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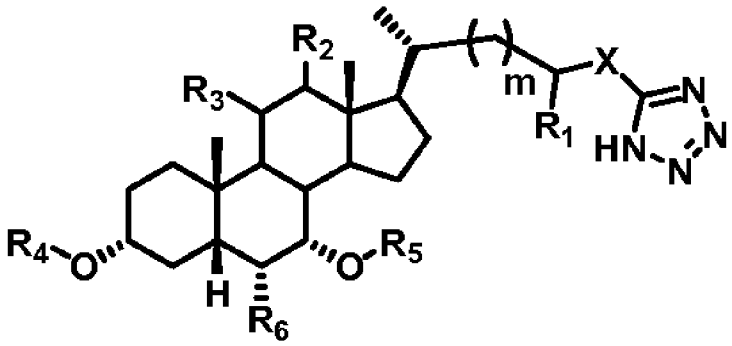
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(54) Title: TETRAZOLE DERIVATIVES OF BILE ACIDS AS FXR/TGR5 AGONISTS AND METHODS OF USE THEREOF



(I)

(57) Abstract: The present invention provides compounds of Formula I: pharmaceutical compositions comprising these compounds and methods of using these compounds to treat or prevent a disease or disorder mediated by FXR and/or TGR5.

TETRAZOLE DERIVATIVES OF BILE ACIDS AS FXR/TGR5 AGONISTS AND METHODS OF USE THEREOF

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/084,769, filed on November 26, 2014, and U.S. Provisional Application No. 62/116,042, filed on February 13, 2015. The entire teachings of the above applications are incorporated herein by reference.

TECHNICAL FIELD

The present invention relates generally to compounds and pharmaceutical compositions useful as FXR/TGR5 modulators. Specifically, the present invention relates to bile acid derivatives and methods for their preparation and use.

BACKGROUND OF THE INVENTION

Farnesoid X Receptor (FXR) is an orphan nuclear receptor initially identified from a rat liver cDNA library (BM. Forman, et al., *Cell*, **1995,** *81(5)*, 687-693) that is most closely related to the insect ecdysone receptor. FXR is a member of the nuclear receptor family of ligand-activated transcription factors that includes receptors for the steroid, retinoid, and thyroid hormones (DJ. Mangelsdorf, et al., *Cell*, **1995,** *83(6)*, 841-850). The relevant physiological ligands of FXR are bile acids (D. Parks et al., *Science*, **1999**, 284(5418), 1362-1365). The most potent one is chenodeoxycholic acid (CDCA), which regulates the expression of several genes that participate in bile acid homeostasis. Farnesol and derivatives, together called farnesoids, are originally described to activate the rat orthologue at high concentration but they do not activate the human or mouse receptor. FXR is expressed in the liver, throughout the entire gastrointestinal tract includingthe esophagus, stomach, duodenum, small intestine, colon, ovary, adrenal gland and kidney. Beyond controlling intracellular gene expression, FXR seems to be also involved in paracrine and endocrine signaling by upregulating the expression of the cytokine Fibroblast Growth Factor (J. Holt et al., *Genes Dev.*, **2003**, *17(13)*, 1581-1591; T. Inagaki et al., *Cell Metab.*, **2005**, *2(4)*, 217-225).

Small molecule compounds which act as FXR modulators have been disclosed in the following publications: WO 2000/037077, WO 2003/015771, WO

2004/048349, WO 2007/076260, WO 2007/092751, WO 2007/140174, WO 2007/140183, WO 2008/051942, WO 2008/157270, WO 2009/005998, WO 2009/012125, WO 2008/025539, WO 2008/025540, WO 2011/020615, and WO 2013/007387.

Further small molecule FXR modulators have been recently reviewed (R. C. Buijsman et al. *Curr. Med. Chem.* **2005**, *12*, 1017-1075).

TGR5 receptor is a G-protein-coupled receptor that has been identified as a cell-surface receptor that is responsive to bile acids (BAs). The primary structure of TGR5 and its responsiveness to bile acids has been found to be highly conserved in TGR5 among human, bovine, rabbit, rat, and mouse, and thus suggests that TGR5 has important physiological functions. TGR5 has been found to be widely distributed in not only lymphoid tissues but also in other tissues. High levels of TGR5 mRNA have been detected in placenta, spleen, and monocytes/macrophages. Bile acids have been shown to induce internalization of the TGR5 fusion protein from the cell membrane to the cytoplasm (Kawamata et al., *J. Bio. Chem.*, **2003**, *278*, 9435). TGR5 has been found to be identical to hGPCR19 reported by Takeda et al., *FEBS Lett.* **2002**,*520*, 97-101.

TGR5 is associated with the intracellular accumulation of cAMP, which is widely expressed in diverse cell types. While the activation of this membrane receptor in macrophages decreases pro-inflammatory cytokine production, (Kawamata, Y., et al., *J. Biol. Chem.* **2003**, *278*, 9435-9440) the stimulation of TGR5 by BAs in adipocytes and myocytes enhances energy expenditure (Watanabe, M., et al. *Nature.* **2006**, *439,* 484-489). This latter effect involves the cAMP-dependent induction of type 2 iodothyronine deiodinase (D2), which by, locally converting T4 into T3, gives rise to increased thyroid hormone activity. Consistent with the role of TGR5 in the control of energy metabolism, female TGR5 knock-out mice show a significant fat accumulation with body weight gain when challenged with a high fat diet, indicating that the lack of TGR5 decreases energy expenditure and elicits obesity (Maruyama, T., et al., *J. Endocrinol.* **2006**, *191,* 197-205). In addition and in line with the involvement of TGR5 in energy homeostasis, bile acid activation of the membrane receptor has also been reported to promote the production of glucagon-like peptide 1 (GLP-1) in murine enteroendocrine cell lines (Katsuma, S., *Biochem. Biophys. Res. Commun.,* **2005**, *329*, 386-390). On the basis of all the above observations, TGR5 is

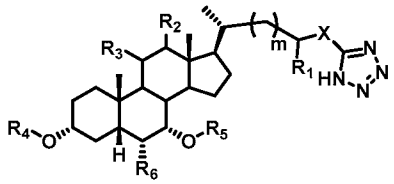
an attractive target for the treatment of disease e.g., obesity, diabetes and metabolic syndrome.

In addition to the use of TGR5 agonists for the treatment and prevention of metabolic diseases, compounds that modulate TGR5 modulators are also useful for the treatment of other diseases *e.g.*, central nervous diseases as well as inflammatory diseases (WO 01/77325 and WO 02/84286). Modulators of TGR5 also provide methods of regulating bile acid and cholesterol homeostasis, fatty acid absorption, and protein and carbohydrate digestion.

There is a need for the development of FXR and/or TGR5 modulators for the treatment and prevention of disease. The present invention has identified compounds, which contain an amino, urea, sulfonyurea or sulfonamide moieties, which modulate FXR and/or TGR as well as methods of using these compounds to treat disease.

SUMMARY OF THE INVENTION

In one aspect, the invention provides compounds represented by Formula I, or pharmaceutically acceptable salts, stereoisomers, solvates, hydrates or combination thereof:



(I)

wherein:

X is absent, –C(O)NH– or –NH–

R_1 is selected from the group consisting of:

1) Hydrogen;
2) Substituted or unsubstituted –C_1-C_8 alkyl;
3) Substituted or unsubstituted –C_2-C_8 alkenyl;
4) Substituted or unsubstituted –C_2-C_8 alkynyl;
5) Substituted or unsubstituted arylalkyl;
6) Substituted or unsubstituted aryl; or

R_1 is selected from the group consisting of:

1) Hydrogen;
2) Substituted or unsubstituted –C_1-C_8 alkyl;

3) Substituted or unsubstituted $-C_2$-C_8 alkenyl;

4) Substituted or unsubstituted $-C_2$-C_8 alkynyl;

5) Substituted or unsubstituted alkylaryl;

6) Substituted or unsubstituted aryl;

preferably R_1 is hydrogen or methyl;

m is 0, 1, 2 or 3; preferably m is 0,1 or 2.

R_2 is hydrogen, hydroxyl, $-OSO_3H$, $-OSO_3^-$, -OAc, $-OPO_3H_2$ or $-OPO_3^{2-}$; preferably R_2 is hydrogen or hydroxyl, more preferably hydrogen.

R_3 is hydrogen, halogen, CN, N_3, hydroxyl, $-OSO_3H$, $-OSO_3^-$, -OAc, $-OPO_3H_2$, $-OPO_3^{2-}$, $-SR_1$ or $-NHR_1$, wherein R_1 is as defined previously; preferably R_3 is hydrogen.

Or R_2 and R_3 are taken together with the carbon atoms to which they are attached to form –CH=CH– or cycloalkyl ring or heterocycloalkyl ring such as, but not limited to cyclopropyl or epoxide.

R_4 and R_5 are independently selected from hydrogen or hydroxyl protecting group such as, but not limited to acetyl, trimethylsilyl, or benzyl; preferably R_4 and R_5 are hydrogen.

R_6 is selected from the group consisting of:

1) Hydrogen;

2) Halogen;

3) Substituted or unsubstituted $-C_1$-C_8 alkyl;

4) Substituted or unsubstituted $-C_2$-C_8 alkenyl;

5) Substituted or unsubstituted $-C_2$-C_8 alkynyl; and

6) Substituted or unsubstituted $-C_3$-C_8 cycloalkyl;

Preferably R_6 is C_1-C_4-alkyl, more preferably R_6 is ethyl.

In another embodiment, the present invention provides a pharmaceutical composition comprising a therapeutically effective amount of a compound or combination of compounds of the present invention, or a pharmaceutically acceptable salt form, stereoisomer, solvate, hydrate or combination thereof, in combination with a pharmaceutically acceptable carrier or excipient.

In another embodiment, the present invention provides a method for the prevention or treatment of an FXR mediated disease or condition. The method comprises administering a therapeutically effective amount of a compound of formula

(I). The present invention also provides the use of a compound of formula (I) for the preparation of a medicament for the prevention or treatment of an FXR mediated disease or condition.

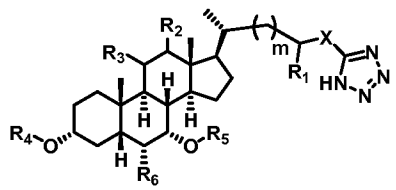
In yet another embodiment, the present invention provides a method for the prevention or treatment of a TGR5 mediated disease or condition. The method comprises administering a therapeutically effective amount of a compound of formula (I). The present invention also provides the use of a compound of formula (I) for the preparation of a medicament for the prevention or treatment of a TGR5 mediated disease or condition.

In certain embodiments, a disease that involves modulation of the TGR5 receptor is selected from metabolic disease, inflammatory disease, liver disease, autoimmune disease, cardiac disease, kidney disease, cancer, and gastrointestinal disease.

DETAILED DESCRIPTION OF THE INVENTION

A first embodiment of the invention is a compound represented by Formula I as described above, or a pharmaceutically acceptable salt, hydrate, solvate, ester or prodrug thereof. In one embodiment of the compounds of Formula I, R_1 is hydrogen or methyl, R_2 is hydrogen or hydroxyl, R_3, R_4, and R_5 are each hydrogen and R_6 is ethyl. In another embodiment of the compounds of Formula I, R_1, R_2, R_3, R_4, and R_5 are each hydrogen and R_6 is ethyl. In certain embodiments of the compounds of Formula I, R_2 is hydrogen or methyl; m is 0, 1 or 2; R_3 is hydrogen or hydroxyl; R_4 is hydrogen; R_5 is hydrogen; R_6 is hydrogen; R_7 is ethyl; R_8 is hydrogen and R_9 is hydrogen hydrogen.

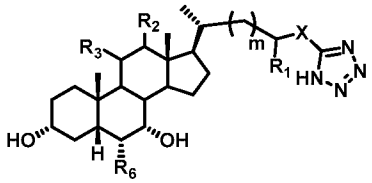
In a preferred embodiment, the compounds of the invention have the stereochemistry set forth in Formula IA:



(IA)

wherein R_1, R_2, R_3, R_4, R_5, R_6, X and m are as defined above.

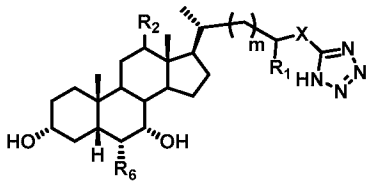
A second embodiment of the invention is a compound represented by Formula II or a pharmaceutically acceptable salt, hydrate, solvate, ester or prodrug thereof:



(II)

wherein R_1, R_2, R_3, R_6, X and m are as previously defined.

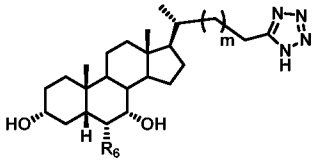
A third embodiment of the invention is a compound represented by Formula III or a pharmaceutically acceptable salt, hydrate, solvate, ester or prodrug thereof:

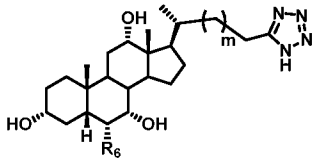


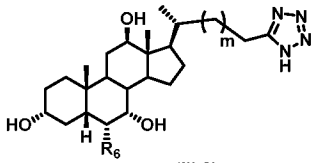
(III)

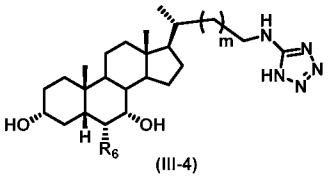
wherein R_1, R_2, R_6, X and m are as previously defined.

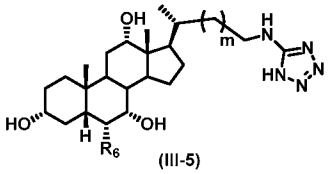
Illustrative structures of formula (III) can be represented, but not limited, by formula (III-1~III-27), where R_6 and m are as previously defined; preferably, R_6 is ethyl and m is 0, 1 or 2.

