

(51) International Patent Classification:
A61K 31/57 (2006.01)(21) International Application Number:
PCT/US2016/017554(22) International Filing Date:
11 February 2016 (11.02.2016)

(25) Filing Language: English

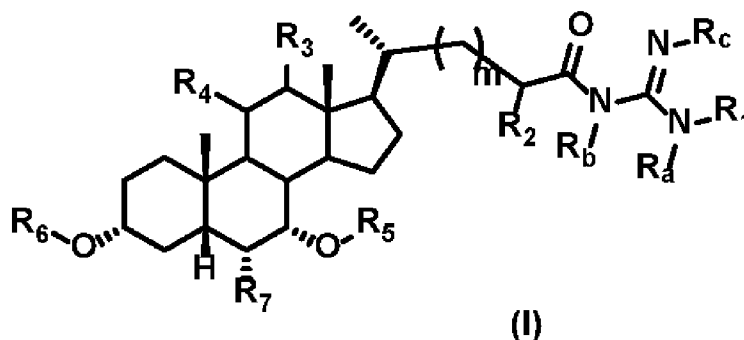
(26) Publication Language: English

(30) Priority Data:
62/114,773 11 February 2015 (11.02.2015) US(71) Applicant: ENANTA PHARMACEUTICALS, INC.
[US/US]; 500 Arsenal Street, Watertown, MA 02472 (US).(72) Inventors: SHEN, Ruichao; 563 LaGrange Street, West
Roxbury, MA 02132 (US). OR, Yat, Sun; 169 Fayette
Street, Watertown, MA 02472 (US). WANG, Guoqiang;
65 Becket Road, Belmont, MA 02478 (US).(74) Agents: HARLAN, Edgar, W. et al.; Elmore Patent Law
Group, 484 Groton Road, Westford, MA 01886 (US).(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ,
TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,
TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,
DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: BILE ACID ANALOGS AS FXR/TGR5 AGONISTS AND METHODS OF USE THEREOF



(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. 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.

BILE ACID ANALOGS AS FXR/TGR5 AGONISTS AND METHODS OF USE THEREOF

RELATED APPLICATION

5 This application claims the benefit of U.S. Provisional Application No. 62/114,773,
filed on February 11, 2015. The entire teachings of the above application 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
10 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
15 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
20 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 including the esophagus, stomach, duodenum,
small intestine, colon, ovary, adrenal gland and kidney. Beyond controlling intracellular
25 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
30 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, and WO

2008/025540. 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.

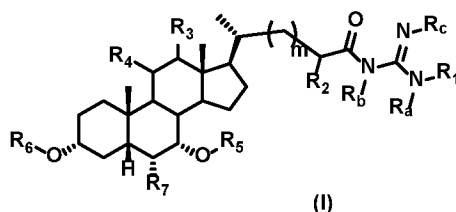
In addition to the use of TGR5 agonists for the treatment and prevention of metabolic diseases, compounds that modulate TGR5 modulators are also useful for the treatment of other diseases e.g., central nervous diseases as well as inflammatory diseases (WO 01/77325 and WO 02/84286). Modulators of TGR5 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 acylguanidine moiety, which modulate FXR and/or TGR as well as methods of using these compounds to treat diseases.

5

SUMMARY OF THE INVENTION

In one aspect, the invention provides compounds represented by Formula I, or pharmaceutically acceptable salts, stereoisomer, solvate, hydrate or combination thereof:



10 wherein:

R₁ is selected from the group consisting of:

- 1) Hydrogen;
- 2) Substituted or unsubstituted -C₁-C₈ alkyl;
- 3) Substituted or unsubstituted -C₂-C₈ alkenyl;
- 15 4) Substituted or unsubstituted -C₂-C₈ alkynyl;
- 5) Substituted or unsubstituted -C₃-C₈ cycloalkyl;
- 6) Substituted or unsubstituted aryl;
- 7) Substituted or unsubstituted arylalkyl;
- 8) Substituted or unsubstituted heterocycloalkyl;
- 20 9) Substituted or unsubstituted heteroaryl;
- 10) Substituted or unsubstituted heteroarylalkyl;

R_a, R_b, and R_c are each independently selected from R₁; preferably R_a, R_b and R_c are hydrogen; or R_a and R_b, or R_a and R_c, or R_b and R_c are taken together with the two nitrogen atoms to which they are attached and the intervening carbon atom to form a heterocyclic, heteroaryl or heterocycloalkenyl ring.

