound having a protected nitrogen atom and then deprotecting. One useful reagent is 2-oxiranylmethyl-isoindole-1,3-dione, commonly epoxypropylphthalimide, which is reacted with intermediate (IV) to form an intermediate in which a phthalimidyl-substituted 2-hydroxy propyl group:



is joined to the nitrogen of the azabicylcooctane ring of formula (IV). The phthalimidyl group is then removed by refluxing in hydrazine to form an intermediate of formula (III) in which R^3 and R^5 are hydrogen.

An intermediate of formula (III) in which R^3 and R^5 are hydrogen can also be prepared by reaction of the azetidine (V) with the anion of phthalimide and subsequent treatment with hydrazine.

In an alternative method of synthesis, an intermediate of formula (III) in which R^3 is hydrogen, can be prepared by reaction of intermediate (IV) with a protected intermediate (VI):

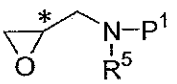


(VI)

followed by a deprotection step. In formula (VI), P^1 is an amino-protecting group, L' is a halo leaving group, and the asterisk denotes a chiral center. The process utilizing an intermediate of formula (VI) is useful for preparing forms of intermediate (III) in which the stereochemistry at the center marked by the aserisk is specifically (*R*) or (*S*) as well as for preparing non-chiral forms of intermediate (III).

Typically, intermediate (IV) is contacted with between about 1 and about 2 equivalents of intermediate (VI) in a polar diluent, such as methanol, in the presence of more than one equivalent of a base, such as *N*,*N*-diisopropylethylamine. The reaction is typically conducted at a temperature of between about 60 °C and about 100 °C for between about 12 and about 24 hours, or until the reaction is substantially complete. The protecting group P^1 is removed by standard procedures to provide an intermediate of formula (III). A useful protecting group P^1 is Boc, which is typically removed by treatment with an acid, such as trifluoroacetic acid.

In yet another alternative process for the preparation of intermediate (III), intermediate (VI) can first be converted to a cyclized form (VII):



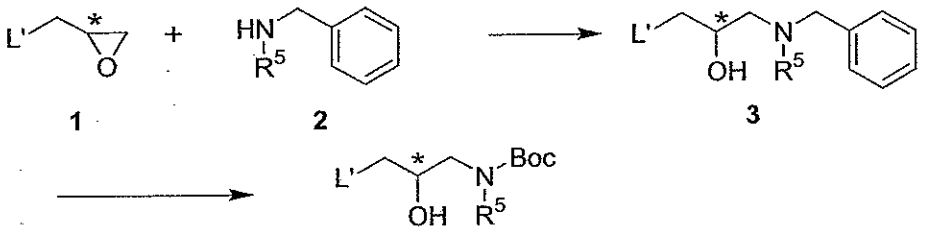
(VII)

before reaction with intermediate (IV) to provide intermediate (III). Intermediate (VII) is typically prepared by dissolving intermediate (VI) in an inert diluent, for example, tetrahydrofuran, in the presence of base, for example sodium hydroxide. The reaction of

(VII) with (IV) to provide intermediate (III) is typically performed by contacting intermediate (IV) with between about 1 and about 4 equivalents of intermediate (VII) in a polar diluent, such as methanol. The reaction is typically conducted at a temperature of between about 60 °C to about 100 °C for between about 1 and about 4 hours, or until the reaction is substantially complete. The protecting group P^1 is removed by standard procedures to provide an intermediate of formula (III).

The protected intermediate (VI) can be prepared from an oxirane as illustrated in Scheme C for the particular example of forming a Boc-protected chiral intermediate (VI') using a chiral oxirane. The reaction is equally useful for the preparation of non-chiral compounds of formula (VI).

Scheme C



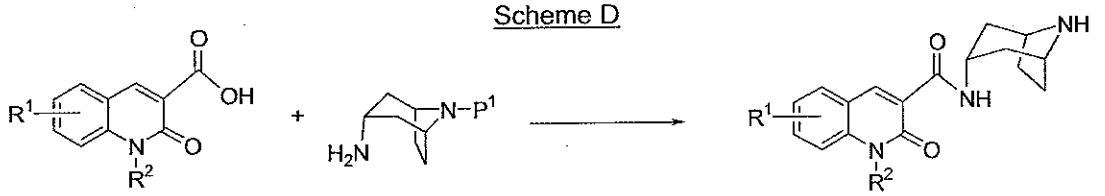
1 2 3

(VI')

As shown in Scheme C, a benzylamine **2** is contacted with at least one equivalent of a chiral oxirane **1** in a non-polar diluent such as hexane or toluene to form the 2-hydroxypropylamine **3**. The reaction is typically conducted at room temperature for between about 12 and about 24 hours, or until the reaction is substantially complete. The intermediate **3** is typically reacted with a slight excess of di-*tert*-butyl dicarbonate (commonly $(Boc)_2O$), for example, about 1.1 equivalents, under a hydrogen atmosphere in the presence of a transition metal catalyst to provide the Boc protected intermediate (VI'). The reaction is typically conducted at ambient temperature for between about 8 to about 24 hours.

A process for preparing intermediates of formula (IV) is shown in Scheme D.

Scheme D



(VIII) 5 (IV)

The protected aminoazabicyclooctane, or commonly, aminotropane **5** is first reacted with the substituted quinolinone carboxylic acid (VIII). Typically, this reaction is conducted by first converting (VIII) to an acid chloride by contacting (VIII) with at least one equivalent, preferably between about 1 and about 2 equivalents of an activating agent, such as thionyl chloride or oxalyl chloride in an aromatic diluent, such as toluene, benzene, xylene, or the like. The reaction is typically conducted at a temperature ranging from about 80 °C to about 120 °C for about 15 minutes to about 4 hours, or until the reaction is substantially complete.

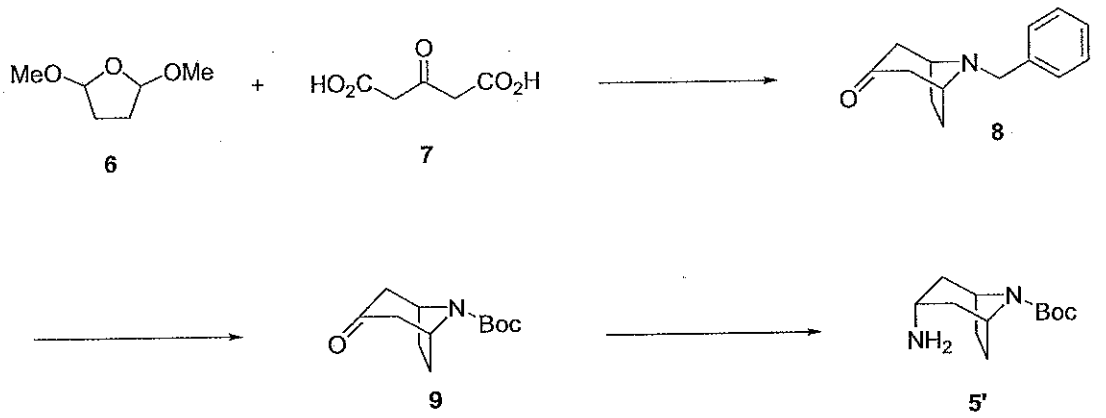
The acid chloride solution is typically added to a biphasic mixture of about 1 equivalent of the aminotropane **5** to form a protected intermediate, which is extracted by standard procedures. The biphasic mixture of **5** is generally prepared by dissolving **5** in an aromatic diluent, such as used above, and adding an aqueous solution containing an excess of base, such as sodium hydroxide or potassium hydroxide, preferably about 2 to 5 equivalents of base.

Alternatively, the amide coupling of intermediate **5** with the carboxylic acid (VIII) can be performed in the presence of a coupling agent such as 1,3 dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC), or benzotriazol-1-yloxytripyrrolidino-phosphonium hexafluorophosphate (PyBop), optionally combined with 1-hydroxy-7-azabenzotriazole (HOAt), as described above for the amide coupling of intermediate (III) with a carboxylic acid. In yet another alternative, the amide coupling of intermediate **5** with the carboxylic acid (VIII) can be performed by converting (VIII) to an activated ester, also described above.

The protecting group P^1 is removed by standard procedures to provide an intermediate of formula (IV). For example when the protecting group is Boc, typically removal is by treatment with an acid, such as trifluoroacetic acid, providing the acid salt of the intermediate. The acid salt of intermediate (IV) can be converted to the free base, if desired, by conventional treatment with base. The protecting group Cbz, for another example, is conveniently removed by hydrogenolysis over a suitable metal catalyst such as palladium on carbon.

The protected aminotropane **5** employed in the reactions described in this application is prepared from readily available starting materials. For example, when the protecting group P^1 is Boc, the protected aminotropane **5'** is prepared by the procedure illustrated in Scheme E.

Scheme E



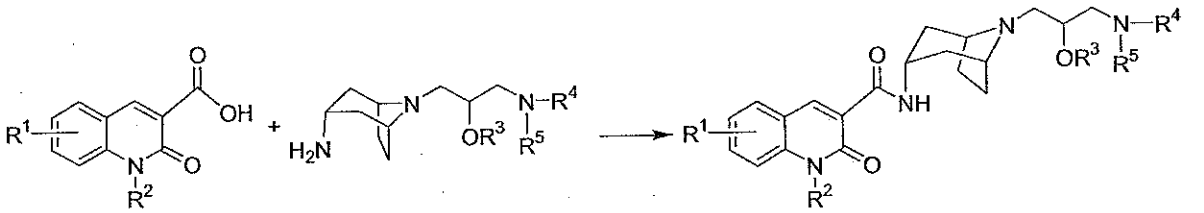
6 7 8 9 5'

As described in detail in Example 1a below, to prepare the protected intermediate **5'**, first, 2,5-dimethoxy tetrahydrofuran **6** is contacted with between about 1 and 2 equivalents, preferably about 1.5 equivalents of benzyl amine and a slight excess, for example about 1.1 equivalents, of 1,3-acetonedicarboxylic acid **7** in an acidic aqueous solution in the presence of a buffering agent such as sodium hydrogen phosphate. The reaction mixture is heated to between about 60 and about 100 °C to ensure decarboxylation of any carboxylated intermediates in the product, 8-benzyl-8-azabicyclo[3.2.1]octan-3-one **8**, commonly *N*-benzyltropanone.

The intermediate **8** is typically reacted with a slight excess of di-*tert*-butyl dicarbonate (commonly $(Boc)_2O$), for example, about 1.1 equivalents, under a hydrogen atmosphere in the presence of a transition metal catalyst to provide the Boc protected intermediate **9**, 3-oxo-8-azabicyclo[3.2.1]octane-8-carboxylic acid *tert*-butyl ester. The reaction is typically conducted at ambient temperature for about 12 to about 72 hours. Finally, intermediate **9** is contacted with a large excess, for example at least about 25 equivalents, of ammonium formate in an inert diluent, such as methanol, in the presence of a transition metal catalyst to provide the product **5'**in the *endo* configuration with high stereospecificity, for example *endo* to *exo* ratio of >99:1. The reaction is typically conducted at ambient temperature for about 12 to about 72 hours or until the reaction is substantially complete. It is advantageous to add the ammonium formate reagent in portions. For example, intermediate **9** is contacted with an initial portion of ammonium formate of about 15 to about 25 equivalents. After an interval of about 12 to about 36 hours, an additional portion of about 5 to about 10 equivalents of ammonium formate is added. The subsequent addition can be repeated after a similar interval. The product **5'** can be purified by conventional procedures, such as alkaline extraction.

In an alternative method of synthesis, compounds of formula (I) are prepared by coupling the substituted quinolinone carboxylic acid (VIII) with an intermediate of formula (IX) as illustrated in Scheme F.