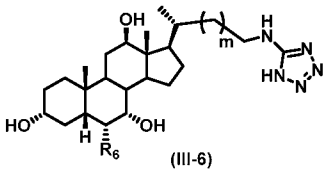

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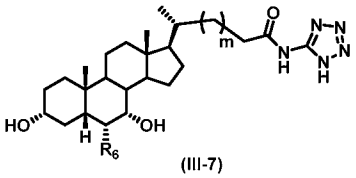

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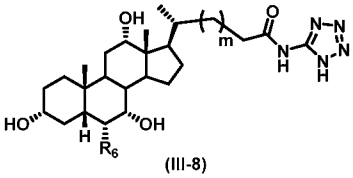

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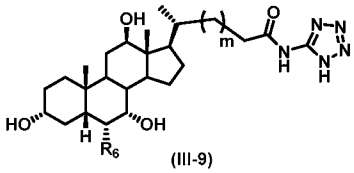

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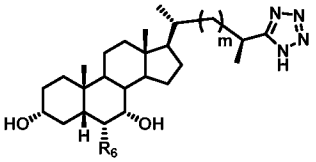

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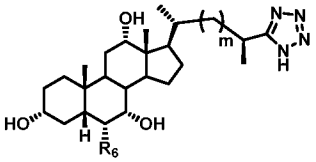

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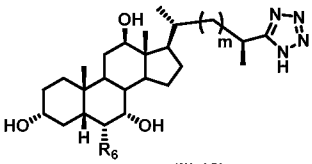

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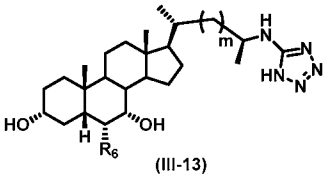

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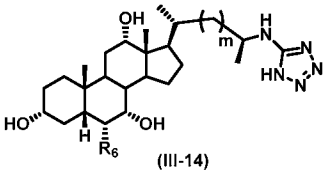

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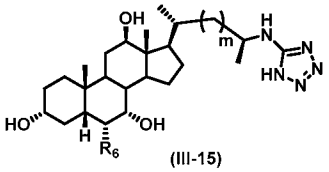

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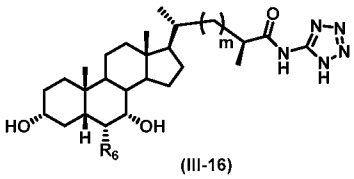

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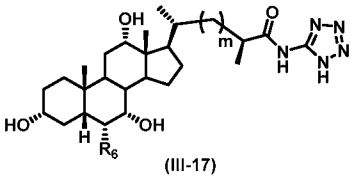

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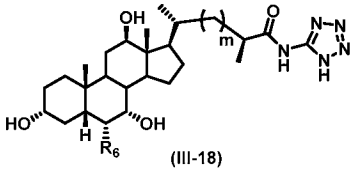

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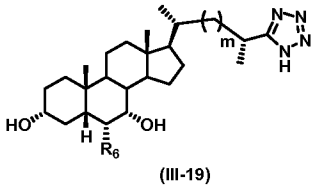

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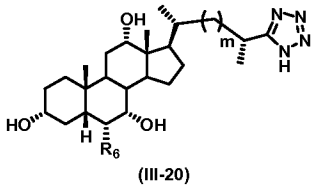

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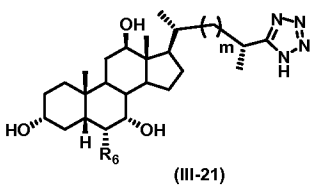

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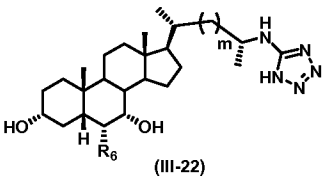

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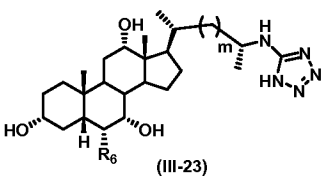

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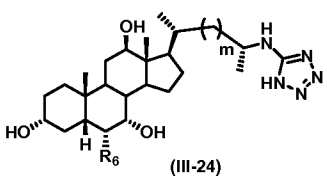

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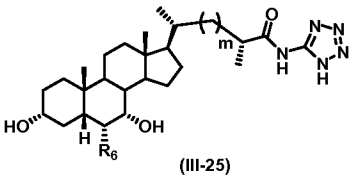

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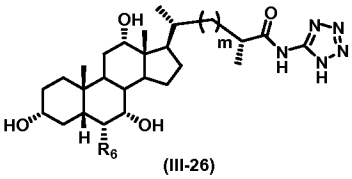

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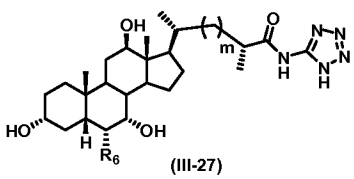

(III-22)


(III-23)

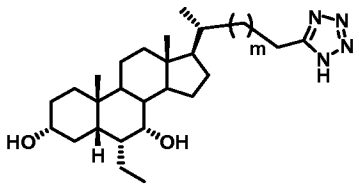

(III-24)

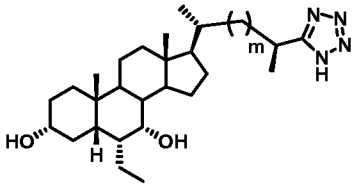

(III-25)


(III-26)


(III-27)

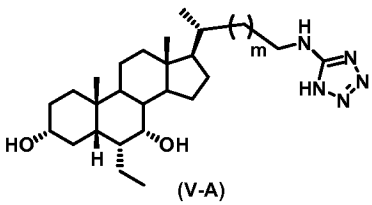
A fourth embodiment of the invention is a compound represented by Formula IV-A or IV-B or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof:

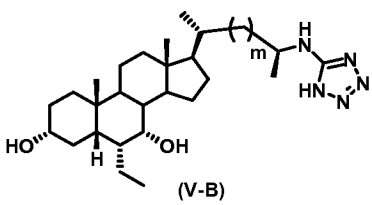

(IV-A)


(IV-B)

wherein m is as previously defined. Preferably, m is 0, 1 or 2.

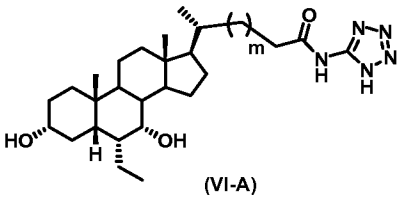
A fifth embodiment of the invention is a compound represented by Formula V-A or V-B or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof:

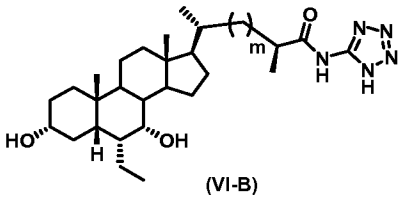

(V-A)


(V-B)

wherein m is as previously defined. Preferably, m is 0, 1 or 2.

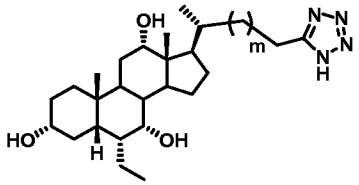
A sixth embodiment of the invention is a compound represented by Formula VI-A or VI-B or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof:

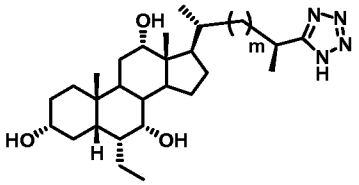

(VI-A)


(VI-B)

wherein m is as previously defined. Preferably, m is 0, 1 or 2.

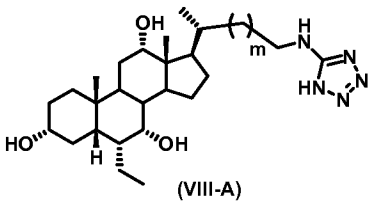
A seventh embodiment of the invention is a compound represented by Formula VII-A or VII-B or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof:

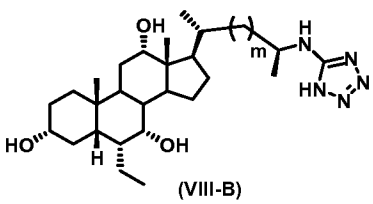

(VII-A)


(VII-B)

wherein m is as previously defined. Preferably, m is 0, 1 or 2.

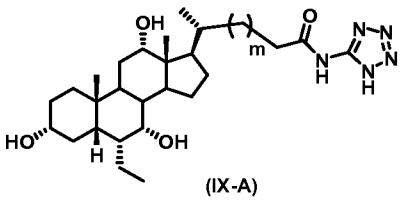
An eighth embodiment of the invention is a compound represented by Formula VIII-A or VIII-B or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof:

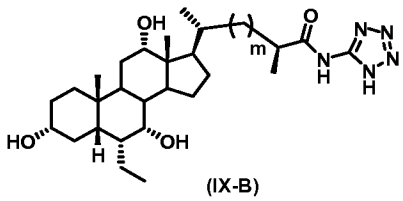

(VIII-A)


(VIII-B)

wherein m is as previously defined. Preferably, m is 0, 1 or 2.

A ninth embodiment of the invention is a compound represented by Formula IX-A or IX-B or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof:


(IX-A)


(IX-B)

wherein m is as previously defined. Preferably, m is 0, 1 or 2.

In certain embodiments, the present invention provides a method for the prevention or treatment of an FXR mediated disease or condition. The method comprises administering a therapeutically effective amount of a compound of formula

(I). The present invention also provides the use of a compound of formula (I) for the preparation of a medicament for the prevention or treatment of an FXR mediated disease or condition.

In certain embodiments, the FXR-mediated disease or condition is cardiovascular disease, atherosclerosis, arteriosclerosis, hypercholesteremia, or hyperlipidemia chronic liver disease, gastrointestinal disease, renal disease, metabolic disease, cancer (i.e., colorectal cancer), or neurological indications such as stroke.

In certain embodiments, the chronic liver disease is primary biliary cirrhosis (PBC), cerebrotendinous xanthomatosis (CTX), primary sclerosing cholangitis (PSC), drug induced cholestasis, intrahepatic cholestasis of pregnancy, parenteral nutrition associated cholestasis (PNAC), bacterial overgrowth or sepsis associated cholestasis, autoimmune hepatitis, chronic viral hepatitis, alcoholic liver disease, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), liver transplant associated graft versus host disease, living donor transplant liver regeneration, congenital hepatic fibrosis, choledocholithiasis, granulomatous liver disease, intra- or extrahepatic malignancy, Sjogren's syndrome, Sarcoidosis, Wilson's disease, Gaucher's disease, hemochromatosis, or alpha 1-antitrypsin deficiency. In certain embodiments, the gastrointestinal disease is inflammatory bowel disease (IBD) (including Crohn's disease and ulcerative colitis), irritable bowel syndrome (IBS), bacterial overgrowth, malabsorption, post-radiation colitis, or microscopic colitis.

In certain embodiments, the renal disease is diabetic nephropathy, focal segmental glomerulosclerosis (FSGS), hypertensive nephrosclerosis, chronic glomerulonephritis, chronic transplant glomerulopathy, chronic interstitial nephritis, or polycystic kidney disease.

In certain embodiments, the cardiovascular disease is atherosclerosis, arteriosclerosis, dyslipidemia, hypercholesterolemia, or hypertriglyceridemia.

In certain embodiments, the metabolic disease is insulin resistance, Type I and Type II diabetes, or obesity.

In yet another embodiment, the invention provides the use of the compound or pharmaceutical composition of the invention, in the manufacture of a medicament for a treating or preventing a disease in a subject that involves modulation of the TGR5 receptor. The invention includes a method of treating or preventing a disease that involves modulation of the TGR5 receptor in a subject by administering a compound or pharmaceutical composition of the invention.

In certain embodiments, a disease that involves modulation of the TGR5 receptor is selected from metabolic disease, inflammatory disease, liver disease, autoimmune disease, cardiac disease, kidney disease, cancer, and gastrointestinal disease.

In one aspect, the invention provides for the use, wherein the disease is an inflammatory disease selected from allergy, osteoarthritis, appendicitis, bronchial asthma, pancreatitis, allergic rash, and psoriasis. The invention includes a method of treating or preventing an inflammatory disease selected from allergy, osteoarthritis, appendicitis, bronchial asthma, pancreatitis, allergic rash, and psoriasis.

In one aspect, the invention provides for the use, wherein the disease is an autoimmune disease selected from rheumatoid arthritis, multiple sclerosis, and type I diabetes. The invention includes a method of treating or preventing an autoimmune disease selected from rheumatoid arthritis, multiple sclerosis, and type I diabetes.

In one aspect, the invention provides for the use, wherein the disease is a gastrointestinal disease selected from inflammatory bowel disease (Crohn's disease, ulcerative colitis), short bowel syndrome (post-radiation colitis), microscopic colitis, irritable bowel syndrome (malabsorption), and bacterial overgrowth. The invention includes a method of treating or preventing a gastrointestinal disease selected from inflammatory bowel disease (Crohn's disease, ulcerative colitis), short bowel syndrome (post-radiation colitis), microscopic colitis, irritable bowel syndrome (malabsorption), and bacterial overgrowth.

In one aspect, the invention provides for the use, wherein the disease is kidney disease selected from diabetic nephropathy, chronic renal failure, hypertensive nephrosclerosis, chronic glomerulonephritis, chronic transplant glomerulopathy, chronic interstitial nephritis, and polycystic kidney disease. The invention includes a method of treating or preventing kidney disease selected from diabetic nephropathy, chronic renal failure, hypertensive nephrosclerosis, chronic glomerulonephritis, chronic transplant glomerulopathy, chronic interstitial nephritis, and polycystic kidney disease.

In one aspect, the invention provides for the use, wherein the disease is cancer selected from colorectal cancer, liver cancer, hepatocellular carcinoma, cholangio carcinoma, renal cancer, gastric cancer, pancreatic cancer, prostate cancer, and insulanoma. The invention includes a method of treating or preventing cancer selected from colorectal cancer, liver cancer, hepatocellular carcinoma, cholangio carcinoma, renal cancer, gastric cancer, pancreatic cancer, prostate cancer, and insulanoma.

In one aspect, the compound is a selective FXR agonist over TGR5 activator.

In one aspect, the compound is a selective TGR5 agonist over FXR activator.

In one aspect, the compound is a dual agonist for both FXR and TGR5.

Yet a further aspect of the present invention is a process of making any of the compounds delineated herein employing any of the synthetic means delineated herein.

DEFINITIONS

Listed below are definitions of various terms used to describe this invention. These definitions apply to the terms as they are used throughout this specification and claims, unless otherwise limited in specific instances, either individually or as part of a larger group.

The term "alkyl", as used herein, refers to a saturated, monovalent straight- or branched-chain hydrocarbon group. Preferred alkyl radicals include C_1-C_6 alkyl and C_1-C_8 alkyl radicals. Examples of C_1-C_6 alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, *n*-butyl, *tert*-butyl, neopentyl, n-hexyl groups; and examples of C_1-C_8 alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, *n*-butyl, *tert*-butyl, neopentyl, n-hexyl, heptyl, and octyl groups.

The term “alkenyl”, as used herein, denote a monovalent group derived from a hydrocarbon moiety by the removal of a single hydrogen atom wherein the hydrocarbon moiety has at least one carbon-carbon double bond. Preferred alkenyl groups include C_2-C_6 alkenyl and C_2-C_8 alkenyl groups. Alkenyl groups include, but are not limited to, for example, ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, heptenyl, octenyl and the like.

The term “alkynyl”, as used herein, denotes a monovalent group derived from a hydrocarbon moiety by the removal of a single hydrogen atom wherein the hydrocarbon moiety has at least one carbon-carbon triple bond. Preferred alkynyl groups include C_2-C_6 alkynyl and C_2-C_8 alkynyl groups. Representative alkynyl groups include, but are not limited to, for example, ethynyl, 1-propynyl, 1-butynyl, heptynyl, octynyl and the like.

The term “carbocycle” refers to a saturated (e.g., “cycloalkyl”), partially saturated (e.g., “cycloalkenyl” or “cycloalkynyl”) or completely unsaturated (e.g., “aryl”) ring system containing zero heteroatom ring atom. “Ring atoms” or “ring members” are the atoms bound together to form the ring or rings. Where a carbocycle group is a divalent moiety linking two other elements in a depicted chemical structure

(such as Z in Formula I_A), the carbocycle group can be attached to the two other elements through any two substitutable ring atoms. A C_4-C_6 carbocycle has 4-6 ring atoms.

The term "cycloalkyl", as used herein, denotes a monovalent group derived from a monocyclic or polycyclic saturated carbocyclic ring compound by the removal of a single hydrogen atom. Preferred cycloalkyl groups include C_3-C_8 cycloalkyl and C_3-C_{12} cycloalkyl groups. Examples of C_3-C_8-cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclopentyl and cyclooctyl; and examples of C_3-C_{12}-cycloalkyl include, but not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, bicyclo [2.2.1] heptyl, and bicyclo [2.2.2] octyl.