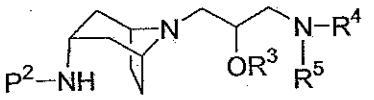
Scheme F



(VIII) (IX) (I)

The reaction of Scheme F is typically conducted under the amide coupling conditions described above for the reaction of the carboxylic acid (VIII) with intermediate **5**.

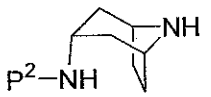
Intermediates of formula (IX) can be prepared by deprotecting an intermediate of formula (X):



(X)

where P^2 represents an amino-protecting group.

Intermediates of formula (X) can be prepared from readily available starting materials using procedures analogous to the alkylation and other reactions described above and/or using alternative reactions well known to those skilled in the art. For example, intermediate (X) can be prepared using an intermediate **10**

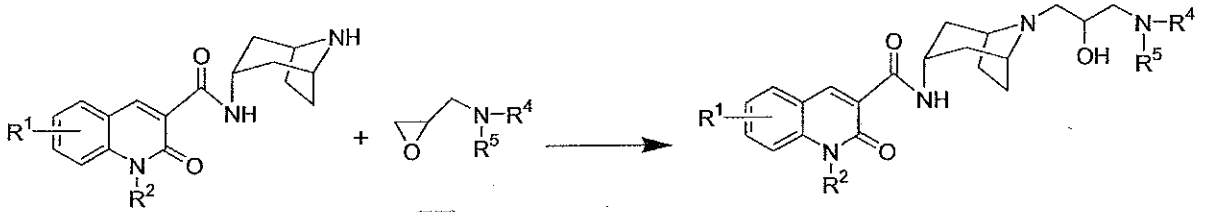


10

which may be formed by protecting the amino nitrogen of the aminoazobicyclooctane **5** with amino-protecting group P^2 and then removing P^1 from the nitrogen of the azabicyclooctane group. Protecting groups P^1 and P^2 are chosen such that they are removed under different conditions. For example when P^1 is chosen as Boc, then Cbz can be used as P^2. Substituting the protected aminotropane **10** for intermediate (IV) in the reactions described above for the preparation of intermediate (III) provides intermediates of formula (X).

In yet another method of synthesis, compounds of formula (I) in which R^3 is hydrogen, represented below as formula (I'), can be prepared as illustrated in Scheme G.

Scheme G



(IV) (XI) (I')

Intermediate (XI) may contain a chiral center, as shown explicitly for the protected oxirane intermediate (VII).

Typically, intermediate (IV) is contacted with between about 1 and about 2 equivalents of the oxirane intermediate (XI) in a polar diluent, such as ethanol to form the product (I'). Intermediate (IV) can be supplied in salt form in which case a slight molar excess of alkaline base is included in the reaction mixture prior to the addition of the oxirane. The reaction is typically conducted at a temperature of about 60 °C to about 100 °C for between about 1 and about 3 hours, or until the reaction is substantially complete. The product can be isolated by crystallization from an inert diluent as the free base or as an acid salt.

Intermediates of formula (XI) can be prepared by reaction of the oxirane intermediate **1**, illustrated in Scheme C, with the secondary amine HNR^4R^5. Typically, an aqueous solution of the amine HNR^4R^5 containing about 1 equivalent of a base, such as sodium hydroxide, lithium hydroxide, cesium hydroxide, or potassium hydroxide, is contacted with between about 1.5 and about 2.5 equivalents of the oxirane intermediate **1**. The reaction is typically conducted at a temperature of between about 0 °C and about 10 °C for between about 12 and about 30 hours, or until the reaction is substantially complete.

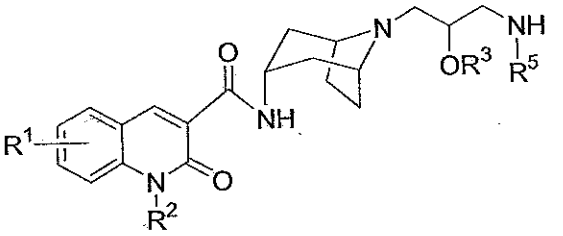
The quinolinone carboxylic acid (VIII) is readily prepared by procedures similar to those reported in the literature in Suzuki *et al, Heterocycles*, **2000**, *53*, 2471-2485 and described in the examples below.

The reagents L'—R^2, L'—R^3, L—R^4, H_2NR^5, and HNR^4R^5 are available commercially or are readily prepared by standard procedures from common starting materials.

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

Accordingly, in a method aspect, the invention provides a process for preparing a compound of formula (I), or a salt or stereoisomer thereof, the process comprising:

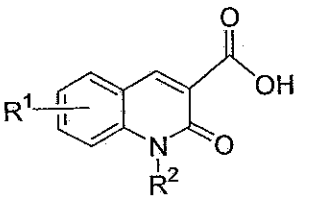
(a) reacting a compound of formula (III):



(III)

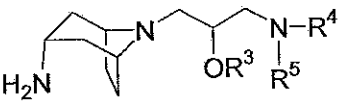
with compound of the formula L—R^4 wherein L is a leaving group, or L—R^4 represents HO-C(O)R^7; or

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



(VIII)

with a compound of formula (IX):

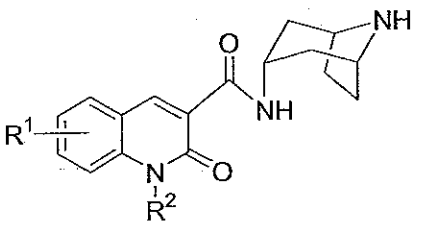


(IX)

to provide a compound of formula (I), or a salt or stereoisomer thereof.

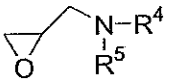
The invention further provides a compound of formula (III), or a salt or stereoisomer or protected derivative thereof, wherein R^1, R^2, R^3, and R^5 are defined as in formula (I).

In an additional method aspect, the invention provides a process for preparing a compound of formula (I') wherein R^1, R^2, R^4, and R^5 are defined as in formula (I), or a salt or stereoisomer thereof, the process comprising reacting a compound of formula (IV):



(IV)

or a salt thereof with a compound of formula (XI):



(XI)

to provide a compound of formula (I') or a salt or stereoisomer thereof.

Pharmaceutical Compositions

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

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

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

Any conventional carrier or excipient may be used in the pharmaceutical compositions of the invention. The choice of a particular carrier or excipient, or combinations of carriers or excipients, will depend on the mode of administration being used to treat a particular patient or type of medical condition or disease state. In this

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

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

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

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

In a preferred embodiment, the pharmaceutical compositions of the invention are suitable for oral administration. Suitable pharmaceutical compositions for oral administration may be in the form of capsules, tablets, pills, lozenges, cachets, dragees, powders, granules; or as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil liquid emulsion; or as an elixir or syrup; and the like; each containing a predetermined amount of a compound of the present invention as an active ingredient.

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

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

phthalate, methacrylic acid!methacrylic acid ester copolymers, cellulose acetate trimellitate (CAT), carboxymethyl ethyl cellulose (CMEC), hydroxypropyl methyl cellulose acetate succinate (HPMCAS), and the like.

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

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

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

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

When administered by inhalation using a pressurized container, the pharmaceutical compositions of the invention will typically comprise the active ingredient

and a suitable propellant, such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas.

Additionally, the pharmaceutical composition may be in the form of a capsule or cartridge (made, for example, from gelatin) comprising a compound of the invention and a powder suitable for use in a powder inhaler. Suitable powder bases include, by way of example, lactose or starch.

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

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

Formulation Example A

Hard gelatin capsules for oral administration are prepared as follows:

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

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

Formulation Example B

Hard gelatin capsules for oral administration are prepared as follows:

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

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

Formulation Example C

Capsules for oral administration are prepared as follows:

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

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

Formulation Example D

Tablets for oral administration are prepared as follows:

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

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

Formulation Example E

Tablets for oral administration are prepared as follows:

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

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

Formulation Example F

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

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

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

Formulation Example G

A suspension for oral administration is prepared as follows:

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

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

Formulation Example H

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

Ingredients	Amount
Compound of the invention	1.0 mg
Lactose	25 mg

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

Formulation Example I

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

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

Formulation Example J

An injectable formulation is prepared as follows:

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

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

Formulation Example K

Capsules for oral administration are prepared as follows:

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

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

Formulation Example L

Capsules for oral administration are prepared as follows:

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

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

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

Utility

The quinolinone-carboxamide compounds of the invention are 5-HT_4 receptor agonists and therefore are expected to be useful for treating medical conditions mediated by 5-HT_4 receptors or associated with 5-HT_4 receptor activity, i.e. medical conditions which are ameliorated by treatment with a 5-HT_4 receptor agonist. Such medical conditions include, but are not limited to, irritable bowel syndrome (IBS), chronic constipation, functional dyspepsia, delayed gastric emptying, gastroesophageal reflux disease (GERD), gastroparesis, post-operative ileus, intestinal pseudo-obstruction, and

drug-induced delayed transit. In addition, it has been suggested that some 5-HT_4 receptor agonist compounds may be used in the treatment of central nervous system disorders including cognitive disorders, behavioral disorders, mood disorders, and disorders of control of autonomic function.

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

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

When used to treat disorders of reduced motility of the GI tract or other conditions mediated by 5-HT_4 receptors, the compounds of the 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 typically be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered and its relative activity, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

Suitable doses for treating disorders of reduced motility of the GI tract or other disorders mediated by 5-HT_4 receptors will range from about 0.0007 to about 20 mg/kg/day of active agent, including from about 0.0007 to about 1 mg/kg/day. For an average 70 kg human, this would amount to from about 0.05 to about 70 mg per day of active agent.

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

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

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

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

The invention also provides a method of treating a mammal having a disease or condition associated with 5-HT_4 receptor activity, the method comprising administering to the mammal a therapeutically effective amount of a compound of the invention or of a pharmaceutical composition comprising a compound of the invention.

As described above, compounds of the invention are 5-HT_4 receptor agonists. The invention further provides, therefore, a method of agonizing a 5-HT_4 receptor in a mammal, the method comprising administering a compound of the invention to the mammal. In addition, the compounds of the invention are also useful as research tools for investigating or studying biological systems or samples having 5-HT_4 receptors, or for discovering new 5-HT_4 receptor agonists. Moreover, since compounds of the invention exhibit binding selectivity for 5-HT_4 receptors as compared with binding to receptors of other 5-HT subtypes, particularly 5-HT_3 receptors, such compounds are particularly useful for studying the effects of selective agonism of 5-HT_4 receptors in a biological system or sample. Any suitable biological system or sample having 5-HT_4 receptors may be employed in such studies which may be conducted either *in vitro* or *in vivo*. Representative biological systems or samples suitable for such studies include, but are not limited to, cells, cellular extracts, plasma membranes, tissue samples, mammals (such as mice, rats, guinea pigs, rabbits, dogs, pigs, etc.) and the like.

In this aspect of the invention, a biological system or sample comprising a 5-HT_4 receptor is contacted with a 5-HT_4 receptor-agonizing amount of a compound of the invention. The effects of agonizing the 5-HT_4 receptor are then determined using conventional procedures and equipment, such as radioligand binding assays and functional assays. Such functional assays include ligand-mediated changes in intracellular cyclic adenosine monophosphate (cAMP), ligand-mediated changes in activity of the enzyme adenylyl cyclase (which synthesizes cAMP), ligand-mediated changes in incorporation of analogs of guanosine triphosphate (GTP), such as [^{35}S]GTPγS (guanosine 5'-O-(γ-thio)triphosphate) or GTP-Eu, into isolated membranes via receptor catalyzed exchange of GTP analogs for GDP analogs, ligand-mediated changes in free intracellular calcium ions (measured, for example, with a fluorescence-linked imaging plate reader or FLIPR® from Molecular Devices, Inc.), and measurement of mitogen activated protein kinase (MAPK) activation. A compound of the invention may agonize or increase the activation of 5-HT_4 receptors in any of the functional assays listed above, or assays of a similar nature. A 5-HT_4 receptor-agonizing amount of a compound of the invention will typically range from about 1 nanomolar to about 500 nanomolar.