The term "cycloalkenyl" as used herein, denote a monovalent group derived from a monocyclic or polycyclic carbocyclic ring compound having at least one carbon-carbon double bond by the removal of a single hydrogen atom. Preferred cycloalkenyl groups include C_3-C_8 cycloalkenyl and C_3-C_{12} cycloalkenyl groups. Examples of C_3-C_8-cycloalkenyl include, but are not limited to, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl, and the like; and examples of C_3-C_{12}-cycloalkenyl include, but not limited to, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl, and the like.

The term "aryl," as used herein, refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like.

The term “arylalkyl,” as used herein, refers to a C_1–C_3 alkyl or C_1–C_6 alkyl residue attached to an aryl ring. Examples include, but are not limited to, benzyl, phenethyl and the like.

The term "heteroaryl," as used herein, refers to a mono-, bi-, or tri-cyclic aromatic radical or ring having from five to ten ring atoms of which at least one ring atom is selected from S, O and N; wherein any N or S contained within the ring may be optionally oxidized. Preferred heteroaryl groups are monocyclic or bicyclic. Heteroaryl groups include, but are not limited to, pyridinyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, benzimidazolyl, benzooxazolyl, quinoxalinyl, and the like.

The term "heteroarylalkyl," as used herein, refers to a C_1–C_3 alkyl or C_1–C_6 alkyl residue residue attached to a heteroaryl ring. Examples include, but are not limited to, pyridinylmethyl, pyrimidinylethyl and the like.

The term "substituted" as used herein, refers to independent replacement of one, two, or three or more of the hydrogen atoms thereon with substituents including, but not limited to, deuterium, -F, -Cl, -Br, -I, -OH, protected hydroxy, -NO_2, -CN, -NH_2, N_3, protected amino, alkoxy, thioalkoxy, oxo, -halo- C_1-C_{12}-alkyl, -halo- C_2-C_{12}-alkenyl, -halo- C_2-C_{12}-alkynyl, -halo-C_3-C_{12}-cycloalkyl, -NH -C_1-C_{12}-alkyl, -NH -C_2-C_{12}-alkenyl, -NH -C_2-C_{12}-alkynyl, -NH -C_3-C_{12}-cycloalkyl, -NH -aryl, -NH -heteroaryl, -NH -heterocycloalkyl, -dialkylamino, -diarylamino, -diheteroarylamino, -O-C_1-C_{12}-alkyl, -O-C_2-C_{12}-alkenyl, -O-C_2-C_{12}-alkynyl, -O-C_3-C_{12}-cycloalkyl, -O-aryl, -O-heteroaryl, -O-heterocycloalkyl, -C(O)- C_1-C_{12}-alkyl, -C(O)- C_2-C_{12}-alkenyl, -C(O)- C_2-C_{12}-alkynyl, -C(O)-C_3-C_{12}-cycloalkyl, -C(O)-aryl, -C(O)-heteroaryl, -C(O)-heterocycloalkyl, -$CONH_2$, -CONH- C_1-C_{12}-alkyl, -CONH- C_2-C_{12}-alkenyl, -CONH- C_2-C_{12}-alkynyl, -CONH-C_3-C_{12}-cycloalkyl, -CONH-aryl, -CONH-heteroaryl, -CONH-heterocycloalkyl, -OCO_2- C_1-C_{12}-alkyl, -OCO_2- C_2-C_{12}-alkenyl, -OCO_2- C_2-C_{12}-alkynyl, -OCO_2-C_3-C_{12}-cycloalkyl, -OCO_2-aryl, -OCO_2-heteroaryl, -OCO_2-heterocycloalkyl, -$OCONH_2$, -OCONH- C_1-C_{12}-alkyl, -OCONH- C_2-C_{12}-alkenyl, -OCONH- C_2-C_{12}-alkynyl, -OCONH- C_3-C_{12}-cycloalkyl, -OCONH- aryl, -OCONH-heteroaryl, -OCONH- heterocycloalkyl, -NHC(O)- C_1-C_{12}-alkyl, -NHC(O)-C_2-C_{12}-alkenyl, -NHC(O)-C_2-C_{12}-alkynyl, -NHC(O)-C_3-C_{12}-cycloalkyl, -NHC(O)-aryl, -NHC(O)-heteroaryl, -NHC(O)-heterocycloalkyl, -$NHCO_2$- C_1-C_{12}-alkyl, -$NHCO_2$-C_2-C_{12}-alkenyl, -$NHCO_2$- C_2-C_{12}-alkynyl, -$NHCO_2$- C_3-C_{12}-cycloalkyl, -$NHCO_2$-aryl, -$NHCO_2$- heteroaryl, -$NHCO_2$- heterocycloalkyl, -$NHC(O)NH_2$, -NHC(O)NH-C_1-C_{12}-alkyl, -NHC(O)NH-C_2-C_{12}-alkenyl, -NHC(O)NH-C_2-C_{12}-alkynyl, -NHC(O)NH-C_3-C_{12}-cycloalkyl, -NHC(O)NH-aryl, -NHC(O)NH-heteroaryl, -NHC(O)NH-heterocycloalkyl, $NHC(S)NH_2$, -NHC(S)NH- C_1-C_{12}-alkyl, -NHC(S)NH-C_2-C_{12}-alkenyl, -NHC(S)NH-C_2-C_{12}-alkynyl, -NHC(S)NH-C_3-C_{12}-cycloalkyl, -NHC(S)NH-aryl, -NHC(S)NH-heteroaryl, -NHC(S)NH-heterocycloalkyl, -$NHC(NH)NH_2$, -NHC(NH)NH- C_1-C_{12}-alkyl, -NHC(NH)NH-C_2-C_{12}-alkenyl, -NHC(NH)NH-C_2-C_{12}-alkynyl, -NHC(NH)NH-C_3-C_{12}-cycloalkyl, -NHC(NH)NH-aryl, -NHC(NH)NH-heteroaryl, -NHC(NH)NH-heterocycloalkyl, -NHC(NH)-C_1-C_{12}-alkyl, -NHC(NH)-C_2-C_{12}-alkenyl, -NHC(NH)-C_2-C_{12}-alkynyl, -NHC(NH)-C_3-C_{12}-

cycloalkyl, -NHC(NH)-aryl, -NHC(NH)-heteroaryl, -NHC(NH)-heterocycloalkyl, -C(NH)NH-C_1-C_{12}-alkyl, -C(NH)NH-C_2-C_{12}-alkenyl, -C(NH)NH-C_2-C_{12}-alkynyl, -C(NH)NH-C_3-C_{12}-cycloalkyl, -C(NH)NH-aryl, -C(NH)NH-heteroaryl, -C(NH)NH-heterocycloalkyl, -S(O)-C_1-C_{12}-alkyl, - S(O)-C_2-C_{12}-alkenyl, - S(O)-C_2-C_{12}-alkynyl, - S(O)-C_3-C_{12}-cycloalkyl, - S(O)-aryl, - S(O)-heteroaryl, - S(O)-heterocycloalkyl - SO_2NH_2, -SO_2NH- C_1-C_{12}-alkyl, -SO_2NH- C_2-C_{12}-alkenyl, -SO_2NH- C_2-C_{12}-alkynyl, -SO_2NH- C_3-C_{12}-cycloalkyl, -SO_2NH- aryl, -SO_2NH- heteroaryl, -SO_2NH-heterocycloalkyl, -NHSO_2-C_1-C_{12}-alkyl, -NHSO_2-C_2-C_{12}-alkenyl, - NHSO_2-C_2-C_{12}-alkynyl, -NHSO_2-C_3-C_{12}-cycloalkyl, -NHSO_2-aryl, -NHSO_2-heteroaryl, -NHSO_2-heterocycloalkyl, -CH_2NH_2, -$CH_2SO_2CH_3$, -aryl, -arylalkyl, -heteroaryl, -heteroarylalkyl, -heterocycloalkyl, -C_3-C_{12}-cycloalkyl, polyalkoxyalkyl, polyalkoxy, -methoxymethoxy, -methoxyethoxy, -SH, -S-C_1-C_{12}-alkyl, -S-C_2-C_{12}-alkenyl, -S-C_2-C_{12}-alkynyl, -S-C_3-C_{12}-cycloalkyl, -S-aryl, -S-heteroaryl, -S-heterocycloalkyl, methylthiomethyl, or –L'–R', wherein L' is C_1-C_6alkylene, C_2-C_6alkenylene or C_2-C_6alkynylene, and R' is aryl, heteroaryl, heterocyclic, C_3-C_{12}cycloalkyl or C_3-C_{12}cycloalkenyl. It is understood that the aryls, heteroaryls, alkyls, and the like can be further substituted. In some cases, each substituent in a substituted moiety is additionally optionally substituted with one or more groups, each group being independently selected from -F, -Cl, -Br, -I, -OH, -NO_2, -CN, or -NH_2.

In accordance with the invention, any of the aryls, substituted aryls, heteroaryls and substituted heteroaryls described herein, can be any aromatic group. Aromatic groups can be substituted or unsubstituted.

It is understood that any alkyl, alkenyl, alkynyl, cycloalkyl and cycloalkenyl moiety described herein can also be an aliphatic group, an alicyclic group or a heterocyclic group. An "aliphatic group" is non-aromatic moiety that may contain any combination of carbon atoms, hydrogen atoms, halogen atoms, oxygen, nitrogen or other atoms, and optionally contain one or more units of unsaturation, e.g., double and/or triple bonds. An aliphatic group may be straight chained, branched or cyclic and preferably contains between about 1 and about 24 carbon atoms, more typically between about 1 and about 12 carbon atoms. In addition to aliphatic hydrocarbon groups, aliphatic groups include, for example, polyalkoxyalkyls, such as polyalkylene glycols, polyamines, and polyimines, for example. Such aliphatic groups may be further substituted. It is understood that aliphatic groups may be used in place of the alkyl, alkenyl, alkynyl, alkylene, alkenylene, and alkynylene groups described herein.

The term "alicyclic," as used herein, denotes a monovalent group derived from a monocyclic or polycyclic saturated carbocyclic ring compound by the removal of a single hydrogen atom. Examples include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, bicyclo [2.2.1] heptyl, and bicyclo [2.2.2] octyl. Such alicyclic groups may be further substituted.

The term "heterocycloalkyl" and "heterocyclic" can be used interchangeably and refer to a non-aromatic 3-, 4-, 5-, 6- or 7-membered ring or a bi- or tri-cyclic group fused system, where: (i) each ring contains between one and three heteroatoms independently selected from oxygen, sulfur and nitrogen, (ii) each 5-membered ring has 0 to 1 double bonds and each 6-membered ring has 0 to 2 double bonds, (iii) the nitrogen and sulfur heteroatoms may optionally be oxidized, (iv) the nitrogen heteroatom may optionally be quaternized, (v) any of the above rings may be fused to a benzene ring, and (vi) the remaining ring atoms are carbon atoms which may be optionally oxo-substituted. Representative heterocycloalkyl groups include, but are not limited to, [1,3]dioxolane, pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, and tetrahydrofuryl. Such heterocyclic groups may be further substituted to give substituted heterocyclic.

It will be apparent that in various embodiments of the invention, the substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, arylalkyl, heteroarylalkyl, and heterocycloalkyl are intended to be monovalent or divalent. Thus, alkylene, alkenylene, and alkynylene, cycloaklylene, cycloalkenylene, cycloalkynylene, arylalkylene, hetoerarylalkylene and heterocycloalkylene groups are to be included in the above definitions, and are applicable to provide the formulas herein with proper valency.

The term "hydroxy activating group", as used herein, refers to a labile chemical moiety which is known in the art to activate a hydroxy group so that it will depart during synthetic procedures such as in a substitution or elimination reactions. Examples of hydroxy activating group include, but not limited to, mesylate, tosylate, triflate, *p*-nitrobenzoate, phosphonate and the like.

The term "activated hydroxy", as used herein, refers to a hydroxy group activated with a hydroxy activating group, as defined above, including mesylate, tosylate, triflate, p-nitrobenzoate, phosphonate groups, for example.

The term "protected hydroxy," as used herein, refers to a hydroxy group protected with a hydroxy protecting group, as defined above, including benzoyl, acetyl, trimethylsilyl, triethylsilyl, and methoxymethyl groups.

The term "hydroxy protecting group," as used herein, refers to a labile chemical moiety which is known in the art to protect a hydroxy group against undesired reactions during synthetic procedures. After said synthetic procedure(s) the hydroxy protecting group as described herein may be selectively removed. Hydroxy protecting groups as known in the are described generally in T.H. Greene and P.G., S. M. Wuts, *Protective Groups in Organic Synthesis*, 3rd edition, John Wiley & Sons, New York (1999). Examples of hydroxy protecting groups include benzyloxycarbonyl, 4-nitrobenzyloxycarbonyl, 4-bromobenzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, methoxycarbonyl, tert-butoxycarbonyl, isopropoxycarbonyl, diphenylmethoxycarbonyl, 2,2,2-trichloroethoxycarbonyl, 2-(trimethylsilyl)ethoxycarbonyl, 2-furfuryloxycarbonyl, allyloxycarbonyl, acetyl, formyl, chloroacetyl, trifluoroacetyl, methoxyacetyl, phenoxyacetyl, benzoyl, methyl, t-butyl, 2,2,2-trichloroethyl, 2-trimethylsilyl ethyl, 1,1-dimethyl-2-propenyl, 3-methyl- 3 -butenyl, allyl, benzyl, para-methoxybenzyldiphenylmethyl, triphenylmethyl (trityl), tetrahydrofuryl, methoxymethyl, methylthiomethyl, benzyloxymethyl, 2,2,2-trichloroethoxymethyl, 2-(trimethylsilyl)ethoxymethyl, methanesulfonyl, para-toluenesulfonyl, trimethylsilyl, triethylsilyl, triisopropylsilyl, and the like. Preferred hydroxy protecting groups for the present invention are acetyl (Ac or -C(O)CH_3), benzoyl (Bz or -C(O)C_6H_5), and trimethylsilyl (TMS or-Si$(CH_3)_3$).

The terms "halo" and "halogen," as used herein, refer to an atom selected from fluorine, chlorine, bromine and iodine.

The compounds described herein contain one or more asymmetric centers and thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)- , or as (D)- or (L)- for amino acids. The present invention is meant to include all such possible isomers, as well as their racemic and optically pure forms. Optical isomers may be prepared from their respective optically active precursors by the procedures described above, or by resolving the racemic mixtures. The resolution can be carried out in the presence of a resolving agent, by chromatography or by repeated crystallization or by some combination of these techniques, which are known to those skilled in the art. Further details regarding resolutions can be found in Jacques, *et al.*, *Enantiomers, Racemates,*

and Resolutions (John Wiley & Sons, 1981). When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers. Likewise, all tautomeric forms are also intended to be included. The configuration of any carbon-carbon double bond appearing herein is selected for convenience only and is not intended to designate a particular configuration unless the text so states; thus a carbon-carbon double bond depicted arbitrarily herein as *trans* may be *cis, trans*, or a mixture of the two in any proportion.

The term "subject" as used herein refers to a mammal. A subject therefore refers to, for example, dogs, cats, horses, cows, pigs, guinea pigs, and the like. Preferably the subject is a human. When the subject is a human, the subject may be referred to herein as a patient.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts of the compounds formed by the process of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art.

Berge, *et al.* describes pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 66: 1-19 (1977). The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or separately by reacting the free base function with a suitable organic acid. Examples of pharmaceutically acceptable salts include, but are not limited to, nontoxic acid addition salts e.g., salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include, but are not limited to, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate,

oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, *p*-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl having from 1 to 6 carbon atoms, sulfonate and aryl sulfonate.

Pharmaceutically acceptable salts can also be prepared by deprotonation of the parent compound with a suitable base, thereby forming the anionic conjugate base of the parent compound. In such salts the counter ion is a cation. Suitable cations include ammonium and metal cations, such as alkali metal cations, including Li^+, Na^+, K^+ and Cs^+, and alkaline earth metal cations, such as Mg^{2+} and Ca^{2+}.

The term “amino protecting group,” as used herein, refers to a labile chemical moiety which is known in the art to protect an amino group against undesired reactions during synthetic procedures. After said synthetic procedure(s) the amino protecting group as described herein may be selectively removed. Amino protecting groups as known in the are described generally in T.H. Greene and P.G. M. Wuts, *Protective Groups in Organic Synthesis*, 3rd edition, John Wiley & Sons, New York (1999). Examples of amino protecting groups include, but are not limited to, t-butoxycarbonyl, 9-fluorenylmethoxycarbonyl, benzyloxycarbonyl, and the like.

As used herein, the term "pharmaceutically acceptable ester" refers to esters of the compounds formed by the process of the present invention which hydrolyze *in vivo* and include those that break down readily in the human body to leave the parent compound or a salt thereof. Suitable ester groups include, for example, those derived from pharmaceutically acceptable aliphatic carboxylic acids, particularly alkanoic, alkenoic, cycloalkanoic and alkanedioic acids, in which each alkyl or alkenyl moiety advantageously has not more than 6 carbon atoms. Examples of particular esters include, but are not limited to, formates, acetates, propionates, butyrates, acrylates and ethylsuccinates.

The term “pharmaceutically acceptable prodrugs” as used herein refers to those prodrugs of the compounds formed by the process of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals with undue toxicity, irritation, allergic

response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the present invention. "Prodrug", as used herein means a compound, which is convertible *in vivo* by metabolic means (e.g. by hydrolysis) to afford any compound delineated by the formulae of the instant invention. Various forms of prodrugs are known in the art, for example, as discussed in Bundgaard, (ed.), *Design of Prodrugs*, Elsevier (1985); Widder, *et al.* (ed.), *Methods in Enzymology*, Vol. 4, Academic Press (1985); Krogsgaard-Larsen, *et al.*, (ed). "Design and Application of Prodrugs, *Textbook of Drug Design and Development*, Chapter 5, 113-191 (1991); Bundgaard, *et al.*, *Journal of Drug Deliver Reviews, 8*:1-38(1992); Bundgaard, J. of *Pharmaceutical Sciences, 77*:285 et seq. (1988); Higuchi and Stella (eds.) Prodrugs as Novel Drug Delivery Systems, *American Chemical Society* (1975); and Bernard Testa & Joachim Mayer, "Hydrolysis In Drug And Prodrug Metabolism: Chemistry, Biochemistry And Enzymology," John Wiley and Sons, Ltd. (2002).

The term "treating", as used herein, means relieving, lessening, reducing, eliminating, modulating, or ameliorating, i.e. causing regression of the disease state or condition. Treating can also include inhibiting, i.e. arresting the development, of a existing disease state or condition, and relieving or ameliorating, i.e. causing regression of an existing disease state or condition, for example when the disease state or condition may already be present.

The term "preventing", as used herein means, to completely or almost completely stop a disease state or condition, from occurring in a patient or subject, especially when the patient or subject is predisposed to such or at risk of contracting a disease state or condition.

Additionally, the compounds of the present invention, for example, the salts of the compounds, can exist in either hydrated or unhydrated (the anhydrous) form or as solvates with other solvent molecules. Nonlimiting examples of hydrates include monohydrates, dihydrates, etc. Nonlimiting examples of solvates include ethanol solvates, acetone solvates, etc.

"Solvates" means solvent addition forms that contain either stoichiometric or non stoichiometric amounts of solvent. Some compounds have a tendency to trap a fixed molar ratio of solvent molecules in the crystalline solid state, thus forming a solvate. If the solvent is water the solvate formed is a hydrate, when the solvent is alcohol, the solvate formed is an alcoholate. Hydrates are formed by the combination of one or more

molecules of water with one of the substances in which the water retains its molecular state as H_2O, such combination being able to form one or more hydrate.

As used herein, the term "analog" refers to a chemical compound that is structurally similar to another but differs slightly in composition (as in the replacement of one atom by an atom of a different element or in the presence of a particular functional group, or the replacement of one functional group by another functional group). Thus, an analog is a compound that is similar to or comparable in function and appearance to the reference compound.

The term "aprotic solvent," as used herein, refers to a solvent that is relatively inert to proton activity, i.e., not acting as a proton-donor. Examples include, but are not limited to, hydrocarbons, such as hexane and toluene, for example, halogenated hydrocarbons, such as, for example, methylene chloride, ethylene chloride, chloroform, and the like, heterocyclic compounds, such as, for example, tetrahydrofuran and N-methylpyrrolidinone, and ethers such as diethyl ether, bis-methoxymethyl ether. Such solvents are well known to those skilled in the art, and individual solvents or mixtures thereof may be preferred for specific compounds and reaction conditions, depending upon such factors as the solubility of reagents, reactivity of reagents and preferred temperature ranges, for example. Further discussions of aprotic solvents may be found in organic chemistry textbooks or in specialized monographs, for example: *Organic Solvents Physical Properties and Methods of Purification*, 4th ed., edited by John A. Riddick *et al.*, Vol. II, in the *Techniques of Chemistry Series*, John Wiley & Sons, NY, 1986.

The terms "protogenic organic solvent" or "protic solvent" as used herein, refer to a solvent that tends to provide protons, such as an alcohol, for example, methanol, ethanol, propanol, isopropanol, butanol, t-butanol, and the like. Such solvents are well known to those skilled in the art, and individual solvents or mixtures thereof may be preferred for specific compounds and reaction conditions, depending upon such factors as the solubility of reagents, reactivity of reagents and preferred temperature ranges, for example. Further discussions of protogenic solvents may be found in organic chemistry textbooks or in specialized monographs, for example: *Organic Solvents Physical Properties and Methods of Purification*, 4th ed., edited by John A. Riddick *et al.*, Vol. II, in the *Techniques of Chemistry Series*, John Wiley & Sons, NY, 1986.

Combinations of substituents and variables envisioned by this invention are only those that result in the formation of stable compounds. The term "stable", as used herein, refers to compounds which possess stability sufficient to allow manufacture and which maintains the integrity of the compound for a sufficient period of time to be useful for the purposes detailed herein (e.g., therapeutic or prophylactic administration to a subject).

The synthesized compounds can be separated from a reaction mixture and further purified by a method such as column chromatography, high pressure liquid chromatography, or recrystallization. Additionally, the various synthetic steps may be performed in an alternate sequence or order to give the desired compounds. In addition, the solvents, temperatures, reaction durations, etc. delineated herein are for purposes of illustration only and variation of the reaction conditions can produce the desired bridged macrocyclic products of the present invention. Synthetic chemistry transformations and protecting group methodologies (protection and deprotection) useful in synthesizing the compounds described herein include, for example, those described in R. Larock, *Comprehensive Organic Transformations*, VCH Publishers (1989); T.W. Greene and P.G.M. Wuts, *Protective Groups in Organic Synthesis*, 2d. Ed., John Wiley and Sons (1991); L. Fieser and M. Fieser, *Fieser and Fieser's Reagents for Organic Synthesis*, John Wiley and Sons (1994); and L. Paquette, ed., *Encyclopedia of Reagents for Organic Synthesis*, John Wiley and Sons (1995).

The compounds of this invention may be modified by appending various functionalities via synthetic means delineated herein to enhance selective biological properties. Such modifications include those which increase biological penetration into a given biological system (e.g., blood, lymphatic system, central nervous system), increase oral availability, increase solubility to allow administration by injection, alter metabolism and alter rate of excretion.

PHARMACEUTICAL COMPOSITIONS

The pharmaceutical compositions of the present invention comprise a therapeutically effective amount of a compound of the present invention formulated together with one or more pharmaceutically acceptable carriers. As used herein, the term "pharmaceutically acceptable carrier" means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Some examples of materials which can serve as pharmaceutically acceptable carriers

are sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols; such a propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator. The pharmaceutical compositions of this invention can be administered to humans and other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), buccally, or as an oral or nasal spray.

The pharmaceutical compositions of this invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir, preferably by oral administration or administration by injection. The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. In some cases, the pH of the formulation may be adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art 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, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive,

castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1, 3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

In order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution, which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of this invention with suitable non-

irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or: a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The active compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed

manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulation, ear drops, eye ointments, powders and solutions are also contemplated as being within the scope of this invention.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to the compounds of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons.

Transdermal patches have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

Unless otherwise defined, all technical and scientific terms used herein are accorded the meaning commonly known to one with ordinary skill in the art. All publications, patents, published patent applications, and other references mentioned herein are hereby incorporated by reference in their entirety.

Abbreviations

Abbreviations which have been used in the descriptions of the schemes and the examples that follow are:

ACN for acetonitrile;

BME for 2-mercaptoethanol;

BOP for benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate;

Bu_3SnN_3 for tributyltin azide;

BzCl for benzoyl chloride;

CDI for carbonyldiimidazole;

COD for cyclooctadiene;

DABCO for 1,4-diazabicyclo[2.2.2]octane;

DAST for diethylaminosulfur trifluoride;

DABCYL for 6-(N-4'-carboxy-4-(dimethylamino)azobenzene)- aminohexyl-1-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite;

DBU for 1, 8-Diazabicycloundec-7-ene;

DCC for N, N'-dicyclohexylcarbodiimide;

DCM for dichloromethane;

DIAD for diisopropyl azodicarboxylate;

DIBAL-H for diisobutylaluminum hydride;

DIPEA for diisopropyl ethylamine;

DMAP for N,N-dimethylaminopyridine;

DME for ethylene glycol dimethyl ether;

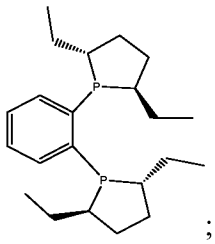
DMEM for Dulbecco's Modified Eagles Media;

DMF for N,N-dimethyl formamide;

DMSO for dimethylsulfoxide;

DSC for N, N'-disuccinimidyl carbonate;

DPPA for diphenylphosphoryl azide;

DUPHOS for ;

EDANS for 5-(2-Amino-ethylamino)-naphthalene-1-sulfonic acid;

EDCI or EDC for 1-(3-diethylaminopropyl)-3-ethylcarbodiimide hydrochloride;

Et_2O for diethyl ether;

EtOAc for ethyl acetate;

EtOH for ethyl alcohol;

HATU for O (7-Azabenzotriazole-1-yl)-N,N,N',N' – tetramethyluronium hexafluorophosphate;

HCl for hydrochloric acid;

Hoveyda's Cat. for Dichloro(*o*-isopropoxyphenylmethylene) (tricyclohexylphosphine)ruthenium(II);

In for indium;

KHMDS for potassium bis(trimethylsilyl) amide;

LAH for lithium aluminium hydride;

Ms for mesyl;

NaCN for sodium cyanide;

NaN_3 for sodium aizde;

NMM for N-4-methylmorpholine;

NMI for N-methylimidazole;

NMO for N-4-methylmorpholine-N-Oxide;

PyBrOP for Bromo-tri-pyrolidino-phosphonium hexafluorophosphate;

Ph for phenyl;

RCM for ring-closing metathesis;

RT for reverse transcription;

RT-PCR for reverse transcription-polymerase chain reaction;

TBME for tert-butyl methyl ether;

TEA for triethyl amine;

Tf_2O for trifluoromethanesulfonic anhydride;

TFA for trifluoroacetic acid;

THF for tetrahydrofuran;

TLC for thin layer chromatography;

$(TMS)_2NH$ for hexamethyldisilazane;

TMSOTf for trimethylsilyl trifluoromethanesulfonate;

$TMSN_3$ for trimethylsilyl azide;

TBS for t-Butyldimethylsilyl;

TMS for trimethylsilyl;

TPAP tetrapropylammonium perruthenate;

TPP or PPh_3 for triphenylphosphine;

TrCl for trityl chloride;

DMTrCl for 4,4'-dimethoxytrityl chloride;

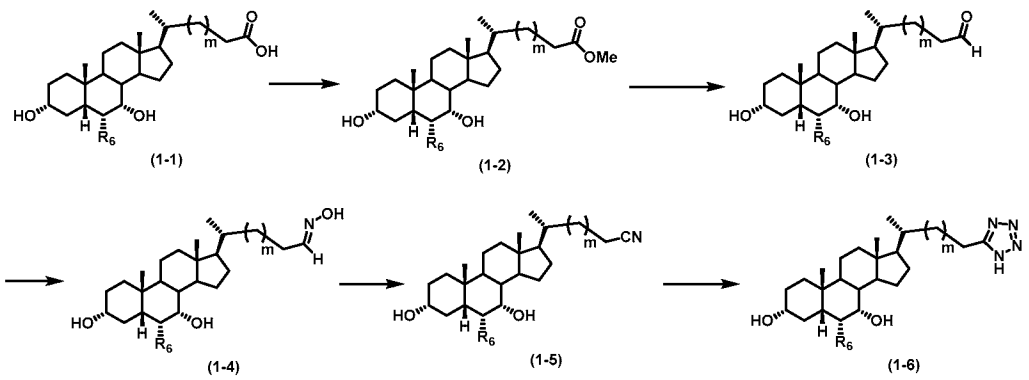
tBOC or Boc for tert-butyloxy carbonyl.

Synthetic Methods

The compounds and processes of the present invention will be better understood in connection with the following synthetic schemes that illustrate the methods by which the compounds of the invention may be prepared, which are intended as an illustration only and not to limit the scope of the invention. Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art and such changes and modifications including, without limitation, those relating to the chemical structures, substituents, derivatives, and/or methods of the invention may be made without departing from the spirit of the invention and the scope of the appended claims.

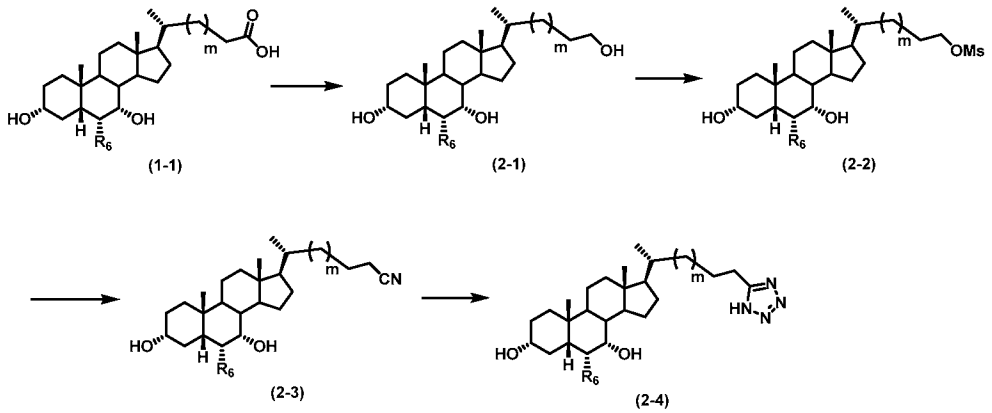
As shown in Scheme 1, novel bile acid analogs of the tetrazole compound of formula (1-6) are prepared from the compound of formula (1-1), wherein m and R_6 are defined as previously. The compound of formula (1-1) is converted to the methyl ester compound of formula (1-2) through esterification. Then the compound of formula (1-2) is partially reduced to the aldehyde compound of formula (1-3) using suitable reagent such as, but not limited to, DIBAL-H. The reaction solvent can be, but not limited to, Et_2O, THF, DCM and toluene. The preferred solvent is Et_2O. The reaction temperature is -78 ^oC. Subsequent reaction of formula (1-3) with hydroxylamine hydrochloride followed by dehydration afford the nitrile compound of formula (1-5), which is then converted to the tetrazole compound of formula (1-6) using suitable reagent such as, but not limited to, NaN_3, $TMSN_3$ and Bu_3SnN_3.