Additionally, the compounds of the invention can be used as research tools for discovering new 5-HT_4 receptor agonists. In this embodiment, 5-HT_4 receptor binding or functional data for a test compound or a group of test compounds is compared to the 5-HT_4 receptor binding or functional data for a compound of the invention to identify test compounds that have superior binding or functional activity, if any. This aspect of the invention includes, as separate embodiments, both the generation of comparison data (using the appropriate assays) and the analysis of the test data to identify test compounds of interest.

Among other properties, compounds of the invention have been found to be potent agonists of the 5-HT_4 receptor and to exhibit substantial selectivity for the 5-HT_4 receptor subtype over the 5-HT_3 receptor subtype in radioligand binding assays. Further, compounds of the invention have demonstrated superior pharmacokinetic properties in a rat model. Compounds of the invention are thus expected to be highly bioavailable upon oral administration. In addition, these compounds have been shown not to exhibit an unacceptable level of inhibition of the potassium ion current in an *in vitro* voltage-clamp model using isolated whole cells expressing the hERG cardiac potassium channel. The voltage-clamp assay is an accepted pre-clinical method of assessing the potential for

pharmaceutical agents to change the pattern of cardiac repolarization, specifically to cause, so-called QT prolongation, which has been associated with cardiac arrhythmia. (Cavero *et al.*, *Opinion on Pharmacotherapy*, **2000**, *1*, 947-73, Fermini *et al.*, *Nature Reviews Drug Discovery*, **2003**, *2*, 439-447) Accordingly, pharmaceutical compositions comprising compounds of the invention are expected to have an acceptable cardiac profile.

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

EXAMPLES

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

Boc	=	*tert*-butoxycarbonyl
$(Boc)_2O$	=	di-*tert*-butyl dicarbonate
DCM	=	dichloromethane
DMF	=	*N*,*N*-dimethylformamide
DMSO	=	dimethyl sulfoxide
EtOAc	=	ethyl acetate
mCPBA	=	*m*-chlorobenzoic acid
MeCN	=	acetonitrile
MTBE	=	*tert*-butyl methyl ether
PyBop	=	benzotriazol-1-yloxytripyrrolidino-phosphonium hexafluorophosphate
R_f	=	retention factor
RT	=	room temperature
TFA	=	trifluoroacetic acid
THF	=	tetrahydrofuran

Reagents (including secondary amines) and solvents were purchased from commercial suppliers (Aldrich, Fluka, Sigma, etc.), and used without further purification. Reactions were run under nitrogen atmosphere, unless noted otherwise. Progress of reaction mixtures was monitored by thin layer chromatography (TLC), analytical high performance liquid chromatography (anal. HPLC), and mass spectrometry, the details of

which are given below and separately in specific examples of reactions. Reaction mixtures were worked up as described specifically in each reaction; commonly they were purified by extraction and other purification methods such as temperature-, and solvent-dependent crystallization, and precipitation. In addition, reaction mixtures were routinely purified by preparative HPLC: a general protocol is described below. Characterization of reaction products was routinely carried out by mass and ^{1}H-NMR spectrometry. For NMR measurement, samples were dissolved in deuterated solvent (CD_3OD, $CDCl_3$, or DMSO-d_6), and ^{1}H-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 content is determined by Karl Fischer titration using a Brinkmann (Westbury, NY) Metrohm Karl Fischer Model 813 coulometer.

Example 1: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

a. Preparation of 8-benzyl-8-azabicyclo[3.2.1]octan-3-one

Concentrated hydrochloric acid (30 mL) was added to a heterogeneous solution of 2,5-dimethoxy tetrahydrofuran (82.2 g, 0.622 mol) in water (170 mL) while stirring. In a separate flask cooled to 0°C (ice bath), concentrated hydrochloric acid (92 mL) was added slowly to a solution of benzyl amine (100 g, 0.933 mol) in water (350 mL). The 2,5-dimethoxytetrahydrofuran solution was stirred for approximately 20 min, diluted with water (250 mL), and then the benzyl amine solution was added, followed by the addition of a solution of 1,3-acetonedicarboxylic acid (100 g, 0.684 mol) in water (400 mL) and then the addition of sodium hydrogen phosphate (44 g, 0.31 mol) in water (200 mL). The pH was adjusted from pH 1 to pH ~ 4.5 using 40% NaOH. The resulting cloudy and pale yellow solution was stirred overnight. The solution was then acidified to pH 3 from pH 7.5 using 50% hydrochloric acid, heated to 85 °C and stirred for 2 hours. The solution was cooled to room temperature, basified to pH 12 using 40% NaOH, and extracted with DCM (3 x 500 mL). The combined organic layers were washed with brine, dried

($MgSO_4$), filtered and concentrated under reduced pressure to produce the crude title intermediate as a viscous brown oil (52 g).

To a solution of the crude intermediate in methanol (1000 mL) was added di-*tert*-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 H_3PO_4 (1000 mL) and washed with dichloromethane (3 x 250 mL) The aqueous layer was basified to pH 12 using aqueous NaOH, and extracted with dichloromethane (3 x 500 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure to produce the title intermediate as a viscous, light brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm) 7.5-7.2 (m, 5H, C_6H_5), 3.7 (s, 2H, CH_2Ph), 3.45 (broad s, 2H, CH-NBn), 2.7-2.6 (dd, 2H, CH_2CO), 2.2-2.1 (dd, 2H, CH_2CO), 2.1-2.0 (m, 2H, CH_2CH_2), 1.6 (m, 2H, CH_2CH_2). (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{17}NO$ 216.14; found, 216.0.

b. Preparation of 3-oxo-8-azabicyclo[3.2.1]octane-8-carboxylic acid *tert*-butyl ester

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, 1.1 eq) in EtOAc (300 mL). The resulting solution and rinse (100 mL EtOAc) was added to a 1 L Parr hydrogenation vessel containing 23 g of palladium hydroxide (20 wt.% Pd, dry basis, on carbon, ~50% wet with water; e.g. Pearlman's catalyst) under a stream of nitrogen. The reaction vessel was degassed (alternating vacuum and N_2 five times) and pressurized to 60 psi of H_2 gas. The reaction solution was agitated for two days and recharged with H_2 as needed to keep the H_2 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 Celite® 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 without further treatment. ^{1}H NMR ($CDCl_3$) δ(ppm) 4.5 (broad, 2H, CH-NBoc), 2.7 (broad, 2H, CH_2CO), 2.4-2.3 (dd, 2H, CH_2CH_2), 2.1 (broad m, 2H, CH_2CO), 1.7-1.6 (dd, 2H, CH_2CH_2), 1.5 (s, 9H, $(CH_3)_3COCON$)).

c. Preparation of (1*S*,3*R*,5*R*)-3-amino-8-azabicyclo[3.2.1]octane-8-carboxylic acid *tert*-butyl ester

To a solution of the product of the previous step (75.4 g, 0.335 mol) in methanol (1 L) was added ammonium formate (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 of N_2 while stirring via mechanical stirrer. After 24 and 48 hours, additional portions of ammonium formate (132g, 2.1 mol) were added each time. Once reaction progression ceased, as monitored by anal. HPLC, Celite® (>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 1M phosphoric acid to a final volume of ~1.5 to 2.0 L at pH 2 and washed with dichloromethane (3 x 700 mL). The aqueous layer was basified to pH 12 using 40% aq. NaOH, and extracted with dichloromethane (3 x 700 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation, then high-vacuum leaving 52 g (70%) of the title intermediate, commonly *N*-Boc-*endo*-3-aminotropane, as a white to pale yellow solid. The isomer ratio of *endo* to *exo* amine of the product was >99:1 based on ^{1}H-NMR analysis (>96% purity by analytical HPLC). ^{1}H NMR ($CDCl_3$) δ (ppm) 4.2-4.0 (broad d, 2H, CHNBoc), 3.25 (t, 1H, $CHNH_2$), 2.1-2.05 (m, 4H), 1.9 (m, 2H), 1.4 (s, 9H, $(CH_3)_3OCON$), 1.2-1.1 (broad, 2H). (*m/z*): $[M+H]^+$ calcd for $C_{12}H_{22}N_2O_2$) 227.18; found, 227.2. Analytical HPLC (isocratic method; 2:98 (A:B) to 90:10 (A:B) over 5 min): retention time = 3.68 min.

d. Preparation of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid

First, acetone (228.2 mL, 3.11 mol) was added to a stirred suspension of 2-aminophenylmethanol (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,-dimethyl-1,4-dihydro-2*H*-benzo[1,3]oxazine (332.2 g, 98 %) as an off-white solid. ^{1}H NMR ($CDCl_3$; 300MHz): 1.48 (s, 6H, $C(C\underline{H}_3)_2$), 4.00 (bs, 1H, N<u>H</u>), 4.86 (s, 2H, $C\underline{H}_2$), 6.66 (d, 1H, Ar<u>H</u>), 6.81 (t, 1H, Ar<u>H</u>), 6.96 (d, 1H, Ar<u>H</u>), 7.10 (t, 1H, Ar<u>H</u>).

A solution of 2,2,-dimethyl-1,4-dihydro-2*H*-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 $LiAlH_4$ in THF (800 mL) at 0 °C. The reaction was quenched by slow portionwise addition of $Na_2SO_4 \cdot 10H_2O$ (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-isopropylaminophenylmethanol (120 g, 95 %) as a yellow oil. ^{1}H NMR ($CDCl_3$; 300MHz): 1.24 (d, 6H, $CH(CH_3)_2$), 3.15 (bs, 1H, OH), 3.61 (sept, 1H, $CH(CH_3)_2$), 4.57 (s, 2H, CH_2), 6.59 (t, 1H, ArH), 6.65 (d, 1H, ArH), 6.99 (d, 1H, ArH), 7.15 (t, 1H, 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 mL) and the reaction mixture was heated to 117 °C for 4 h. The reaction mixture was allowed to cool to room 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-isopropylaminobenzaldehyde (105 g, 90 %) as an orange oil. ^{1}H NMR ($CDCl_3$; 300MHz): 1.28 (d, 6H, $CH(CH_3)_2$), 3.76 (sept, 1H, $CH(CH_3)_2$), 6.65 (t, 1H, ArH), 6.69 (d, 1H, ArH), 7.37 (d, 1H, ArH), 7.44 (t, 1H, ArH), 9.79 (s, 1H, CHO).