Scheme 1



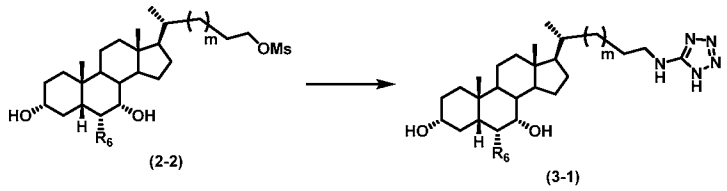
An alternative procedure to prepare tetrazole compound of formula (2-4) is illustrated in scheme 2, wherein m and R_6 are defined as previously. The compound of formula (1-1) is converted to the alcohol compound of formula (2-1) using suitable reagent such as, but not limited to, LAH. Subsequent mesylation followed by replacement reaction provide the nitrile compound of formula (2-3), which is then converted to the tetrazole compound of formula (2-4) using suitable reagent such as, but not limited to, NaN_3, $TMSN_3$ and Bu_3SnN_3.

Scheme 2



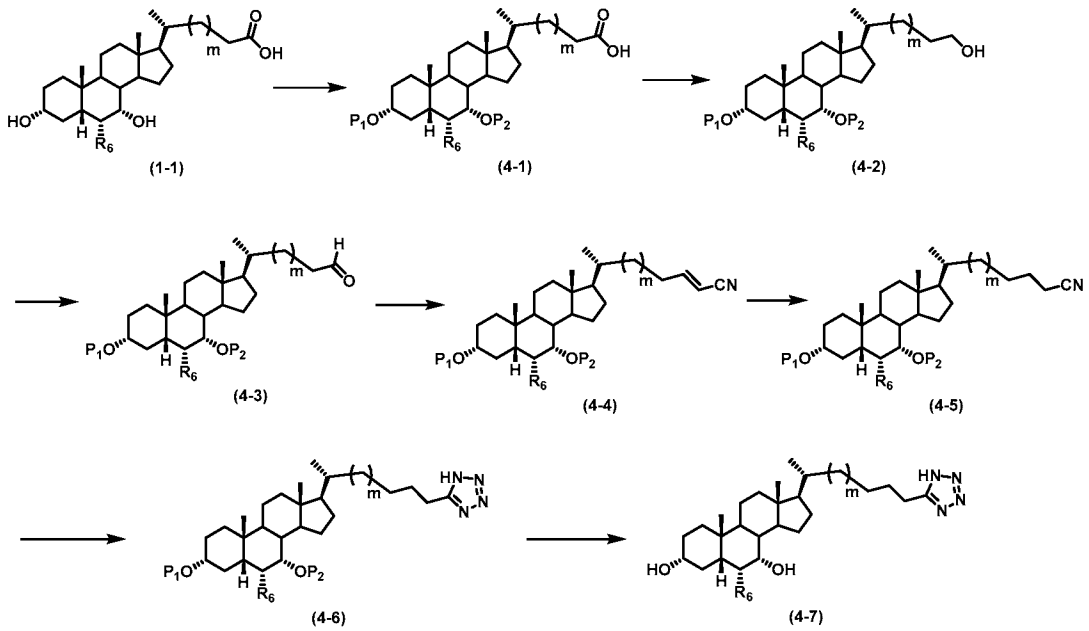
Scheme 3 illustrates the preparation of the amino tetrazole compound of formula (3-1) from the compound of formula (2-2), wherein m and R_6 are defined as previously. The compound of formula (2-2) is converted to the compound of formula (3-1) by replacement of 5-aminotetrazole.

Scheme 3



Another procedure to prepare the tetrazole compound of formula (4-7) is illustrated in scheme 4, wherein m and R_6 are defined as previously, P_1 and P_2 are hydroxyl protecting groups. Thus, the two hydroxyl groups of the compound of formula (1-1) are protected with P_1 and P_2 groups to afford the compound of formula (4-1). P_1 and P_2 can be same or different. P_1 and P_2 can be any hydroxyl protecting group such as, but not limited to Ac, Bz, chloroacetyl, TES, TBS, MOM and Bn. The compound of formula (4-1) is subjected to suitable reduction conditions to give the compound of formula (4-2), which then is converted to the compound of formula (4-3) using suitable oxidation conditions followed by olefination to afford compound of formula (4-4). The subsequent hydrogenation provides compound of formula (4-5), which is then converted to the tetrazole compound of formula (4-6) using suitable reagent such as, but not limited to, NaN_3, $TMSN_3$ and Bu_3SnN_3. Further deprotection of the compound of formula of (4-6) gives the tetrazole compound of formula (4-7). A more detailed discussion of the procedures, reagents and conditions for deprotection of hydroxyl protecting groups is described in literature, for example, by T.W. Greene and P.G.M. Wuts in *"Protective Groups in Organic Synthesis"* 3rd ed., John Wiley & Son, Inc., 1999.

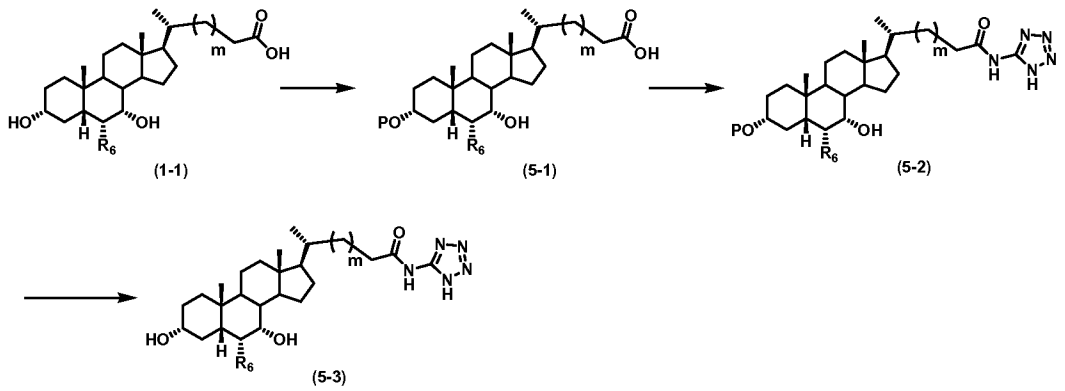
Scheme 4



Scheme 5 illustrates the preparation of the tetrazole amide compound of formula (5-3) from the compound of formula (1-1), wherein m and R_6 are defined as previously, P is hydroxyl protecting groups. Thus, the indicated hydroxyl group of the compound of formula (1-1) are protected with P group to afford the compound of formula (5-1). P can be any hydroxyl protecting group such as, but not limited to Ac, Bz, chloroacetyl, TES, TBS, MOM and Bn. A more detailed discussion of the procedures, reagents and conditions for protection of hydroxyl group is described in literature, for example, by T.W. Greene and P.G.M. Wuts in *"Protective Groups in Organic Synthesis"* 3rd ed., John Wiley & Son, Inc., 1999. The compound of formula (5-1) is coupled with 5-aminotetrazole using suitable coupling condition to provide tetrazole amide compound of formula (5-2). The coupling reagent can be selected from, but not limited to, DCC, EDC, CDI, di-isopropyl carbodiimide, BOP-Cl, PyBOP, PyAOP, TFFH and HATU. Suitable bases include, but are not limited to, triethylamine, diisopropylethylamine, DBU, N-methylmorpholine and DMAP. The coupling reaction is carried out in an aprotic solvent such as, but not limited to, CH_2Cl_2, DMF or THF. The reaction temperature can vary from 0 °C to about 50 °C. Then deprotection of P groups affords the compound of formula (5-3). A more detailed discussion of the procedures, reagents and conditions for deprotection of hydroxyl protecting groups is described in literature, for example, by T.W. Greene and

P.G.M. Wuts in *"Protective Groups in Organic Synthesis"* 3rd ed., John Wiley & Son, Inc., 1999.

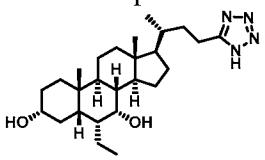
Scheme 5



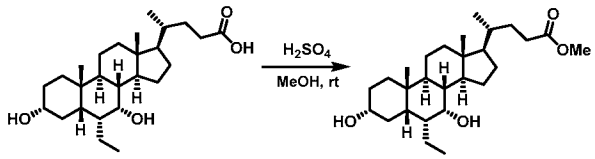
Examples

The compounds and processes of the present invention will be better understood in connection with the following examples, which are intended as an illustration only and not limiting of the scope of the invention. Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art and such changes and modifications including, without limitation, those relating to the chemical structures, substituents, derivatives, formulations and/or methods of the invention may be made without departing from the spirit of the invention and the scope of the appended claims.

Example 1:



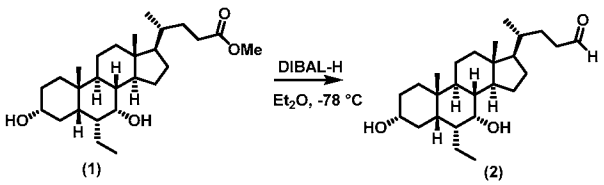
Step 1-1:



To a 250 mL round bottle flask were added 6(α)-ethyl-chenodeoxycholic acid (6-ECDCA) (960 mg, 2.28 mmol), MeOH (30 mL) and conc. H_2SO_4 (0.1 mL) respectively and the reaction mixture was stirred at room temperature for 22 h.

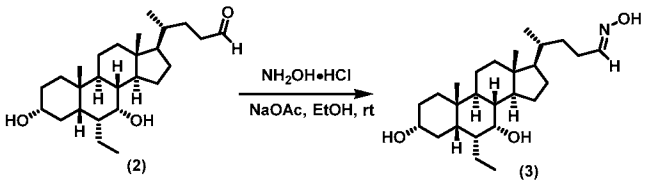
Transferred to a separation funnel, diluted with EtOAc (150 mL) and washed with aqueous $NaHCO_3$ (100 mL). The aqueous layer was extracted with EtOAc (100 mL x 2). The combined organic layers were washed with brine-water (100 mL, 1:1 v/v), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting white solid was directly used in the subsequent reaction without any further purification.

Step 1-2:



Methyl ester (1) (848 mg, 1.95 mmol) was dissolved in Et_2O (20 mL) and cooled to -78 °C. To the solution was slowly added DIBAL-H (1.0 M in hexane, 5.95 mL, 5.95 mmol). The mixture was allowed to stir at -78 °C for 1 h before carefully diluted with EtOAc (50 mL) and quenched with 10% aqueous solution of Rochelle's salt (50 mL) at 0 °C. The solution was then allowed to warm to room temperature and stir for additional 1 hour. Then the aqueous layer was then separated and extracted with EtOAc (100 mL x 3). The combined organic layers were washed with brine-water (100 mL, 1:1 v/v), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting pale yellow oil was purified by CombiFlash (40 g SiO_2, EtOAc/hexanes = 0->50%) to give the aldehyde compound (2) as a white solid, 755.2 mg, 95% yield.

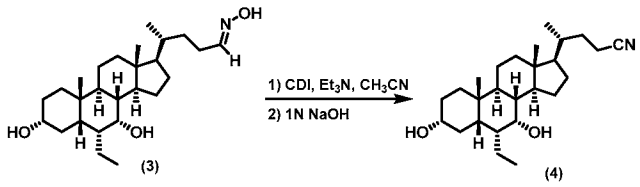
Step 1-3:



The aldehyde compound (2) (84.7 mg, 0.209 mmol), hydroxylamine hydrochloride (32.0 mg, 0.461 mmol) and NaOAc (39.4 mg, 0.48 mmol) was dissolved in anhydrous EtOH (2.1 mL). The reaction was allowed to stir for 1 hour. The solvent was removed under reduced pressure. Water (20 mL) was then added and extracted with DCM (40 mL x 3). The combined organic layers were washed with brine-water (30 mL, 1:1 v/v), dried over Na_2SO_4, filtered and concentrated under

reduced pressure. The residue was directly used in the subsequent reaction without any further purification.

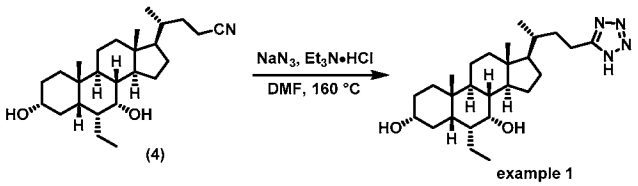
Step 1-4:



(3) (4)

The oxime compound (3) obtained from previous step was dissolved in CH_3CN (2.0 mL) followed by addition of Et_3N (116.5 µL, 0.836 mmol) and CDI (135.6 mg, 0.836 mmol) at room temperature. The mixture was allowed to stir at 80 °C for 90 min. The reaction was diluted with water (2 mL) and cooled to 0 °C, followed by addition of 1.0 N NaOH (0.2 mL). The reaction mixture was allowed to slowly warm to room temprerature over 90 min. CH_3CN was then removed under reduced pressure. The resulting aqueous solution was extracted with EtOAc (20 mL x 4). The combined organic layers were washed with brine-water (30 mL, 1:1 v/v), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting pale yellow oil was purified by CombiFlash (12 g SiO_2, EtOAc/Hexanes = 0->50%) to give the nitrile compound (4) as a white solid, 58.6 mg, 70% yield over 2 steps.

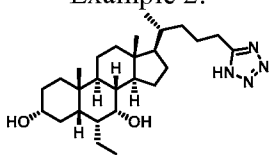
Step 1-5:



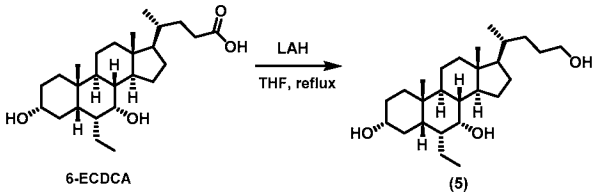
(4) example 1

The nitrile compound (4) (70 mg, 0.174 mmol), NaN_3 (45.3 mg, 0.697 mmol) and triethylamine hydrochloride (71.9 mg 0.522 mmol) was dissolved in DMF (3.0 mL). The reaction was allowed to stir at 160 °C for 30 hours. The reaction was cooled to room temperature and diluted with EtOAc (20 mL) and washed with water (20 mL x 4). The organic layer was washed with brine-water (10 mL, 1:1 v/v), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting pale yellow oil was purified by CombiFlash (4 g SiO_2, MeOH/DCM = 0->5%) to give the tetrazole compound of example 1 as a white solid, 18.7 mg, 24% yield.

Example 2:

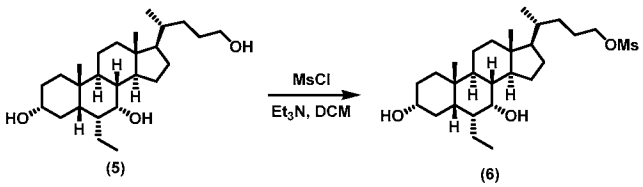


Step 2-1:



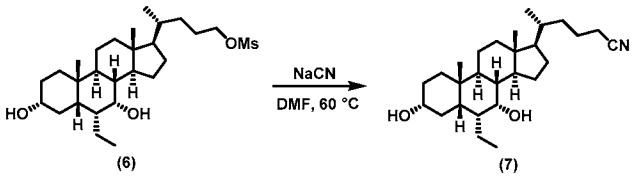
To a solution of 6(α)-ethyl-chenodeoxycholic acid (6-ECDCA) (5.3 g, 12.6 mmol) in THF (160 mL) was slowly added 1.0 M LAH in Et_2O solution (25.2 mL, 25.2 mmol) at room temperature. Then the solution was allowed to stir for 6 hours at reflux before carefully quenched with water (1.0 mL) at 0 °C followed by 15% aqueous solution of NaOH (1.0 mL). The solution was then allowed to stir for 15 min before addition of another portion of water (2.5 mL). After additional 15 min stirring, the crude was dried over $MgSO_4$, filtered and washed with EtOAc. The solvent was then removed under reduced pressure to afford the compound (5) as a white solid, which was directly used in the subsequent reaction without any further purification.

Step 2-2:



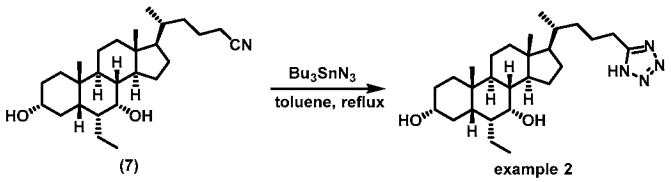
The compound (5) obtained from previous step was dissolved in DCM (100 mL) followed by addition of Et_3N (2.64 mL, 18.9 mmol) and MsCl (0.9 mL, 11.6 mmol) at 0°C. The reaction was allowed to stir for additional 1.5 hr at the same temperature. The solvent was removed under reduced pressure. Water (50 mL) was then added and extracted with EtOAc (100 mL x 3). The combined organic layers were washed with brine-water (50 mL, 1:1 v/v), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was directly used in the subsequent reaction without any further purification.

Step 2-3:



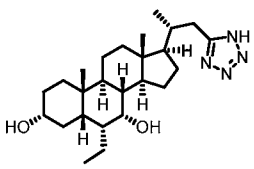
The compound (6) obtained from previous step was dissolved in DMF (50 mL) followed by addition of NaCN (1.14 g, 23.2 mmol) at room temperature. The reaction was allowed to stir for 2 days at 60 °C. The reaction was then cooled to temperature, diluted with EtOAc (150 mL) and washed with water (125 mL x 4). The organic layer was washed with brine-water (30 mL, 1:1 v/v), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting pale yellow oil was purified by CombiFlash (120 g SiO_2, EtOAc/hexanes = 0->50%) to give the nitrile compound (7) as a white solid, 1.944 g, 54% overall yield.

Step 2-4:

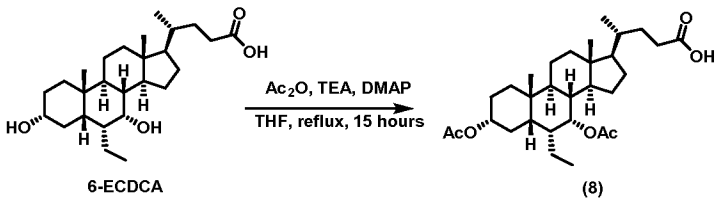


To a pressure released vial was charged compound (7) (128.7 mg, 0.31 mmol) and Bu_3SnN_3 (0.339 mL, 1.24 mmol) in toluene (2.1 mL) at room temperature. The reaction was allowed to stir for 20 hours at reflux behind blast shield. The reaction was then cooled to temperature, diluted with EtOAc (20 mL) and washed with water (50 mL). The aqueous layer was acidified to pH =2 using 1.0 N HCl and then extracted with EtOAc (50 mL x 3). The combined organic layers were then washed with brine-water (50 mL, 1:1 v/v), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting pale yellow oil was purified by CombiFlash (12 g SiO_2, MeOH/DCM = 0->10%) to give the tetrazole compound of example 2 as a white solid, 83.7 m g, 59% yield.

Example 3:

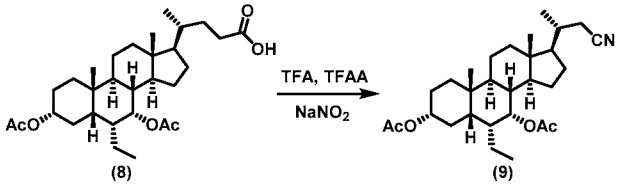


Step 3-1:



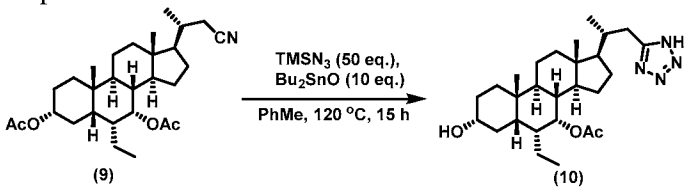
Into a 1000 mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed a solution of 6-ECDCA (18.0 g, 0.04 mol, 1.00eq) in THF (200 mL), TEA (86.5 g, 0.86 mol, 20.0 eq), 4-dimethylaminopyridine (0.63 g, 0.004 mol, 0.1eq), and acetic anhydride (87.3 g, 0.86 mol, 20.0 eq). The resulting solution was stirred at 90 °C for 15 hours. After being cooled to rt, it was concentrated and the residue was dissolved in ethyl acetate (500 mL), then was washed with water (100 mL x 2), saturated NaCl (100 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash silica chromatography, elution gradient 0 to 20 % EtOAc in petroleum ether to give the desired compound (8) as a yellow solid, 20.0 g, 93% yield.

Step 3-2:



Into a solution of compound (8) (20.0 g, 40 mmol) in TFA (150 mL) was added TFAA (63.0 g, 300 mmol). Then $NaNO_2$ was added in 5 portions over 45 minutes at 0 °C. After stirred at 0 °C for 1 hour, the solution was moved to 40 °C for 40 minutes. The solution was quenched with water after being cooled to rt, then extracted with ethyl acetate (200 mL x 3). The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash silica chromatography, elution gradient 10 to 30% EtOAc in petroleum ether to give the desired compound (9) as a yellow solid, 12.2 g, 67% yield.

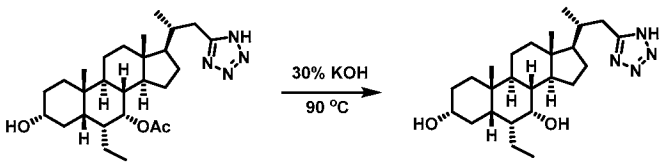
Step 3-3:



Into a 50-mL round-bottom flask, was placed a solution of compound (9) (300

mg, 0.64 mmol), azidotrimethylsilane (3.7 g, 32 mmol), dibutylstannanone (1.5 g, 6.4 mmol) in toluene (15 mL). The resulting solution was stirred at 120 °C for 15 hours. After cooling to rt the solution was diluted with 100 mL of water and extracted with ethyl acetate (50 mL x 3). The combined organic layer was concentrated under vacuum to give the desired compound (10) as a yellow oil, which was used for next step without further purification.

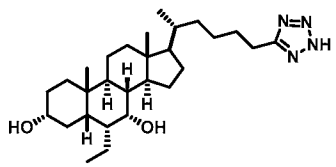
Step 3-4:



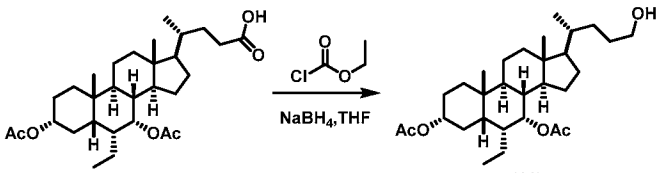
(10) example 3

Into a 50-mL round-bottom flask, was placed a solution of compound (10) (200 mg, 0.42 mmol), methanol (15 mL) and 30 % potassium hydroxide (15 mL). The resulting solution was stirred at 90 °C for 15 hours. After cooling to rt the pH value of the solution was adjusted to 6 with con HCl and extracted with ethyl acetate (20 mL x 3). The combined organic layer was concentrated and purified by Flash-Prep-HPLC ((IntelFlash-1): Column, C18; mobile phase, MeCN/H_2O, Detector, UV 220 nm) to give the tetrazole compound of example 3 as a white solid, 26 mg, 14% yield.

Example 4:



Step 4-1:

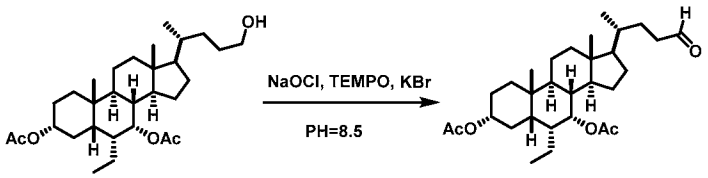


(8) (11)

Ethyl carbonochloridate (1.41g, 13 mmol) was added dropwise to a solution of compound (8) (5.1 g, 10 mmol) and Et_3N (1.32 g, 13 mmol) in dry THF (50 mL) at RT over a period of 10 minutes under nitrogen. The resulting mixture was stirred at RT for 0.5 hour. The reaction mixture was cooled to 0 °C and $NaBH_4$ (1.9 g, 50 mmol) was added slowly. Then MeOH (15 mL) was added slowly and stirred for 2 hours at 0 °C. The resulting mixture was added water (100 mL), adjusted pH 6 with

2N HCl and extracted by EtOAc (200 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 20 to 50% EtOAc in petroleum ether. Pure fractions were evaporated to dryness to afford compound (11) as a yellow oil, 4.1 g, 68 % yield.

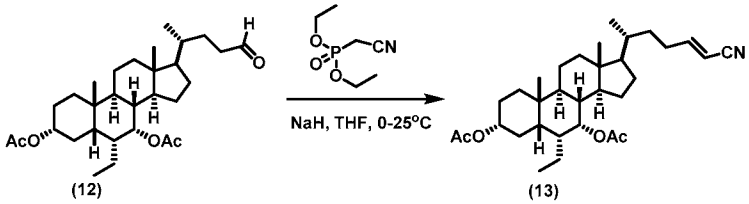
Step 4-2:



(11) (12)

To a solution of compound (11) (3.9 g, 8 mmol) in CH_2Cl_2 (100 mL), 2,2,6,6-tetramethylpiperidine1-oxyl free radical (TEMPO) (31 mg, 0.2mol) and KBr (105 mg) were added gradually with ice-bath cooling. To the above mixture, a solution of NaOCl (8 mL) dissolved in a buffer solution (pH 8.5, 60 mL) prepared from 0.5 M sodium bicarbonate, and 0.05 M sodium carbonate was added. The mixture was stirred at 0 oC for 20 min. The reaction was monitored by TLC using hexane–EtOAc (1:1, v/v) as the developing solvent. After adding methanol (10 mL), the reaction product was extracted with CH_2Cl_2 (100 mL). The combined organic phase was washed with water and evaporated to dryness to give the crude compound. The crude product was purified by flash silica chromatography, elution gradient 20 to 50% EtOAc in petroleum ether. Pure fractions were evaporated to dryness to afford compound (12) as a yellow oil, 3 g, 77 % yield.

Step 4-3:

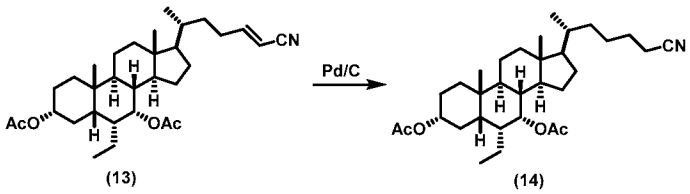


(12) (13)

Into a 50-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed diethyl(cyanomethyl)phosphonate (4.43 g, 25.01 mmol) and THF (20 mL). Then sodium hydride (1 g, 41.67 mmol) was added slowly at 0 °C. The solution was stirred for 30 minutes. Then a solution of compound (12) (1.22 g, 2.50 mmol) in THF (10 mL) was added at 0 °C. The resulting solution was stirred at 25 °C for 2 hours. The resulting solution was diluted with 50 mL of water

and extracted with EtOAc (50 mL x 3). The combined organic layer was washed sequentially with water (50 mL) and saturated brine (50 mL), then dried over Na_2SO_4, filtered and evaporated. The residue was purified by flash silica chromatography, elution gradient 20 to 50% EtOAc in petroleum ether. Pure fractions were evaporated to dryness to afford the desired compound (13) as yellow oil, 0.7 g, 54 % yield.

Step 4-4:



(13) (14)

Into a 50-mL round-bottom flask, was placed a solution of compound (13) (52 mg, 0.10 mmol) in methanol (10 mL), then followed by the addition of Pd/C (0.2 g). The resulting solution was stirred under H_2 (1 atm) at rt for 2 hours. Then Pd/C was removed by filtration and the filtrate was evaporated to give the desired compound (14) as a yellow oil, which was used for the next step without further purification.

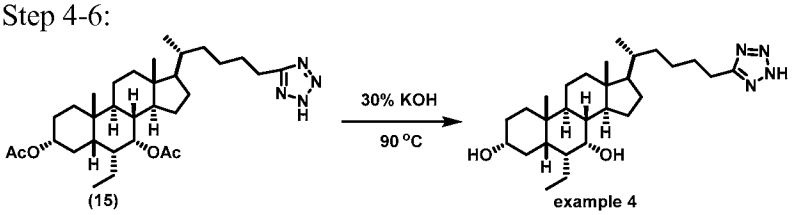
Step 4-5:



(14) (15)

Into a 50-mL round-bottom flask, was placed a solution of compound (14) (300 mg, 0.58 mmol), azidotrimethylsilane (3.4 g, 29.51 mmol), and dibutylstannanone (1.5 g, 6.03 mmol) in toluene (20 mL). The resulting solution was stirred at 120°C for 15 hours. After cooling to rt the resulting solution was diluted with 200 mL of water and extracted with ethyl acetate (30 mL x 3). The organic layer was concentrated under vacuum to give the desired compound (15) as a yellow oil, which was used for next step without further purification.

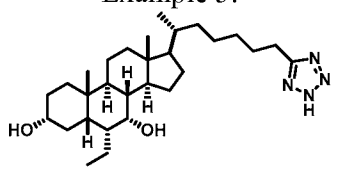
Step 4-6:



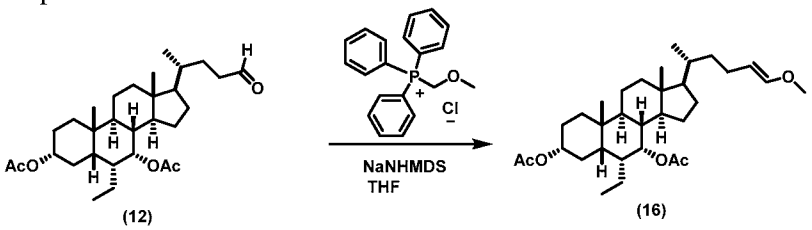
(15) example 4

Into a 50-mL round-bottom flask, was placed a solution of compound (15) (200 mg, 0.39 mmol), methanol (15 mL) and 30 % potassium hydroxide (15 mL). The resulting solution was stirred at 90°C for 15 hours. After cooling to rt the pH value of the solution was adjusted to 6 with con HCl and extracted with ethyl acetate (20 mL x 3). The combined organic layer was concentrated and purified by Flash-Prep-HPLC ((IntelFlash-1): Column, C18; mobile phase, MeCN/H_2O, Detector, UV 220 nm) to give the tetrazole compound of example 4 as a white solid, 27 mg, 15% yield.