2,2-Dimethyl-[1,3]dioxane-4,6-dione, commonly Meldrum'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 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. ^{1}H NMR ($CDCl_3$; 300MHz): 1.72 (d, 6H, $CH(CH_3)_2$), 5.50 (bs, 1H, $CH(CH_3)_2$), 7.44 (t, 1H, ArH), 7.75-7.77 (m, 2H, ArH), 7.82 (d, 1H, ArH), 8.89 (s, 1H, CH).

e. Preparation of (1*S*,3*R*,5*R*)-3-[1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carbonyl)amino]-8-azabicyclo[3.2.1]octane-8-carboxylic acid *tert*-butyl ester

Thionyl chloride (36.6 mL, 0.52 mol) was added to a stirred suspension of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (80 g, 0.35 mol) in toluene (600 mL) at 85 °C and the reaction mixture then heated to 95 °C for 2 h. The reaction mixture was cooled to room temperature and then added over 25 min to a vigorously stirred biphasic solution of (1*S*,3*R*,5*R*)-3-amino-8-azabicyclo[3.2.1]octane-8-carboxylic acid *tert*-butyl ester (78.2 g, 0.35 mol) and sodium hydroxide (69.2 g, 1.73 mol) in

toluene/water (1:1) (1L) at °C. After 1 h, the layers were allowed to separate and the organic phase concentrated under reduced pressure. The aqueous phase was washed with EtOAc (1 L) and then (500 mL) and the combined organic extracts used to dissolve the concentrated organic residue. This solution was washed with 1M H_3PO_4 (500 mL), sat. aq. $NaHCO_3$ (500 mL) and brine (500 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield the title intermediate (127.9 g, approx. 84 %) as a yellow solid. ^{1}H NMR ($CDCl_3$): 1.47 (s, 9H), 1.67 (d, 6H), 1.78-1.84 (m, 2H), 2.04-2.18 (m, 6H), 4.20-4.39 (m, 3H), 5.65 (bs, 1H), 7.26 (dd. 1H), 7.63 (m, 2H), 7.75 (dd, 1H), 8.83 (s, 1H), 10.63 (d, 1H).

f. Preparation of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-azabicyclo[3.2.1]oct-3-yl}amide

TFA (300 mL) was added to a stirred solution of the product of the previous step (127.9 g) in CH_2Cl_2 (600 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 1 h and then concentrated under reduced pressure. The oily brown residue was then poured into a vigorously stirred solution of ether (3 L) and a solid precipitate formed immediately. The suspension was stirred overnight and then the solid collected by filtration and washed with ether to yield the title intermediate as its trifluoroacetic acid salt (131.7 g, 86% over two steps) as a light yellow solid. ^{1}H NMR ($CDCl_3$): 1.68 (d, 6H), 2.10 (d, 2H), 2.33-2.39 (m, 4H), 2.44-2.61 (m, 2H), 4.08 (bs, 2H), 4.41 (m, 1H), 5.57 (bs, 1H), 7.31 (m. 1H), 7.66 (m, 2H), 7.77 (d, 1H), 8.83 (s, 1H), 9.38 (bd, 2H), 10.78 (d, 1H).

g. Preparation of 3-hydroxy-3'-{[1-isopropyl-2-oxo-1,2-dihydroquinolin-3-yl)carbonyl]amino}spiro[azetidine-1,8'-(1*S*,3*R*,5*R*)-8-azabicyclo[3.2.1]octane (Intermediate (V) with R^1 = H, R^2 = isopropyl)

2-Bromomethyloxirane (10.72 mL, 129.5 mmol) was added to a stirred solution of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-aza-bicyclo[3.2.1]oct-3-yl}amide trifluoroacetic acid salt (14.65 g, 43.2 mmol) in ethanol (150 mL) at room temperature. The reaction mixture was stirred for 36 h, at which time a solid precipitate formed. The solid was collected by filtration and washed with ethanol (70 mL) to yield the title intermediate as the bromide salt (8.4 g). (*m/z*): $[M]^+$ calcd for $C_{23}H_{30}N_3O_3$ 396.23; found, 396.5. Retention time (anal. HPLC: 2-50% MeCN/H_2O over 5 min) = 4.13 min.

h. Preparation of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-methylaminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

3-Hydroxy-3'-{[1-isopropyl-2-oxo-1,2-dihydroquinolin-3-yl)carbonyl]amino}spiro[azetidine-1,8'-(1*S*,3*R*,5*R*)-8-aza-bicyclo[3.2.1]octane bromide (678 mg, 1.4 mmol) was dissolved in ethanol (10 mL), and then methylamine (41 % solution in water) (510 µL, 8.0 mmol) was added. The mixture was heated at 80 °C for 16 h, and then concentrated under reduced pressure to give the title intermediate as a crude oil, which was used directly in the following step.

i. Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

The product of the previous step was dissolved in dichloromethane (10 mL), and then 1,8-diazabicyclo[5.4.0]undec-7-ene (763 µL, 5.1mmol) was added, and the mixture was stirred under nitrogen and cooled to 0 °C. Methanesulfonylchloride (132 µL, 1.7 mmol) was added and the mixture was stirred at 0 °C for 30 min. The reaction was quenched by the addition of water, and concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (10 mL) and purified by HPLC chromatography. The purified fractions were lyophilized yielding the title compound as the trifluoroacetic acid salt (340 mg). (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{36}N_4O_5S$, 505.25; found, 505.4. Retention time (anal. HPLC: 2-50% MeCN/H_2O over 5 min) = 4.17 min.

Example 2: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-methoxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

a. Preparation of 3-methoxy-3'-{[1-isopropyl-2-oxo-1,2-dihydroquinolin-3-yl)carbonyl]amino}spiro[azetidine-1,8'-(1*S*,3*R*,5*R*)-8-aza-bicyclo[3.2.1]octane (Intermediate (V') with R^1 = H, R^2 = isopropyl, R^3 =methyl)

Potassium-*tert*-butoxide (1.63 g, 14.5 mmol) was added to a stirred suspension of 3-hydroxy-3'-{[1-isopropyl-2-oxo-1,2-dihydroquinolin-3-yl)carbonyl]amino}spiro[azetidine-1,8'-(1*S*,3*R*,5*R*)-8-azabicyclo[3.2.1]octane bromide (3.45 g, 7.25 mmol) in dichloromethane (100 mL) at room temperature. After 2 min, methyl iodide (0.477 mL, 7.61 mmol) was added to the reaction mixture. After 30 min, water (2 mL) was added to quench the reaction and the reaction mixture concentrated under reduced pressure. The residue was dissolved in a minimal volume of acetic acid/water (1:1) and purified by preparative HPLC to yield the title intermediate as a

trifluoroacetic acid salt (2.1 g). (*m/z*): $[M]^+$ calcd for $C_{24}H_{32}N_3O_3$, 410.24; found 410.5. Retention time (anal. HPLC: 2-50% MeCN/H_2O over 5 min) = 4.36min.

b. Preparation of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-methoxy-3-methylaminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

3-Methoxy-3'-{[1-isopropyl-2-oxo-1,2-dihydroquinolin-3-yl)carbonyl]amino}spiro[azetidine-1,8'-(1*S*,3*R*,5*R*)-8-aza-bicyclo[3.2.1]octane trifluoroacetic acid salt (410 mg, 0.84 mmol) was dissolved in ethanol (10 mL), and then methylamine (41% solution in water, 320 µL, 5 mmol) was added. The mixture was heated at 80 °C for 16 h, and then concentrated under reduced pressure to give the product as a crude oil which was used directly in the following step.

c. Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1S,3R,5R)-8-[2-methoxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

The product of the previous step (53.6 mg, 0.12 mmol) was dissolved in dichloromethane (1.0 mL), and then 1,8-diazabicyclo[5.4.0]undec-7-ene (89.7 µL, 0.6 mmol) was added, and the mixture was stirred under nitrogen and cooled to 0°C. Methanesulfonylchloride (18.6 µL, 0.24 mmol) was added and the mixture was stirred at 0°C for 30 min. The mixture was quenched by the addition of water, and concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (1.5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the title compound as the trifluoroacetic acid salt (45.8 mg). (*m/z*): $[M+H]^+$ calcd for $C_{26}H_{38}N_4O_5S$, 519.27; found 519.2. Retention time (anal. HPLC: 5-40% MeCN/H_2O over 4 min) = 2.72 min.

Example 3: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(methanesulfonyl-pyridin-3-ylmethyl-amino)-2-methoxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

a. Preparation of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-{2-methoxy-3-[(pyridin-3-ylmethyl)amino]propyl}-8-azabicyclo[3.2.1]oct-3-yl}amide

3-Methoxy-3'-{[1-isopropyl-2-oxo-1,2-dihydroquinolin-3-yl)carbonyl]amino}spiro[azetidine-1,8'-(1*S*,3*R*,5*R*)-8-aza-bicyclo[3.2.1]octane (410 mg, 0.84 mmol) was dissolved in ethanol (10 mL), and then 3-aminomethylpyridine (153 µL, 1.5 mmol) was added. The mixture was heated at 60 °C for 16 h, and then concentrated under reduced pressure to give the title intermediate as a crude oil which was used directly in the next step.

b. Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(methanesulfonyl-pyridin-3-ylmethyl-amino)-2-methoxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

The product of the previous step (102.6 mg, 0.2 mmol) was dissolved in dichloromethane (1.0 mL) and then 1,8-diazabicyclo[5.4.0]undec-7-ene (119.6 μL, 0.8 mmol) was added, and the mixture was stirred under nitrogen and cooled to 0°C. Methanesulfonylchloride (30.1 μL, 0.4 mmol) was added and the mixture was stirred at 0°C for 30 min. The reaction was quenched by the addition of water, and the mixture was concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (1.5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the title compound as the trifluoroacetic acid salt (63.5 mg). (*m/z*): $[M+H]^+$ calcd for $C_{31}H_{41}N_5O_5S$, 596.29; found 596.2. Retention time (anal. HPLC: 5-40% MeCN/H_2O over 4 min) = 2.35 min.

Example 4: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-methanesulfonylamino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

a. Preparation of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-2-hydroxypropy]-8-azabicyclo[3.2.1]oct-3-yl}amide

1-Isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-aza-bicyclo[3.2.1]oct-3-yl}amide (3.39 g, 10mmol) was dissolved in ethanol (40 mL), and then 2-oxiranylmethylisoindole-1,3-dione, commonly, epoxypropylphthalimide, (3.05 g, 15 mmol) was added. The mixture was heated at 80 °C for 36 h, cooled, and then concentrated under reduced pressure. The resulting oil was flash chromatographed (SiO_2, eluting with a 9:1 solution of dichloromethane/methanol) to give the title intermediate (4.95 g) as a white solid which was used directly in the next step.

b. Preparation of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-aminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

The product of the previous step (4.95 g, 9.13 mmol) was dissolved in ethanol (40 mL), and then hydrazine (860 μL, 27.4 mmol) was added. The mixture was refluxed for 16 h, and then cooled to room temperature. The mixture was filtered, and the filtrate concentrated to give the title intermediate as a crude oil, which was used directly without further purification.

c. Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1S,3R,5R)-8-[2-hydroxy-3-methanesulfonylamino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

The product of the previous step (82 mg, 0.2 mmol) was dissolved in dichloromethane (1.0 mL), then 1,8-diazabicyclo[5.4.0]undec-7-ene (60 μL, 0.4 mmol) was added, and the mixture was stirred under nitrogen and cooled to -78°C. Methanesulfonylchloride (15.5 μL, 0.2 mmol) was added and the mixture was stirred and allowed to warm to room temperature over 30 min. The reaction was quenched by the addition of water, and the mixture was concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (1.5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the title compound as the trifluoroacetic acid salt (70.7 mg). (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{34}N_4O_5S$, 491.23; found 491.2. Retention time (anal. HPLC: 5-40% MeCN/H_2O over 4 min) = 2.43 min.

Example 5: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(methanesulfonyl-pyridin-3-ylmethyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

a. Preparation of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-{2-hydroxy-3-[(pyridin-3-ylmethyl)amino]propyl}-8-azabicyclo[3.2.1]oct-3-yl}amide

3-Hydroxy-3'-{[1-isopropyl-2-oxo-1,2-dihydroquinolin-3-yl)carbonyl]amino}spiro[azetidine-1,8'-(1*S*,3*R*,5*R*)-8-aza-bicyclo[3.2.1]octane bromide (505 mg, 1.27 mmol) was dissolved in ethanol (10 mL), and then 3-(aminomethyl)-pyridine (193 μL, 1.9 mmol) was added. The mixture was heated at 80 °C for 16 h, and then concentrated under reduced pressure to give the title intermediate as a crude oil which was used directly in the following step.

b. Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(methanesulfonyl-pyridin-3-ylmethyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

The product of the previous step (42.5 mg, 0.08 mmol) was dissolved in dichloromethane (1.0 mL), 1,8-diazabicyclo[5.4.0]undec-7-ene (74.8 μL, 0.5 mmol) was added, and the mixture was stirred under nitrogen and cooled to 0 °C. Methanesulfonylchloride (6.1 μL, 0.08 mmol) was added and the mixture was stirred at 0 °C for 30 min. The reaction was quenched by the addition of water, and concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (1.5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the title compound as the trifluoroacetic acid salt (22.8 mg). (*m/z*): $[M+H]^+$ calcd

for $C_{30}H_{39}N_5O_5S$, 582.28; found 582.2. Retention time (anal. HPLC: 5-40% MeCN/H_2O over 4 min) = 2.78 min.

Example 6: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide

a. Preparation of (*S*)-1-(benzyl-methyl-amino)-3-chloropropan-2-ol

N-Benzylmethylamine (13.95 mL, 108.1mmol) and (*S*)-2-chloromethyloxirane, commonly (*S*)-epichlorohydrin (8.48 mL, 108.1 mmol) were dissolved in hexane (40 mL), and stirred for 16 h. The solution was then flash chromatographed (SiO_2, eluting with 10 % methanol/90 % dichloromethane). Fractions containing product were concentrated to give the title intermediate as an oil (19.7 g). ^{1}H-NMR (DMSO *d6*, 299.96 MHz): δ (ppm) 2.01 (s, 3H), 2.2-2.4 (m, 2H), 3.21-3.5 (m, 3H), 3.53-3.6 (m, 1H), 3.65-3.75 (m, 1H), 4.95 (d, 1H), 7.0-7.25 (m, 5H). (*m/z*): $[M+H]^+$ calcd for $C_{11}H_{16}ClNO$, 214.10; found 214.1.

b. Preparation of ((*S*)-3-chloro-2-hydroxypropyl)methylcarbamic acid *tert*-butyl ester

(*S*)-1-(benzyl-methyl-amino)-3-chloropropan-2-ol (8.4 g, 39.3 mmol) was dissolved in ethyl acetate (75 mL), and then di-*tert*-butyl dicarbonate (9.3 g, 43.23 mmol) and palladium hydroxide (2.5 g) were added. The mixture was shaken for 12 h under hydrogen (60 atm). The mixture was filtered through a bed of Celite®, and concentrated to dryness under reduced pressure. The resulting oil was filtered through silica, eluting with hexane, followed by dichloromethane, followed by diethyl ether. The ether layer was concentrated to give the title intermediate as an oil (7.1 g). ^{1}H-NMR (DMSO d_6, 299.96 MHz): δ (ppm) 1.35-1.46 (s, 9H), 2.81-2.85 (s, 3H), 2.95-3.1 (m, 1H), 3.3-3.6 (m, 3H), 3.67-3.85 (m, 1H), 5.25-5.4 (m, 1H). (*m/z*): $[M+H\text{-}Boc]^+$ calcd for $C_9H_{18}ClNO_3$, 123.10; found 123.1.

c. Preparation of ((*R*)-2-hydroxy-3-{3-[(1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carbonyl)amino]-(1*S*,3*R*,5*R*)-8-azabicyclo[3.2.1]oct-8-yl}propyl)methylcarbamic acid *tert*-butyl ester

((*S*)-3-chloro-2-hydroxypropyl)methylcarbamic acid *tert*-butyl ester (335 mg, 1.5 mmol) and 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-aza-bicyclo[3.2.1]oct-3-yl}amide (339 mg, 1.0 mmol), were dissolved in methanol (5 mL) and then *N,N*-diisopropylethylamine (523 µL, 3.0 mmol) was added. The mixture was heated at 80 °C for 16h, and then concentrated under reduced pressure. The resulting oil

was flash chromatographed (SiO_2, eluting with 10 % methanol/90 % dichloromethane). Fractions containing product were concentrated to give the title intermediate as a white solid (0.5 g). (*m/z*): $[M+H]^+$ calcd for $C_{29}H_{42}N_4O_5$, 527.33; found 527.6. Retention time (anal. HPLC: 2-50% MeCN/H_2O over 5 min) = 4.75 min.

d. Preparation of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*S*)-2-hydroxy-3-methylaminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

((*R*)-2-hydroxy-3-{3-[(1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carbonyl)amino]-(1*S*,3*R*,5*R*)-8-azabicyclo[3.2.1]oct-8-yl}propyl)methylcarbamic acid *tert*-butyl ester (575 mg, 1.09 mmol) was dissolved in dichloromethane (5 mL) and then trifluoroacetic acid (5 mL) was added slowly. The mixture was stirred for 30 min, and then concentrated under reduced pressure. The resulting oil was triturated with diethyl ether, and then filtered. The precipitate was dried under vacuum to give the title intermediate as the trifluoroacetic acid salt (0.68 g). (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{34}N_4O_3$, 427.27; found 427.2. Retention time (anal. HPLC: 2-50% MeCN/H_2O over 5 min) = 3.40 min.

e. Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*S*)-2-hydroxy-3-methylaminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide (1.03 g, 1.57 mmol) was dissolved in dichloromethane (6.0 mL), 1,8-diazabicyclo[5.4.0]undec-7-ene (747 µL, 5.0 mmol) was added, and the mixture was stirred under nitrogen and cooled to 0 °C. Methanesulfonylchloride (124.4 µL, 1.6 mmol) was added and the mixture was stirred at 0 °C for 30 min. The reaction was quenched by the addition of water, and the mixture was concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the title compound as the trifluoroacetic acid salt (0.38 g).

(*m/z*): $[M+H]^+$ calcd for $C_{25}H_{36}N_4O_5S$, 505.25; found 505.4. Retention time (anal. HPLC: 2-50% MeCN/H_2O over 5 min) = 4.17 min. Free base: ^{1}H-NMR (DMSO *d6*, 299.96 MHz): δ (ppm) 1.40-1.68 (d, 6H), 1.81-2.02 (br s, 4H), 2.02-2.18 (br m, 2H), 2.22-2.36 (d, 2H), 2.78-2.90 (2 s, 6H), 2.91-3.04 (m, 1H), 3.10-3.30 (m, 4H), 3.61-3.78 (br s, 1H), 4.02-4.17 (m, 1H), 4.71-4.79 (br s, 1H), 5.2-5.8 (br s, 1H), 7.3-7.4 (t, 1H), 7.67-7.78 (t, 1H), 7-82-7.94 (d, 1H), 7.98-8.02 (d, 1H), 8.80 (s, 1H), 10.37-10.40 (d, NH).

Example 7: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*S*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide

Following the procedure of Example 6, with the substitution of (*R*)-2-chloromethyloxirane for (*S*)-2-chloromethyloxirane in step a, the following intermediates and the title compound were prepared.

(*R*)-1-(benzyl-methyl-amino)-3-chloropropan-2-ol: ^{1}H-NMR (DMSO d_6, 299.96 MHz): δ (ppm) 2.01 (s, 3H), 2.2-2.4 (m, 2H), 3.21-3.5 (m, 3H), 3.53-3.6 (m, 1H), 3.65-3.75 (m, 1H), 4.95 (d, 1H), 7.0-7.25 (m, 5H). (*m/z*): $[M+H]^+$ calcd for $C_{11}H_{16}ClNO$, 214.10; found 214.1.

((*R*)-3-chloro-2-hydroxy-propyl)methylcarbamic acid *tert*-butyl ester: ^{1}H-NMR (DMSO d_6, 299.96 MHz): δ (ppm) 1.35-1.46 (s, 9H), 2.81-2.85 (s, 3H), 2.95-3.1 (m, 1H), 3.3-3.6 (m, 3H), 3.67-3.85 (m, 1H), 5.25-5.4 (m, 1H). (*m/z*): $[M+H\text{-Boc}]^+$ calcd for $C_9H_{18}ClNO_3$, 123.10; found 123.1.

((S)-2-hydroxy-3-{3-[(1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carbonyl)amino]-(1*S*,3*R*,5*R*)-8-azabicyclo[3.2.1]oct-8-yl}propyl)methylcarbamic acid *tert*-butyl ester: (*m/z*): $[M+H]^+$ calcd for $C_{29}H_{42}N_4O_5$, 527.33; found 527.6. Retention time (anal. HPLC: 2-50% MeCN/H_2O over 5 min) = 4.75 min.

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-methylaminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide: (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{34}N_4O_3$, 427.27; found 427.2. Retention time (anal. HPLC: 2-50% MeCN/H_2O over 5 min) = 3.40 min.

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*S*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide: (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{36}N_4O_5S$, 505.25; found 505.4. Retention time (anal. HPLC: 2-50% MeCN/H_2O over 5 min) = 4.17 min.

Example 8: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-[methyl-(pyridine-4-carbonyl)amino]propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-methylaminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide (71 mg, 0.17 mmol) was dissolved in DMF (0.5 mL), and then *N,N*-diisopropylethylamine (88.9 μL,

0.51 mmol) was added. Next, a solution of isonicotinic acid (41.8 mg, 0.34 mmol) and PyBOP (177 mg, 0.34 mmol) in DMF was added. The resulting mixture was shaken at room temperature for 30 min, and then concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (1.5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the title compound as the trifluoroacetic acid salt (30.8 mg). (*m/z*): $[M+H]^+$ calcd for $C_{30}H_{37}N_5O_4$, 532.29; found 532.7. Retention time (anal. HPLC: 5-40% MeCN/H_2O over 4 min) = 2.11 min.

Example 9: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-[(pyridine-4-carbonyl)amino]propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

1-Isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-aminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide (82.4 mg, 0.2 mmol) was dissolved in DMF (0.5 mL), and then *N,N*-diisopropylethylamine (69.7 μL, 0.4 mmol) was added. Next, a solution of isonicotinic acid (49.2 mg, 0.4 mmol) and PyBOP (208.2 mg, 0.4 mmol) in DMF was added. The resulting mixture was shaken at room temperature for 30 min, and then concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (1.5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the title compound as the trifluoroacetic acid salt (82.1 mg). (*m/z*): $[M+H]^+$ calcd for $C_{29}H_{35}N_5O_4$, 518.28; found 518.2. Retention time (anal. HPLC: 5-40% MeCN/H_2O over 4 min) = 2.20 min.

Example 10: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(acetyl-methyl-amino)-2-methoxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

1-Isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-methoxy-3-methylaminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide (53.6 mg, 0.12 mmol) was dissolved in DMF (1.0 mL), and then *N,N*-diisopropylethylamine (104. μL, 0.6 mmol) was added. The mixture was stirred under nitrogen and cooled to 0°C. Acetyl chloride (17.1 μL, 0.24 mmol) was added and the mixture was stirred at 0°C for 30 min, and then concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (1.5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the title compound as the trifluoroacetic acid salt (40.4 mg). (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{38}N_4O_4$, 483.30; found 483.2. Retention time (anal. HPLC: 5-40% MeCN/H_2O over 4 min) = 2.54 min.

Example 11. Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-methoxy-3-[methyl-(pyridine-4-carbonyl)amino]propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

1-Isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-methoxy-3-methylaminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide (53.6 mg, 0.12mmol) was dissolved in DMF (1.0 mL), and then *N,N*-diisopropylethylamine (104.5 μL, 0.6 mmol) was added. The mixture was stirred under nitrogen and cooled to 0°C. Isonicotinyl chloride (42.7 mg, 0.24 mmol) was added and the mixture was stirred at 0 °C for 30 min, and then concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (1.5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the title compound as the trifluoroacetic acid salt (54.4 mg). (*m/z*): $[M+H]^+$ calcd for $C_{31}H_{39}N_5O_4$, 546.31; found 546.2. Retention time (anal. HPLC: 5-40% MeCN/H_2O over 4 min) = 2.29 min.