Example 5:

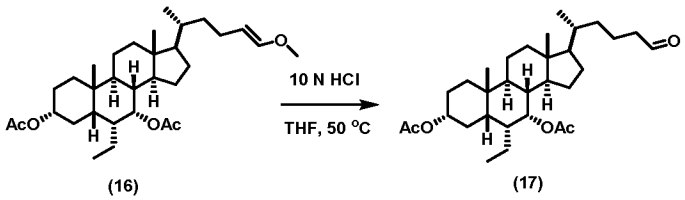


Step 5-1:



To a solution of the (methoxymethyl)triphenylphosphonium chloride (3.5g, 10 mmol) in THF (20 mL), NaNHMDS (1M in THF, 10 mL) was added with ice-bath cooling. After the mixture was stirred at 0 °C for 20 min, a solution of compound (12) (976 mg, 2 mmol) in THF (5 mL) was added slowly at 0 °C and stirred for 2 hours at rt. After adding water (100 mL), the reaction product was extracted with EtOAc (100 mL). The organic layer was washed with water (50 mL) and evaporated to dryness to give the crude compound. The crude product was purified by flash silica chromatography, elution gradient 20 to 50% EtOAc in petroleum ether. Pure fractions were evaporated to dryness to afford compound (16) as a yellow oil, 520 mg, 50 % yield.

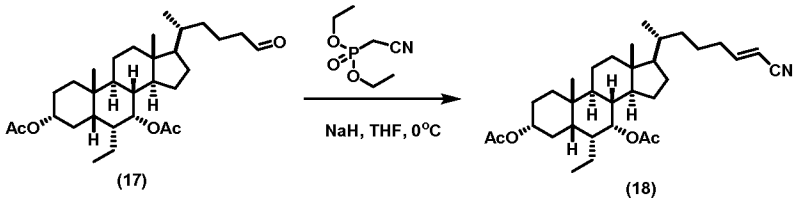
Step 5-2:



A solution of compound (16) (1.03 g, 2 mmol) and 10N HCl (20 mL) in THF (20 mL) was stirred at 50 °C for 2 hours. Then this mixture was cooled to rt, extracted

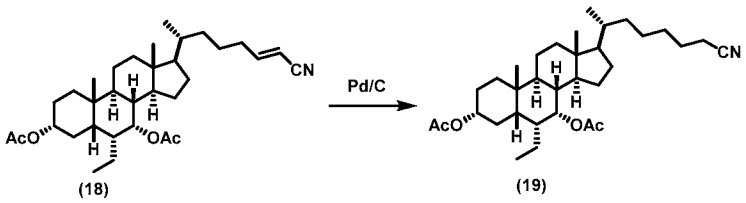
by EtOAc (80 mL). The organic layer was dried by Na_2SO_4 and condensed to get the desired compound (17) as a yellow oil, which was used in the next step without further purification.

Step 5-3:



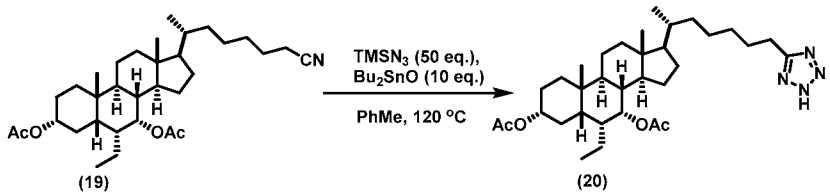
Into a 50-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed diethyl (cyanomethyl)phosphonate (2.12 g, 12 mmol) and THF (20 mL). Then sodium hydride (0.48 g, 12 mmol) was added slowly at 0 °C. This solution was stirred for 30 minutes. Then a solution of compound (17) (0.6 g, 1.2 mmol) in THF (10 mL) was added. The resulting solution was stirred for 2 hours at 25 °C. After cooling to rt the solution was diluted with 50 mL of water and extracted with ethyl acetate (20 mL x 3). The combined organic layer was washed with water (50 mL) and saturated brine (50 mL), then dried over Na_2SO_4, filtered and evaporated. The residue was purified by flash silica chromatography, elution gradient 20 to 50% EtOAc in petroleum ether. Pure fractions were evaporated to dryness to afford the desired compound (18) as a yellow oil, 0.35g, 55 % yield.

Step 5-4:



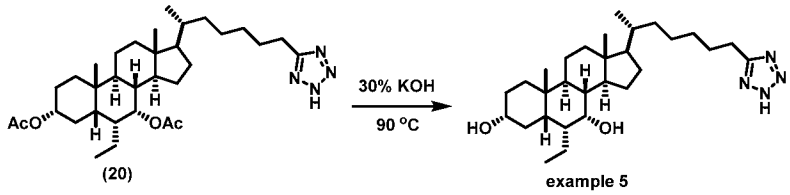
Into a 50-mL round-bottom flask, was placed a solution of compound (18) (600 mg, 1.2 mmol) in methanol (10 mL). This resulting mixture was followed by the addition of Pd/C (0.3 g). The resulting solution was stirred under H_2 (1 atm) at 25 °C for 2 hours. Then Pd/C was removed by filtration and the filtrate was evaporated to give the desired compound (19) as a yellow oil, which was used for the next step without further purification.

Step 5-5:



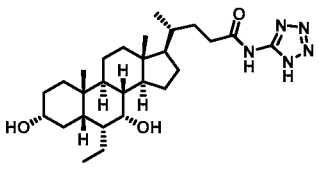
Into a 50-mL round-bottom flask, was placed a solution of compound (19) (106 mg, 0.2 mmol), azidotrimethylsilane (1.2 g, 10 mmol), and dibutylstannanone (0.5 g, 2 mmol) in toluene (10 mL). The resulting solution was stirred at 120 °C for 15 hours. After cooling to rt the resulting solution was diluted with 100 mL of water and extracted with ethyl acetate (50 mL x 3). The combined organic layer was concentrated under vacuum to give the desired compound (20) as a yellow oil, which was used for next step without further purification.

Step 5-6:

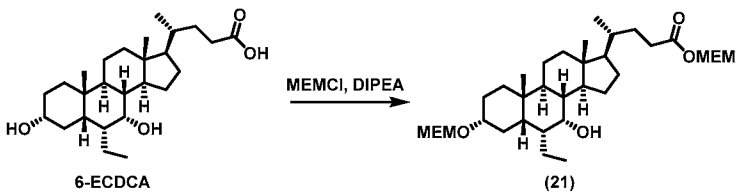


Into a 50-mL round-bottom flask, was placed a solution of compound (20) (200 mg, 0.35 mmol), methanol (15 mL) and 30 % potassium hydroxide (15 mL). The resulting solution was stirred at 90 °C for 15 hours. After cooling to rt the pH value of the solution was adjusted to 6 with conc. HCl and extracted with ethyl acetate (20 mL x 3). The combined organic layer was concentrated and purified by Flash-Prep-HPLC ((IntelFlash-1): Column, C18; mobile phase, MeCN/H_2O, Detector, UV 220 nm) to give the title tetrazole compound of example 5 as a white solid, 23 mg, 14% yield.

Example 6:

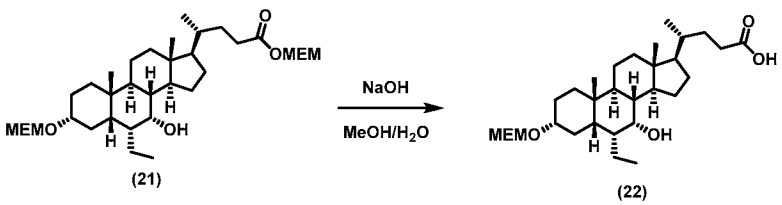


Step 6-1:



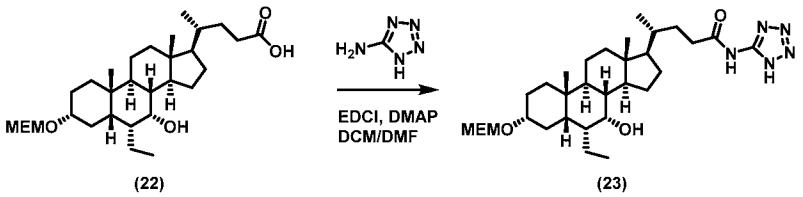
To a solution of 6-ECDCA (4.21 g, 10.0 mmol) in DCM at 0 °C (100 mL) was added DIPEA (7.75 g, 60 mmol) followed by MEMCl (3.69 mL, 30 mmol). The reaction mixture was allowed to warm up to RT and stirred overnight, quenched with 1N HCl/brine (1/1), and extracted with EtOAc. The combined organic layers were washed with brine and concentrated. The resulting crude oil was used for next step without further purification.

Step 6-2:



To a stirred solution of above obtained crude compound (21) in $MeOH/H_2O$ (v:v = 21/1, 22 mL) at RT was added 50% aq. NaOH (2 mL, 24 mmol). The reaction mixture was heated to 50 °C and stirred for 3h, then quenched with 1N HCl/brine (1/1), and extracted with EtOAc. The combined organic layers were washed with brine and concentrated. The residue was purified by chromatography on silica gel using acetone /hexane (0→40%) to afford compound (22) as a pale yellow solid, 4.7 g, 93% yield over 2 steps.

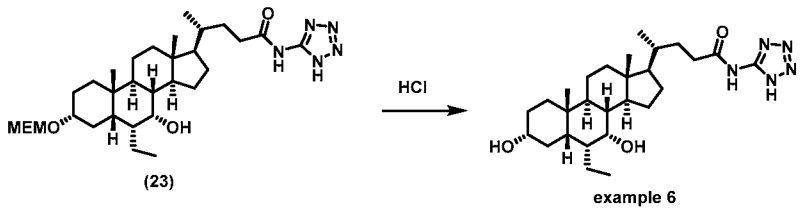
Step 6-3:



To a solution of compound (22) (65 mg, 0.128 mmol) in 2 mL DMF/CH_2Cl_2 solution (1:1, v/v) was charged 5-Amino-1,2,3,4-tetrazole (32.6 mg, 0.383 mmol), EDCI (49 mg, 0.256 mmol) and DMAP (31.2 mg, 0.256 mmol). The resulting solution was allowed to stir at 60 °C until completion indicated by TLC. The solvent was removed under reduced pressure. Water (15 mL) and 1N HCl aq. solution (2

mL) was added. The solution was then extracted with ethyl acetate (30 mL x 3). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by CombiFlash (12 g SiO_2, MeOH/CH_2Cl_2 = 0→10%) to give the compound (23) as a white solid, 30.9 mg, 42% yield.

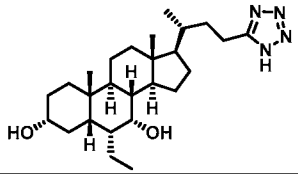
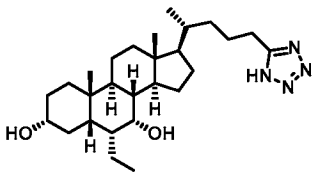
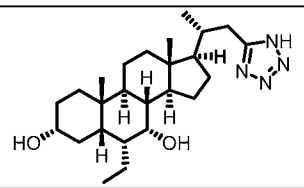
Step 6-4:

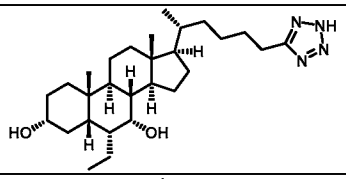
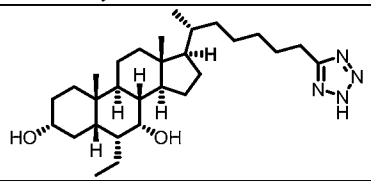
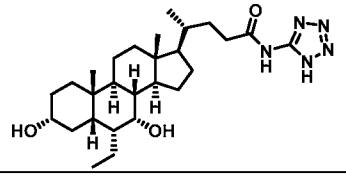


(23) example 6

The above obtained compound (23) (30.9 mg) was dissolved in THF (0.9 mL) followed by addition of 0.1 mL of 37% conc. aq. HCl. The mixture was stirred at room temperature for 1 hour, quenched with sat. $NaHCO_3$, and extracted with EtOAc (3 x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was by CombiFlash (4 g SiO_2, MeOH/CH_2Cl_2 = 0→20%) to provide the compound of example 6 as white solid, 13.3 mg, 51% yield.

Table 1

Example	structure	HRMS:	^{1}H NMR (500MHz)
1		489.2849 $(M+formate)^-$	
2		457.2944 $(M-H)^-$	3.72 (1H, s), 3.46 (1H, br s), 3.03 (1H, m), 2.95 (1H, m), 1.87 (5H, m), 1.70 (4H, m), 1.59 (2H, m), 1.39 (9H, m), 1.22 (3H, m), 1.10 (3H, m), 1.01 (1H, t, J = 8.0 Hz), 0.92 (3H, d, J = 7.0 Hz), 0.89 (6H, s), 0.63 (3H, s).
3		429.40 $(M-H)^-$	

4		471.45 $(M-H)^-$	
5		485.50 $(M-H)^-$	
6		486.28 $(M-H)^-$	

ASSAYS

Human FXR (NR1H4) Assay

Determination of a ligand mediated Gal4 promoter driven transactivation to quantify ligand binding mediated activation of FXR. FXR Reporter Assay kit purchased from Indigo Bioscience (Catalogue number: IB00601) to determine the potency and efficacy of compound developed by Enanta that can induce FXR activation. The principal application of this reporter assay system is to quantify functional activity of human FXR. The assay utilizes non-human mammalian cells, CHO (Chinese hamster ovary) cells engineered to express human NR1H4 protein (referred to as FXR). Reporter cells also incorporate the cDNA encoding beetle luciferase which catalyzes the substrates and yields photon emission. Luminescence intensity of the reaction is quantified using a plate-reading luminometer, Envision. Reporter Cells include the luciferase reporter gene functionally linked to an FXR responsive promoter. Thus, quantifying changes in luciferase expression in the treated reporter cells provides a sensitive surrogate measure of the changes in FXR activity. EC_{50} and efficacy (normalize to CDCA set as 100%) is determined by XLFit. The assay is according to the manufacturer's instructions. In brief, the assay was performed in white, 96 well plates using final volume of 100ul containing cells with different doses of compounds. Retrieve Reporter Cells from -80°C storage. Perform a rapid thaw of the frozen cells by transferring a 10 ml volume of 37°C cell recovery medium into the tube of frozen cells. Recap the tube of Reporter Cells and

immediately place it in a 37°C water bath for 5 - 10 minutes. Retrieve the tube of Reporter Cell Suspension from the water bath. Sanitize the outside surface of the tube with a 70% alcohol swab, and then transfer it into the cell culture hood. Dispense 90 µl of cell suspension into each well of the 96-well Assay Plate. Transfer the plate into 37°C incubator, allowing the cells adherent to the bottom of the well. Dilute compounds in Dilution Plate (DP), and administrate to cells at Assay Plate (AP). DMSO content of the samples was kept at 0.2%. Cells were incubated for additional 22 hours before luciferase activities were measured. Thirty minutes before intending to quantify FXR activity, remove Detection Substrate and Detection Buffer from the refrigerator and place them in a low-light area so that they may equilibrate to room temperature. Remove the plate's lid and discard all media contents by ejecting it into an appropriate waste container. Gently tap the inverted plate onto a clean absorbent paper towel to remove residual droplets. Cells will remain tightly adhered to well bottoms. Add 100 µl of luciferase detection reagent to each well of the assay plate. Allow the assay plate to rest at room temperature for at least 5 minutes following the addition of LDR. Set the instrument (Envision) to perform a single 5 second "plate shake" prior to reading the first assay well. Read time may be 0.5 second (500mSec) per well. EC_{50} and Efficacy (normalize to CDCA set as 100%) is determined by XLFit.