Example 12. Synthesis of 1-isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(acetyl-pyridin-3-ylmethyl-amino)-2-methoxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

1-Isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (8-{2-methoxy-3-[(pyridin-3-ylmethyl)amino]propyl}-8-azabicyclo[3.2.1]oct-3-yl)amide (102.6 mg, 0.2 mmol) was dissolved in DMF (1.0 mL), and then *N,N*-diisopropylethylamine (139.4 μL, 0.8 mmol) was added. The mixture was stirred under nitrogen and cooled to 0°C. Acetyl chloride (28.5 μL, 0.4 mmol) was added and the mixture was stirred at 0°C for 30 min, and then concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (1.5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the title compound as the trifluoroacetic acid salt (33.2 mg). (*m/z*): $[M+H]^+$ calcd for $C_{32}H_{41}N_5O_4$, 560.33; found 560.4. Retention time (anal. HPLC: 5-40% MeCN/H_2O over 4 min) = 2.27 min.

Example 13. Synthesis of 1-isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-acetylamino-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

1-Isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-aminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide (82.4 mg, 0.2mmol) was dissolved in DMF (0.5 mL), and then *N,N*-diisopropylethylamine (69.7 μL, 0.4 mmol) was added. Next, a solution of acetic acid (22.7 μL, 0.4 mmol) and PyBOP (208.2 mg, 0.4 mmol) in DMF was added. The resulting mixture was shaken at room temperature for

30 min, and then concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (1.5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the title compound as the trifluoroacetic acid salt (79.2 mg). (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{34}N_4O_4$, 455.27; found 455.2. Retention time (anal. HPLC: 5-40% MeCN/H_2O over 4 min) = 2.33 min.

Example 14. Synthesis of 1-isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(acetyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

1-Isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-methylaminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide (43 mg, 0.1 mmol) was dissolved in DMF (1.0 mL), and then *N,N*-diisopropylethylamine (87.1 μL, 0.5 mmol) was added. The mixture was stirred under nitrogen and cooled to 0 °C. Acetyl chloride (17.8 μL, 0.25 mmol) was added and the mixture was stirred at 0 °C for 30 min. The mixture was concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (1.5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the title intermediate as the trifluoroacetic acid salt (17.1 mg). (*m/z*): $[M+H]^+$ calcd for $C_{26}H_{36}N_4O_4$, 469.28; found 469.2. Retention time (anal. HPLC: 5-40% MeCN/H_2O over 4 min) = 2.49 min.

Example 15. Synthesis of 1-isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(formyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

1-Isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-methylaminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide (43 mg, 0.1 mmol) was dissolved in ethyl formate (1.0 mL). The mixture was heated at 65 °C for 16 h, and then concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (1.5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the title intermediate as the trifluoroacetic acid salt (22.7 mg). (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{34}N_4O_4$, 455.27; found 455.2. Retention time (anal. HPLC: 5-40% MeCN/H_2O over 4 min) = 2.37 min.

Example 16. Synthesis of 1-isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-formylamino-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

1-Isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-aminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide (41.2 mg, 0.1 mmol) was dissolved in ethyl formate (1.0 mL). The mixture was heated at 65°C for 16 h, and then concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (1.5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the title intermediate as the trifluoroacetic acid salt (37.5 mg). (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{32}N_4O_4$, 441.25; found 441.2. Retention time (anal. HPLC: 5-40% MeCN/H_2O over 4 min) = 2.89 min.

Example 17. Synthesis of 1-isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-3-(acetyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

1-Isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*S*)-2-hydroxy-3-methylaminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide (200 mg, 0.3 mmol) was dissolved in DMF (1.0 mL), and then *N,N*-diisopropylethylamine (160.3 µL, 0.92 mmol) was added. Next, a solution of acetic acid (17.3 µL, 0.3 mmol) and PyBOP (159 mg, 0.3 mmol) in DMF was added. The resulting mixture was shaken at room temperature for 30 min, and then concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (1.5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the title intermediate as the trifluoroacetic acid salt (130 mg). (*m/z*): $[M+H]^+$ calcd for $C_{26}H_{36}N_4O_4$, 469.28; found 469.5. Retention time (anal. HPLC: 2-50% MeCN/H_2O over 5 min) = 3.94 min.

Example 18. Synthesis of 1-isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-3-(formyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

1-Isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*S*)-2-hydroxy-3-methylaminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide (235mg, 0.36mmol) was dissolved in ethyl formate (5.0 mL). The mixture was heated at 65 °C for 16 h, and then concentrated to dryness under reduced pressure. The product was taken up in acetic acid/water (1:1) (1.5 mL) and purified by HPLC chromatography. The purified fractions were lyophilized to give the the title intermediate as the trifluoroacetic acid salt

(97.5 mg). (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{34}N_4O_4$, 455.27; found 455.2. Retention time (anal. HPLC: 2-50% MeCN/H_2O over 5 min) = 3.87 min.

Example 19: Synthesis of 5-bromo-1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

To a solution of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide (500 mg, 0.99 mmol) dissolved in a mixture of acetonitrile (5 mL) and acetic acid (10 mL) was added bromine (0.30 mL, 5.9 mmol). The mixture was stirred at ambient temperature for 12 h, and concentrated under reduced pressure, yielding a pale yellow oily residue. The residue was dissolved in 20% acetonitrile in water (0.5% TFA) (5 mL), and purified by HPLC. The title compound was obtained as a major product and isolated as a tifluoroacetic acid salt (200 mg) ^{1}H-NMR (CD_3OD): δ (ppm) 8.64 (s, 1H), 7.97 (s, 1H), 7.72-7.70 (m, 2H), 4.18 (br m, 2H), 4.0 (br s, 1H), 3.2-3.0 (m), 2.88 (s, 3H), 2.79 (s, 3H), 2.5-2.2 (m, 2H), 1.56 (d, 6H). (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{35}BrN_4O_5S$, 583.16; found 583.4. Retention time (anal. HPLC: 10-50% MeCN/H_2O over 6 min) = 3.90 min.

Example 20: Alternative synthesis of ((*R*)-2-hydroxy-3-{3-[(1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carbonyl)amino]-(1*S*,3*R*,5*R*)-8-azabicyclo[3.2.1]oct-8-yl}propyl)methylcarbamic acid *tert*-butyl ester

a. Preparation of methyl-(*S*)-1-oxiranylmethylcarbamic acid *tert*-butyl ester

((*S*)-3-chloro-2-hydroxypropyl)-methylcarbamic acid *tert*-butyl ester (2.23 g, 10 mmol) was dissolved in THF (30 mL), and then an aqueous sodium hydroxide solution (0.48 g in 10 mL water) was added. The mixture was stirred for 2 h. The mixture was then concentrated under reduced pressure to remove most of the THF, and the remaining aqueous solution was extracted into ethyl acetate, washing with water. The product was dried over sodium sulfate, filtered and concentrated under reduced pressure to give the title intermediate as an oil (1.6 g). (*m/z*): $[M+Na]^+$ calcd for $C_9H_{17}NO_3$, 210.10; found 210.1. ^{1}H-NMR (DMSO *d6*, 299.96 MHz): δ (ppm) 1.32-1.42 (s, 9H), 2.69-2.73 (m, 1H), 2.75-2.85 (s, 3H), 2.95-3.05 (br s, 1H), 3.10-3.15 (dm, 1H), 3.16-3.21 (d, 1H), 3.37-3.51 (m, 2H).

b. Synthesis of ((*R*)-2-hydroxy-3-{3-[(1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carbonyl)amino]-(1S,3*R*,5*R*)-8-azabicyclo[3.2.1]oct-8-yl}propyl)methylcarbamic acid tert-butyl ester

Methyl-(*S*)-1-oxiranylmethylcarbamic acid tert-butyl ester (9.53 g, 51.1 mmol), and 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-aza-bicyclo[3.2.1]oct-3-yl}amide (8.7 g, 25.6 mmol), were dissolved in methanol (100 mL). The mixture was heated at 80 °C for 2h, and then concentrated under reduced pressure. The resulting oil was taken up in ethyl acetate and washed with saturated aqueous sodium bicarbonate, followed by saturated aqueous sodium chloride. The organics were dried over sodium sulfate, filtered, and concentrated under reduced pressure. The resulting oil was flash chromatographed (SiO_2, eluting with 10% methanol/90% dichloromethane). Fractions containing product were concentrated to give the title intermediate as a white solid (13.5 g). (*m/z*): $[M+H]^+$ calcd for $C_{29}H_{42}N_4O_5$, 527.33; found 527.6. Retention time (anal. HPLC: 2-50% MeCN/H_2O over 5 min) = 4.75 min.

Example 21: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(methanesulfonyl-ethylamino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

Following the procedure of Example 1 replacing methylamine with ethylamine in step h, the title compound was prepared. (*m/z*): $[M+H]^+$ calcd for $C_{26}H_{38}N_4O_5S$, 519.28; found 519.2. Retention time (anal. HPLC: 10-70% MeCN/H_2O over 6 min) = 2.91 min.

Example 22: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonylamino)-propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

a1. Preparation of (*S*)-2-oxiranylmethylisoindole-1,3-dione

To a cold solution of (S)-1-oxiranylmethanol (5 g, 67.5 mmol) and phthalimide (9.9 g, 67.3 mmol) in tetrahydrofuran (200 mL) in ice bath was added triphenylphosphine (17.9 g, 68.2 mmol) and diethyl azodicarboxylate (12.3 g, 70.6 mmol). The mixture was stirred at 0°C for 2 h and at ambient temperature for 48 h. The mixture was concentrated under vacuum, and the oily residue was purified by flash silica column chromatography, yielding the desired product (10.1 g) as pale yellow solid: R_f= 0.51 in 1:1 EtOAc/hexane. ^{1}H-NMR ($CDCl_3$, 300MHz): δ (ppm) 7.76-7.64 (m, 2H), 7.63-7.60 (m, 2H), 3.9-3.8 (dd, 1H), 3.70-3.65 (dd, 1H), 3.15 (m, 1H), 2.70-2.67 (dd, 1H), 2.58-2.55 (dd, 1H).

b1. Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1S,3R,5R)-8-[(*R*)-2-hydroxy-3-methanesulfonylamino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

Following the procedure of Example 4, replacing racemic 2-oxiranylmethyl-isoindole-1,3-dione with the chiral intermediate of the previous step, the trifluoroacetate salt of the title compound was prepared. (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{34}N_4O_5S$, 491.24; found 491.4. Retention time (anal. HPLC: 10-50% MeCN/H_2O over 6 min) = 3.69 min. ^{1}H-NMR (CD_3OD, 300MHz): δ (ppm) 8.67 (s, 1H), 7.74-7.73 (m, 3H), 7.7-7.6 (dt, 1H), 7.3-7.2 (t, 1H), 4.2 (br s, 2H), 4.0 (br m, 2H), 3.2-2.9 (m, 4H), 2.8 (s, 3H), 2.6-2.3 (br m, 6H), 2.2-2.1 (br m, 2H), 1.57-1.55 (d, 6H).

The title compound was also prepared by the following procedure.

a2. Preparation of *N*-((*S*)-oxiranylmethyl)methanesulfonamide

To a cold solution of methanesulfonamide (10 g, 0.105 mol) in water (100 mL) in an ice bath was added sodium hydroxide as pellets (8.4 g, 0.21 mol) and then (*S*)-2-chloromethyloxirane (12.4 g, 0.158 mol). The mixture was stirred at the same temperature for 2 h, and at room temperature for 12 h and then concentrated hydrochloric acid (18 mL) was added. The product was isolated by extracting the aqueous layer with dichloromethane (2 x 300 mL). The organic layer was dried over $MgSO_4$ and then evaporated to dryness, yielding a colorless liquid (2.5 g), which was used directly in the next step.

b2. Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonylamino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

To a solution of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-aza-bicyclo[3.2.1]oct-3-yl}amide (TFA salt; 4 g, 8.8 mmol) in methanol (150 mL) was added *N,N*-diisopropylethylamine (1.7 mL, 9.5 mmol), and the product of the previous step (2.5 g, 18.2 mmol). The mixture was stirred at 80 °C for 2 days. After being concentrated under vacuum, the residue was purified by preparative HPLC, yielding the trifluoroacetate salt of the title compound (1.3 g). (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{34}N_4O_5S$, 491.24; found 491.4. Retention time (anal. HPLC: 10-50% MeCN/H_2O over 6 min) = 3.69 min.

Example 23: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(1,1-dioxo-2-isothiazolidinyl)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

a. Preparation of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(3-chloropropanesulfonylamino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

To a cold solution of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-aminopropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide TFA salt (0.125 g, 0.195 mmol) in dichloromethane (2 mL) in an ice bath was added *N,N*-diisopropylethylamine (0.119 mL, 0.683 mmol) and 3-chloropropanesulfonyl chloride (0.025 mL, 0.205 mmol). After stirring at 0 °C for 2 h, the mixture was stirred at room temperature overnight. It was diluted with dichloromethane (50 mL), and washed with brine and saturated $NaHCO_3$ solution. After drying over $MgSO_4$, the organic solution was evaporated to dryness, yielding an oily residue. The crude product was used directly in next step.

b. 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(1,1-dioxo-2-isothiazolidinyl)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide

To a solution of 1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(3-chloropropanesulfonylamino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide (100 mg, 0.18 mmol) in anhydrous DMF (3 mL) was added potassium carbonate (75 mg, 0.56 mmol). The reaction mixture was shaken at 85 °C for 12 h, and concentrated under vacuum. The residue was dissolved in dichloromethane (50 mL), and washed with saturated $NaHCO_3$. After drying over $MgSO_4$, the filtrate was evaporated to dryness, and the residue was purified by preparative HPLC to yield the title compound. (*m/z*): $[M+H]^+$ calcd for $C_{26}H_{36}N_4O_5S$, 517.26; found 517.3.

Example 24: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide

a. Preparation of (1*S*,3*R*,5*R*)-3-[1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carbonyl)amino]-8-azabicyclo[3.2.1]octane-8-carboxylic acid *tert*-butyl ester

In a 3 L flask, 1-isopropyl-2-oxo-1,2-dihydroquinoline-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 min. The mixture was heated at 95 °C for 1.5 h with stirring and then allowed to cool to room temperature.

In a separate 12 L flask, (1*S*,3*R*,5*R*)-3-amino-8-azabicyclo[3.2.1]octane-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 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-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-azabicyclo[3.2.1]oct-3-yl}amide

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 h. 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 mL), a viscous brown oil, contained the crude intermediate.

To a 12 L flask charged with MTBE (2.8 L), the crude brown oil was added over 1 h at 1-2 °C with stirring. The suspension was stirred at the same temperature for 1 h and then filtered. The filtrate was washed with MTBE (2 x 300 mL) and dried under vacuum at room temperature for 4 days to provide the trifluoroacetate salt of the title intermediate (163.3 g) as a pale yellow powder.

c. Preparation of *N*-methyl-*N*-[(*S*)-2-oxiran-2-ylmethyl]methanesulfonamide

A 12 L flask was charged with water (1 L) followed by the addition NaOH (50 % 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-chloromethyloxirane (339.6 g, 3.67 mol) was added. The mixture was stirred for 20 h at 3-4 °C. Dichloromethane (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 H_3PO_4 (800 mL) and brine (800 mL). Dichloromethane was removed by rotary

evaporation. To the crude product, toluene (400 mL) was added and removed by rotary evaporation. After three additional cycles of the toluene process, the title intermediate was obtained (228.2 g) which was used without further purification in the next step.

d. Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide

In a 3 L flask, 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-azabicyclo[3.2.1]oct-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 the washings were added to the reaction mixture. After 30 min of stirring, a solution of *N*-methyl-*N*-[(*S*)-2-oxiran-2-ylmethyl]methanesulfonamide (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 of the 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 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 %). ^{1}H NMR ($CDCl_3$) δ ppm 10.52 (d, 1H), 8.83 (s, 1H), 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, 1H), 2.30-1.81(m, 11H), 1.68 (d, 6H).

The seed crystals were obtained from a previous preparation of the title compound by the method of this example at smaller scale, in which crystallization occurred spontaneously.

Example 25: Synthesis of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide hydrochloride

In a 1 L flask, 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-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 (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 26: Synthesis of citric acid salt of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide

1-Isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide (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 resulting solid was filtered and washed with cold ethanol to give the title compound as a solid (74.3 mg).

Example 27: Synthesis of acid salts of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide

Following the procedure of Example 26, the acid salts of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide listed below in Table III were prepared in solid form using the indicated equivalents of acid.

Table III: Acid Salts

Acid	No. of equivalents of acid	Product weight (mg)
adipic	0.5	48.5
phosphoric	0.5	86.6
sulfuric	0.5	27.0
tartaric	0.5	66.3
malic	0.5	25.3
hydrobromic	1	62.9

Example 28: Synthesis of methanesulfonic acid salt of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide

To a solution of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide (0.1 g, 0.2 mmol) in 50 % acetonitrile/water (1 mL) was added a 1M solution of methanesulfonic acid in ethanol (0.2 mL, 0.2 mmol, 1 eq). The 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).

Example 29: Synthesis of acid salts of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide

Following the procedure of Example 28, the acid salts of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide listed below in Table IV were prepared in solid form using the indicated equivalents of acid.

Table IV: Acid Salts

Acid	No. of equivalents of acid	Product weight (mg)
fumaric	1	107.2
benzoic	1	105.0
(*R*)-mandelic	1	96.1

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

a. Membrane Preparation 5-HT$_{4(c)}$

HEK-293 (human embryonic kidney) cells stably-transfected with human 5-HT$_{4(c)}$ receptor cDNA (Bmax = ~ 6.0 pmol/mg protein, as determined using [^{3}H]-GR113808 membrane radioligand binding assay) were grown in T-225 flasks in Dulbecco's Modified Eagles Medium (DMEM) containing 4,500 mg/L D-glucose and pyridoxine hydrochloride (GIBCO-Invitrogen Corp., Carlsbad CA: Cat #11965) supplemented with 10% fetal bovine serum (FBS) (GIBCO-Invitrogen Corp.: Cat #10437), 2 mM L-glutamine and (100 units) penicillin-(100 µg) streptomycin/ml (GIBCO-Invitrogen Corp.: Cat #15140) in

a 5% CO_2, humidified incubator at 37 °C. Cells were grown under continuous selection pressure by the addition of 800 μg/mL geneticin (GIBCO-Invitrogen Corp.: Cat #10131) to the medium.

Cells were grown to roughly 60-80% confluency (< 35 subculture passages). At 20-22 hours prior to harvesting, cells were washed twice and fed with serum-free DMEM. All steps of the membrane preparation were performed on ice. The cell monolayer was lifted by gentle mechanical agitation and trituration with a 25 mL pipette. Cells were collected by centrifugation at 1000 rpm (5 min).

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

b. Radioligand Binding Assays

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

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

of 1 μM unlabeled GR113808. Assays were incubated for 60 min at room temperature, and then the binding reactions were terminated by rapid filtration over 96-well GF/B glass fiber filter plates (Packard BioScience Co., Meriden, CT) presoaked in 0.3% polyethyleneimine. Filter plates were washed three times with filtration buffer (ice-cold 50mM HEPES, pH7.4) to remove unbound radioactivity. Plates were dried, 35 μL Microscint-20 liquid scintillation fluid (Packard BioScience Co., Meriden, CT) was added to each well and plates were counted in a Packard Topcount liquid scintillation counter (Packard BioScience Co., Meriden, CT).

Binding data were analyzed by nonlinear regression analysis with the GraphPad Prism Software package (GraphPad 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 μM GR113808. K_i values for test compounds were calculated, in Prism, from the best-fit IC_{50} values, and the K_d value of the radioligand, using the Cheng-Prusoff equation (Cheng and Prusoff, *Biochemical Pharmacology,* **1973**, *22,* 3099-108): $K_i = IC_{50} / (1 + [L]/K_d)$ where [L] = concentration [^{3}H]-GR113808. Results are expressed as the negative decadic logarithm of the K_i values, pK_i.

Test compounds having a higher pK_i value in this assay have a higher binding affinity for the 5-HT_4 receptor. The compounds of the invention which were tested in this assay had a pK_i value ranging from about 6.3 to about 9.0, typically ranging from about 6.5 to about 8.5.

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

a. Membrane Preparation 5-HT_{3A}

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

#SH30070.03) and (50 units) penicillin-(50 µg) streptomycin/ml (GIBCO-Invitrogen Corp.: Cat #15140) in a 5% CO_2, humidified incubator at 37 °C.

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

b. Radioligand Binding Assays

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

Binding data were analyzed using the non-linear regression procedure described above to determine K_i values. The BOTTOM (curve minimum) was fixed to the value for

nonspecific binding, as determined in the presence of 10 μM MDL72222. The quantity [L] in the Cheng-Prusoff equation was defined as the concentration [^{3}H]-GR65630.

Selectivity for the 5-HT_4 receptor subtype with respect to the 5-HT_3 receptor subtype was calculated as the ratio K_i(5-HT_{3A})/K_i(5-$HT_{4(c)}$). The compounds of the invention which were tested in this assay had a 5-HT_4/5-HT_3 receptor subtype selectivity ranging from about 50 to about 8000, typically ranging from about 100 to about 4000.

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

In this assay, the functional potency of a test compound was determined by measuring the amount of cyclic AMP produced when HEK-293 cells expressing 5-HT_4 receptors were contacted with different concentrations of test compound.

a. Cell Culture

HEK-293 (human embryonic kidney) cells stably-transfected with cloned human 5-$HT_{4(c)}$ receptor cDNA were prepared expressing the receptor at two different densities: (1) at a density of about 0.5-0.6 pmol/mg protein, as determined using a [^{3}H]-GR113808 membrane radioligand binding assay, and (2) at a density of about 6.0 pmol/mg protein. The cells were grown in T-225 flasks in Dulbecco's Modified Eagles Medium (DMEM) containing 4,500 mg/L D-glucose (GIBCO-Invitrogen Corp.: Cat #11965) supplemented with 10% fetal bovine serum (FBS) (GIBCO-Invitrogen Corp.: Cat #10437) and (100 units) penicillin-(100 μg) streptomycin/ml (GIBCO-Invitrogen Corp.: Cat #15140) in a 5% CO_2, humidified incubator at 37°C. Cells were grown under continuous selection pressure by the addition of geneticin (800 μg/mL: GIBCO-Invitrogen Corp.: Cat #10131) to the medium.

b. Cell Preparation

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

(37°C) stimulation buffer (10mL equivalent per 2-3 T-225 flasks). This time was noted and marked as time zero. The cells were counted with a Coulter counter (count above 8 μm, flask yield was 1-2 x 10^7 cells/flask). Cells were resuspended at a concentration of 5 x 10^5 cells/ml in pre-warmed (37°C) stimulation buffer (as provided in the flashplate kit) and preincubated at 37°C for 10 min.

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

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

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

Test compounds exhibiting a higher pEC_{50} value in this assay have a higher potency for agonizing the 5-HT_4 receptor. The compounds of the invention which were tested in this assay, for example, in the cell line (1) having a density of about 0.5-0.6 pmol/mg protein, had a pEC_{50} value ranging from about 7.0 to about 9.0, typically ranging from about 7.5 to about 8.5.