In vitro Human TGR5 (GPBAR1) activity assay

The potency and efficacy of the compounds of the invention on TGR5 receptor can be evaluated using *in vitro* assays which carried out using the express kit from DiscoverX (cAMP Hunter™ eXpress GPBAR1 CHO-K1 GPCR Assay; Catalogue number: 95-0049E2CP2S)GPBAR1 (G protein-coupled bile acid receptor 1) encodes a member of the G protein-coupled receptor (GPCR) superfamily. GPBAR1 activation following ligand binding initiates a series of second messenger cascades that result in a cellular response. Treatment of CHO cells expressing GPBAR1 with bile acids induces the production of intracellular cAMP and internalization of the receptor. The potency and efficacy of compound for GPBAR1 activation by measuring cyclic adenosine monophosphate (cyclic AMP or cAMP) levels in live cells using a competitive immunoassay based on Enzyme Fragment Complementation (EFC).

In briefly, following seeding the cells into the white, 96 well microplate, place it in a 37°C, 5% CO2 in a humidified incubator for 18-24 hours prior to testing. On second day, proceed to the appropriate cAMP Hunter eXpress Protocol according to the manufacturer's instructions. Dissolve agonist compound in DMSO at the desired stock concentration, and prepare 3-fold serial dilutions of agonist compound in Cell Assay Buffer. The concentration of each dilution should be prepared at 4X of the final screening concentration (i.e. 15 µL compound + 45 µL Cell Assay Buffer/cAMP Antibody Reagent). For each dilution, the final concentration of solvent should remain constant. Transfer 15 µL diluted compound the assay plate and incubate the plate for 30 minutes at 37°C. Following agonist incubation, add 60 µL of working cAMP detection reagents/cAMP Solution mixture (cAMP Lysis Buffer, Substrate Reagent 1, cAMP Solution D) to the appropriate wells. Incubate for 1 hour at room temperature (23°C), protected from light. Add 60 µl of cAMP Solution A to the appropriate wells. Incubate for 3 hours at room temperature (23°C), protected from light. Read samples on Envision standard luminescence plate reader. Calculate of average EC_{50} after logarithm transformation.

To assess the FXR agonistic potency of the example compounds as well as for reference compound, potency ranges were determined in the Human FXR (NR1H4) Assay as listed below in Table 2. The efficacy was normalized to CDCA set as 100%. (A= 0.1 µM < EC50 < 1.0 µM; B=1.0 µM < EC50 < 10 µM; C= EC50 > 10 µM)

Table 2

Example	EC50 (µM)	Efficacy (%)
CDCA	C	100
6-ECDCA	A	430
1	B	397
2	A	354
3	B	192
4	A	234
5	A	191
6	B	105

CDCA = chenodoxycholic acid; 6-ECDCA = 6-α-ethylchenodoxycholic acid.

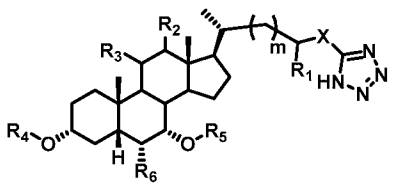
While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

5

CLAIMS

WHAT IS CLAIMED IS:

1. A compound represented by Formula I or a pharmaceutically acceptable salt, ester, stereoisomer, tautomer, solvate, or combination thereof:



(I)

wherein:

X is absent, –C(O)NH– or –NH–;

R_1 is selected from the group consisting of:

1) Hydrogen;

2) Substituted or unsubstituted –C_1-C_8 alkyl;

3) Substituted or unsubstituted –C_2-C_8 alkenyl;

4) Substituted or unsubstituted –C_2-C_8 alkynyl;

5) Substituted or unsubstituted arylalkyl; and

6) Substituted or unsubstituted aryl;

m is 0, 1, 2 or 3;

R_2 is hydrogen, hydroxyl, -OSO_3H, -OSO_3^-, -OAc, -OPO_3H_2 or –OPO_3^{2-}; preferably R_2 is hydrogen or hydroxyl;

R_3 is hydrogen, halogen, CN, N_3, hydroxyl, -OSO_3H, -OSO_3^-, -OAc, -OPO_3H_2, –OPO_3^{2-}, -SR_1 or –NHR_1, wherein R_1 is as defined previously;

Or R_2 and R_3 are taken together with the carbon atoms to which they are attached to form –CH=CH- or cycloalkyl ring or heterocycloalkyl ring such as, but not limited to cyclopropyl, or epoxide;

R_4 and R_5 are independently selected from hydrogen or hydroxyl protecting group; and

R_6 is selected from the group consisting of:

1) Hydrogen;

2) Halogen;

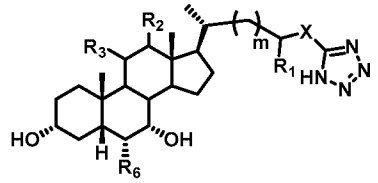
3) Substituted or unsubstituted –C_1-C_8 alkyl;

4) Substituted or unsubstituted –C_2-C_8 alkenyl;

5) Substituted or unsubstituted $-C_2$-C_8 alkynyl; and

6) Substituted or unsubstituted $-C_3$-C_8 cycloalkyl.

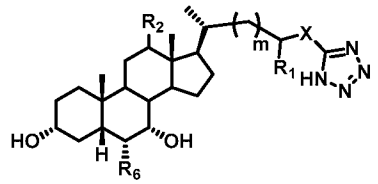
2. The compound of claim 1, represented by Formula II or a pharmaceutically acceptable salt, ester, stereoisomer, tautomer, solvate, or combination thereof:



(II) ,

wherein R_1, R_2, R_3, R_6, X and m are as defined in claim 1.

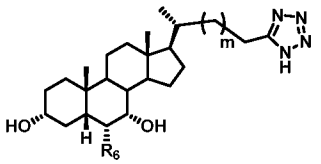
3. The compound of claim 1, represented by Formula III or a pharmaceutically acceptable salt, ester, stereoisomer, tautomer, solvate, or combination thereof:

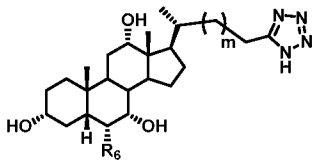


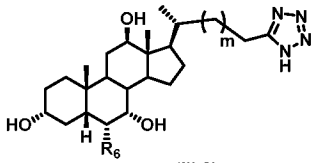
(III) ,

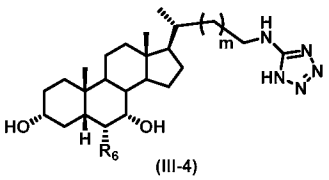
wherein R_1, R_2, R_6, X and m are as defined in claim 1.

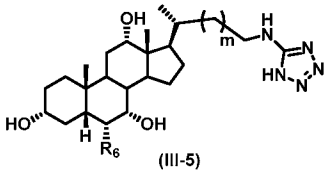
4. The compound of claim 1, represented by formula (III-1~III-18), where R_6 and m are as defined in claim 1:

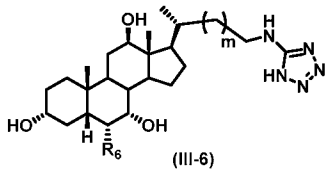

(III-1)

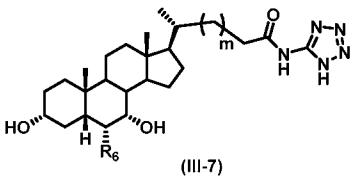

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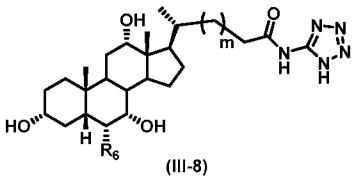

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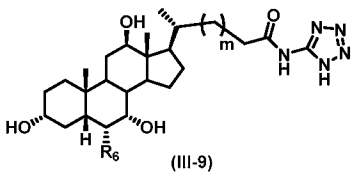

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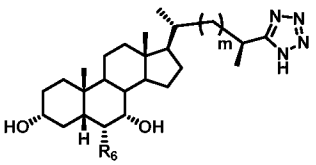

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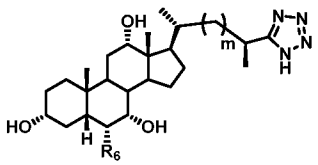

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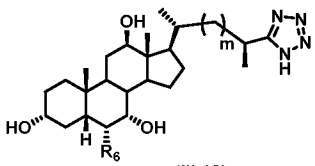

(III-7)

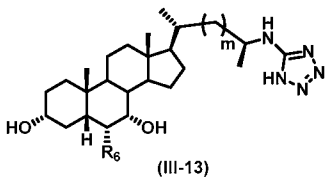

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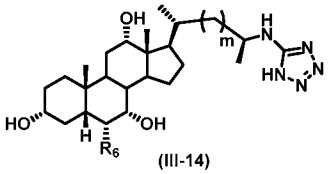

(III-9)

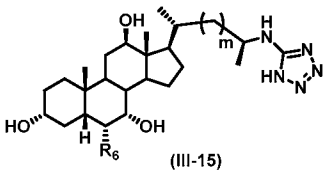

(III-10)

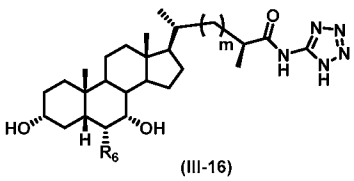

(III-11)

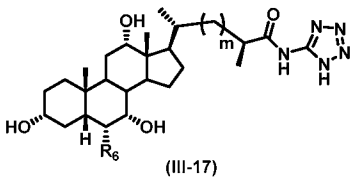

(III-12)

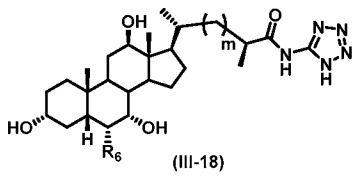

(III-13)


(III-14)

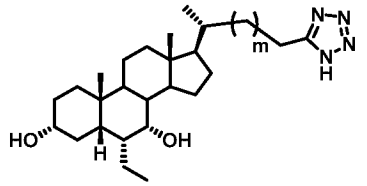

(III-15)


(III-16)


(III-17)


(III-18)

5. The compound of claim 1, represented by Formula IV or a pharmaceutically acceptable salt thereof:

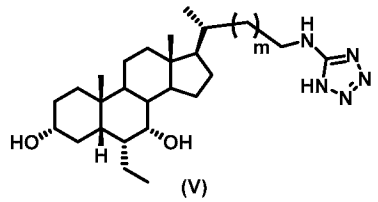


(IV)

,

wherein m is as defined in claim 1.

6. The compound of claim 1, represented by Formula V or a pharmaceutically acceptable salt thereof:

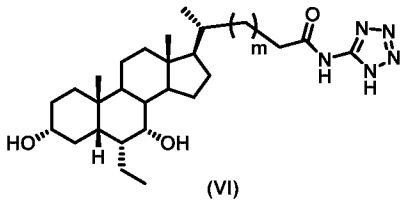


(V)

,

wherein m is as defined in claim 1.

7. The compound of claim 1, represented by Formula VI or a pharmaceutically acceptable salt thereof:



(VI)

,

wherein m is as defined in claim 1.

8. A method for the prevention or treatment of an FXR-mediated disease or condition in a mammal comprising administering to the mammal suffering from an FXR-mediated disease or condition a therapeutically effective amount of a compound of formula (I) according to any one of claims 1-6.

9. The method according to claim 8, wherein the FXR-mediated disease or condition is selected from the group consisting of chronic liver disease, gastrointestinal disease, renal disease, cardiovascular disease, and metabolic disease.

10. The method according to claim 9, wherein the chronic liver disease is selected from the group consisting of primary biliary cirrhosis (PBC),

cerebrotendinous xanthomatosis (CTX), primary sclerosing cholangitis (PSC), drug induced cholestasis, intrahepatic cholestasis of pregnancy, parenteral nutrition associated cholestasis (PNAC), bacterial overgrowth or sepsis associated cholestasis, autoimmune hepatitis, chronic viral hepatitis, alcoholic liver disease, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), liver transplant associated graft versus host disease, living donor transplant liver regeneration, congenital hepatic fibrosis, choledocholithiasis, granulomatous liver disease, intra- or extrahepatic malignancy, Sjogren's syndrome, Sarcoidosis, Wilson's disease, Gaucher's disease, hemochromatosis, and alpha 1-antitrypsin deficiency.

11. The method according to claim 9, wherein the renal disease is selected from the group consisting of diabetic nephropathy, focal segmental glomerulosclerosis (FSGS), hypertensive nephrosclerosis, chronic glomerulonephritis, chronic transplant glomerulopathy, chronic interstitial nephritis, and polycystic kidney disease.

12. The method according to claim 9, wherein the cardiovascular disease is selected from the group consisting of atherosclerosis, arteriosclerosis, dyslipidemia, hypercholesterolemia, and hypertriglyceridemia.

13. The method according to claim 9, wherein the metabolic disease is selected from the group consisting of insulin resistance, Type I and Type II diabetes, and obesity.

14. A method for the prevention or treatment of an TGR5-mediated disease or condition in a mammal comprising administering to the mammal suffering from an TGR5-mediated disease or condition a therapeutically effective amount of a compound of formula (I) according to any one of claims 1-6.

15. A pharmaceutical composition comprising a compound of formula (I) according to any one of claims 1-6 and a pharmaceutically acceptable carrier.

16. Use of a compound of any one of claims 1-6 for the preparation of pharmaceutical compositions for the prevention or treatment of FXR-mediated or TGR5-mediated diseases or conditions.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/62641

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/58; C07J 9/00, 43/00 (2015.01)
CPC - C07J 9/00, 41/00, 43/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 31/58; C07J 9/00, 43/00 (2015.01)
CPC: C07J 9/00, 41/00, 43/00 USPC: 514/176; 540/107; 552/553

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PATSEER (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, Other Countries (INPADOC), RU, AT, CH, TH, BR, PH); EBSCO; Google Scholar; IP.com; SureChEMBL; KEYWORDS: FXR modulat*, bile acid*, steroid*, cholic acid* derivative*, treat* FXR mediated disease*, liver disease*, primary biliary cirrhosis, metabolic disease*, insulin resistance, diabetes, obesity, tetrazol*, TGR5 modulat*

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/0057886 A1 (INTERCEPT PHARMACEUTICALS) 27 February 2014; paragraphs [0002], [0066]-[0068], [0081], [0084]-[0085]; claims 1 and 8-9	1-5, 8/1-5, 9/8/1-5, 10/9/8/1-5, 11/9/8/1-5, 12/9/8/1-5, 13/9/8/1-5, 14/1-5, 15/1-5, 16/1-5
Y --- A	US 2010/0324004 A1 (MCLANE, M et al.) 23 December 2010; paragraphs [0002], [0008], [0023]; page 18, table 1, see compound 1486; page 20, table 1, see compound 1561; page 50, table 1, see compound 2490	1-5, 8/1-5, 9/8/1-5, 10/9/8/1-5, 11/9/8/1-5, 12/9/8/1-5, 13/9/8/1-5, 14/1-5, 15/1-5, 16/1-5 --- 6-7, 8/6, 9/8/6, 10/9/8/6, 11/9/8/6, 12/9/8/6, 13/9/8/6, 14/6, 15/6, 16/6
Y --- A	US 2011/0172198 A1 (PELLICCIARI, R) 14 July 2011; paragraphs [0007], [0016], [0040], [0063]-[0066], [0113]	1-5, 14/1-5 --- 6-7, 8/6, 9/8/6, 10/9/8/6, 11/9/8/6, 12/9/8/6, 13/9/8/6, 14/6, 15/6, 16/6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 January 2016 (04.01.2016)	19 FEB 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)