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

CHO-K1 cells stably transfected with hERG cDNA were obtained from Gail Robertson at the University of Wisconsin. Cells were held in cryogenic storage until needed. Cells were expanded and passaged in Dulbecco's Modified Eagles Medium/F12 supplemented with 10 % fetal bovine serum and 200 μg/mL geneticin. Cells were seeded onto poly-D-lysine (100 μg/mL) coated glass coverslips, in 35 mm^2 dishes (containing 2 mL medium) at a density that enabled isolated cells to be selected for whole cell voltage-clamp studies. The dishes were maintained in a humidified, 5% CO_2 environment at 37°C.

Extracellular solution was prepared at least every 7 days and stored at 4°C when not in use. The extracellular solution contained (mM): NaCl (137), KCl (4), $CaCl_2$ (1.8), $MgCl_2$ (1), Glucose (10), 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) (10), pH 7.4 with NaOH. The extracellular solution, in the absence or presence of test compound, was contained in reservoirs, from which it flowed into the recording chamber at approximately 0.5 mL/min. The intracellular solution was prepared, aliquoted and stored at -20°C until the day of use. The intracellular solution contained (mM): KCl (130), $MgCl_2$ (1), ethylene glycol-bis(beta-aminoethyl ether) *N,N,N',N'*-tetra acetic acid salt (EGTA) (5), MgATP (5), 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) (10), pH 7.2 with KOH. All experiments were performed at room temperature (20-22°C).

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

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

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

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

The smaller the percentage inhibition of the potassium ion current in this assay, the smaller the potential for test compounds to change the pattern of cardiac repolarization when used as therapeutic agents. The compounds of the invention which were tested in this assay at a concentration of 3 μM exhibited an inhibition of the potassium ion current of less than about 20 %, typically, less than about 15 %.

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

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

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

control. The air manifold delivered 95% O_2/5% CO_2 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 μM and with ^{14}C-mannitol to monitor the integrity of the monolayer. All experiments were conducted at 37 °C for 60 min. Samples were taken at 0, 30 and 60 min from both the donor and receiver sides of the chamber. Samples were analyzed by HPLC or liquid scintillation counting for test compound and mannitol concentrations. The permeation coefficient (K_p) in cm/sec was calculated.

In this assay, a K_p value greater than about 10 x 10^{-6} cm/sec is considered indicative of favorable bioavailability. The compounds of the invention that were tested in this assay exhibited K_p values of between about 10 x 10^{-6} cm/sec and about 50 x 10^{-6} cm/sec, typically between about 20 x 10^{-6} cm/sec and about 40 x 10^{-6} cm/sec.

Assay 6: Pharmacokinetic Study in the Rat

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

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

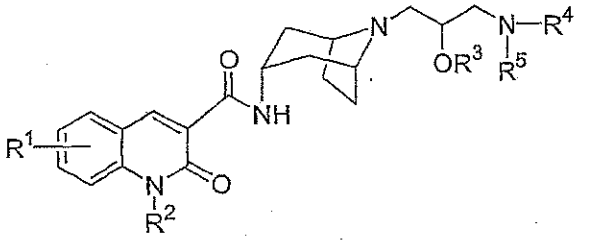
$$F(\%) = AUC_{PO}/AUC_{IV} \times Dose_{IV}/Dose_{PO} \times 100\%$$

Test compounds which exhibit larger values of the parameters C_{max}, AUC(0-t), and F(%) in this assay are expected to have greater bioavailability when administered orally. The compounds of the invention that were tested in this assay had C_{max} values typically ranging from about 0.1 to about 0.25 μg/mL and AUC(0-t) values typically ranging from about 0.4 to about 0.9 μg·hr/mL. By way of example, the compound of Example 1 had a C_{max} value of 0.17 μg/mL, an AUC(0-t) value of 0.66 μg·hr/mL and oral bioavailability (F(%)) in the rat model of about 35 %.

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 may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto. Additionally, all publications, patents, and patent documents cited hereinabove are incorporated by reference herein in full, as though individually incorporated by reference.

WHAT IS CLAIMED IS:

1. A compound of formula (I):



(I)

wherein:

R^1 is hydrogen, halo, hydroxy, C_{1-4}alkyl, or C_{1-4}alkoxy;

R^2 is C_{3-4}alkyl, or C_{3-6}cycloalkyl;

R^3 is hydrogen or C_{1-3}alkyl;

R^4 is —$S(O)_2R^6$ or —$C(O)R^7$;

R^5 is hydrogen, C_{1-3}alkyl, C_{2-3}alkyl substituted with –OH or C_{1-3}alkoxy, or —CH_2—pyridyl;

R^6 is C_{1-3}alkyl;

or, R^5 and R^6 taken together form C_{3-4}alkylenyl; and

R^7 is hydrogen, C_{1-3}alkyl, or pyridyl;

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

2. The compound of Claim 1 wherein

R^5 is hydrogen, C_{1-3}alkyl, C_{2-3}alkyl substituted with –OH or C_{1-3}alkoxy, or —CH_2—pyridyl; and

R^6 is C_{1-3}alkyl.

3 The compound of Claim 2 wherein R^1 is hydrogen or halo.

4. The compound of Claim 2 wherein R^2 is C_{3-4}alkyl.

5. The compound of Claim 2 wherein R^4 is —$S(O)_2R^6$.

6. The compound of Claim 5 wherein R^4 is —$S(O)_2CH_3$.

7. The compound of Claim 2 wherein R^4 is —$C(O)R^7$.

8. The compound of Claim 7 wherein R^7 is hydrogen or methyl.

9. The compound of Claim 2 wherein R^5 is hydrogen or methyl.

10. The compound of Claim 2 wherein:

R^1 is hydrogen;

R^2 is C_{3-4}alkyl or C_{4-5}cycloalkyl;

R^3 is hydrogen;

R^4 is —$S(O)_2R^6$ or —$C(O)R^7$;

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

R^6 is C_{1-3}alkyl; and

R^7 is hydrogen or C_{1-3}alkyl.

11. The compound of Claim 1 wherein the compound is selected from:

1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-methoxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(methanesulfonyl-pyridin-3-ylmethyl-amino)-2-methoxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-methanesulfonylamino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(methanesulfonyl-pyridin-3-ylmethyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*S*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-[methyl-(pyridine-4-carbonyl)amino]propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-[(pyridine-4-carbonyl)amino]propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(acetyl-methyl-amino)-2-methoxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-methoxy-3-[methyl-(pyridine-4-carbonyl)amino]propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(acetyl-pyridin-3-ylmethyl-amino)-2-methoxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-acetylamino-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(acetyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid {(1S,3R,5R)-8-[3-(formyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amid;

1-isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-formylamino-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-3-(acetyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-3-(formyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

5-bromo-1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(methanesulfonyl-ethylamino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonylamino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide; and

1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(1,1-dioxo-2-isothiazolidinyl)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide; and

pharmaceutically-acceptable salts and solvates and stereoisomers thereof.

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

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(methanesulfonylamino)propyl]-8-aza-bicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*S*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(acetyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[3-(formyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-3-(acetyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-3-(formyl-methyl-amino)-2-hydroxypropyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinolinone-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonylamino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide; and

pharmaceutically-acceptable salts and solvates and stereoisomers thereof.

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

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*S*)-2-hydroxy-3-(methanesulfonyl-methyl-amino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide;

1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid {(1*S*,3*R*,5*R*)-8-[(*R*)-2-hydroxy-3-(methanesulfonylamino)propyl]-8-azabicyclo[3.2.1]oct-3-yl}amide; and

pharmaceutically-acceptable salts and solvates and stereoisomers thereof.

14. A pharmaceutical composition comprising a therapeutically effective amount of the compound of any one of Claims 1 to 13 and a pharmaceutically acceptable carrier.

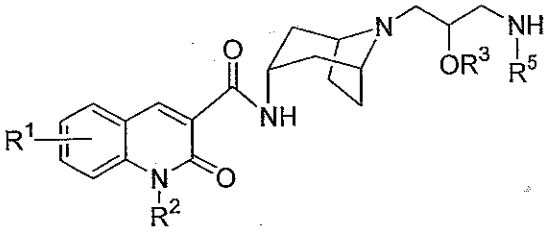
15. A compound as claimed in any one of Claims 1 to 13 for use in therapy.

16. Use of a compound of any one of Claims 1 to 13 for manufacture of a medicament for the treatment of a disorder of reduced motility of the gastrointestinal tract in a mammal.

17. The use of Claim 16, wherein the disorder of reduced motility is selected from the group consisting of chronic constipation, constipation-predominant irritable bowel syndrome, diabetic and idiopathic gastroparesis, and functional dyspepsia.

18. A process for preparing a compound of any one of Claims 1 to 13, the process comprising:

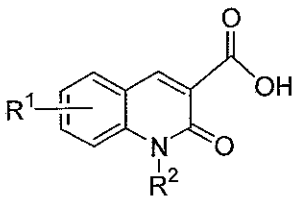
(a) reacting a compound of formula (III):



(III)

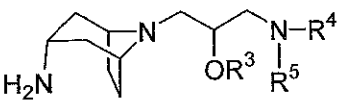
with compound of the formula L—R^4 wherein L is a leaving group, or L—R^4 represents HO-C(O)R^7; or

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



(VIII)

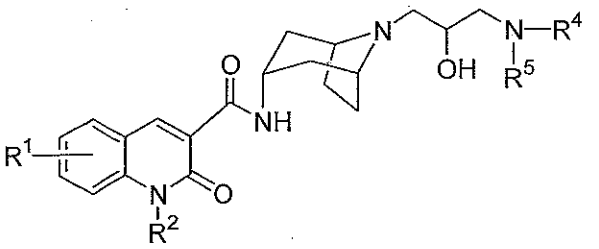
with a compound of formula (IX):



(IX)

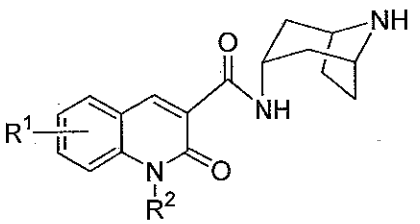
to provide a compound of formula (I), or a pharmaceutically-acceptable salt or solvate or stereoisomer thereof.

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



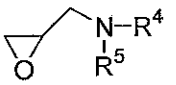
(I')

wherein R^1, R^2, R^4, and R^5 are defined as in Claim 1; or a pharmaceutically-acceptable salt or solvate or stereoisomer thereof, the process comprising reacting a compound of formula (IV):



(IV)

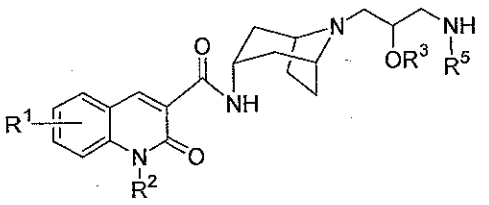
or a salt thereof with a compound of formula (XI):



(XI)

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

20. A compound of formula (III):



(III)

wherein:

R^1 is hydrogen, halo, hydroxy, C_{1-4}alkyl, or C_{1-4}alkoxy;

R^2 is C_{3-4}alkyl, or C_{3-6}cycloalkyl;

R^3 is hydrogen or C_{1-3}alkyl;

R^4 is —$S(O)_2R^6$ or —$C(O)R^7$; and

R^5 is hydrogen, C_{1-3}alkyl, C_{2-3}alkyl substituted with –OH or C_{1-3}alkoxy, or —CH_2—pyridyl;

or a salt or stereoisomer or protected derivative thereof.

21. An in vitro method of studying a biological system or sample comprising a 5-HT_4 receptor, the method comprising:

(a) contacting the biological system or sample with a compound of any one of Claims 1 to 13; and

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

ABSTRACT

The invention provides novel quinolinone-carboxamide 5-HT_4 receptor agonist compounds. The invention also provides pharmaceutical compositions comprising such compounds, methods of using such compounds to treat diseases associated with 5-HT_4 receptor activity, and processes and intermediates useful for preparing such compounds.