25

R₂ 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; and
 - 5) Substituted or unsubstituted $-C_2-C_8$ alkynyl;
 - 6) Substituted or unsubstituted arylalkyl; and
 - 5 7) Substituted or unsubstituted aryl;
- preferably R_2 is hydrogen or methyl.
 m is 0, 1, 2 or 3, preferably 0, 1 or 2.
 R_3 is hydrogen, hydroxyl, $-OSO_3H$, $-OSO_3^-$, $-OAc$, $-OPO_3H_2$ or $-OPO_3^{2-}$; preferably R_3 is hydrogen or hydroxyl, more preferably hydrogen.
- 10 R_4 is hydrogen, halogen, CN , N_3 , hydroxyl, $-OSO_3H$, $-OSO_3^-$, $-OAc$, $-OPO_3H_2$, $-OPO_3^{2-}$, $-SR_2$ or $-NHR_2$, wherein R_2 is as defined previously; preferably R_4 is hydrogen.
 Or R_3 and R_4 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.
 - 15 R_5 and R_6 are each independently selected from hydrogen and hydroxyl protecting groups, such as, but not limited to acetyl, trimethyl silyl, or benzyl; preferably R_5 and R_6 are hydrogen.
 R_7 is selected from the group consisting of:
- 20 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
 - 25 6) Substituted or unsubstituted $-C_3-C_8$ cycloalkyl;
- preferably R_7 is C_1-C_4 -alkyl; more preferably R_7 is ethyl.

In another embodiment, the present invention provides a pharmaceutical composition comprising a therapeutically effective amount of a compound or combination of compounds

- 30 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 in a subject in need thereof. The method comprises administering to the subject 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.

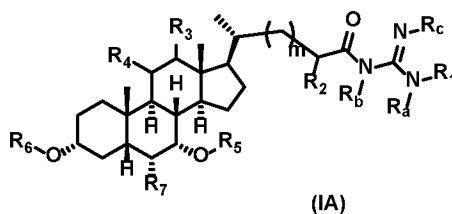
In yet another embodiment, the present invention provides a method for the prevention or treatment of a TGR5 mediated disease or condition. The method comprises administering a therapeutically effective amount of a compound of formula (I). The present invention also provides the use of a compound of 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_4 , R_5 , and R_6 are each hydrogen; R_2 is hydrogen or methyl; R_3 is hydrogen or hydroxyl and R_7 is ethyl. In another embodiment of the compounds of Formula I, R_a , R_b and R_c are each hydrogen. In another embodiment of the compounds of Formula I, R_a and R_c , together with the nitrogen atoms to which they are attached and the intervening carbon atoms, form a heterocyloalkenyl ring or a heteroaryl ring, such as an imidazolyl or dihydroimidazolyl ring, for example 2,5-dihydro-1H-imidazolyl.

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

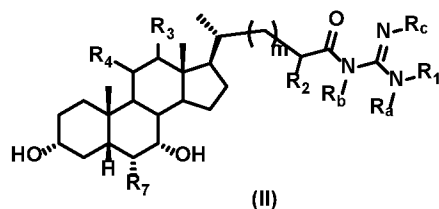


where m , R_a , R_b , R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and R_7 have the meanings given for these variables above.

In certain embodiments of the compounds of the invention, R_1 is C_1 - C_4 -alkyl; halogenated C_1 - C_4 -alkyl; C_1 - C_4 -alkenyl; phenyl- C_1 - C_4 -alkyl; substituted or unsubstituted C_3 - C_6 -cycloalkyl; C_1 - C_6 -cycloalkyl- C_1 - C_4 -alkyl; heteroaryl, such as 5- or 6-membered heteroaryl; or substituted or unsubstituted aryl, such as substituted or unsubstituted phenyl or naphthyl.

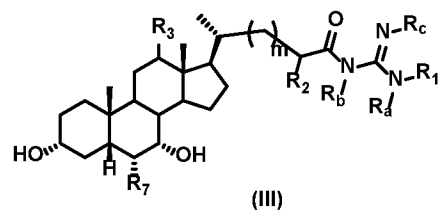
In certain embodiments of the compounds of the invention, R_1 is selected from the group consisting of hydrogen, methyl, ethyl, isopropyl, butyl, t-butyl, propyl, benzyl, allyl, CF_3 , cyclohexyl, cyclopentyl, , , , , , , , , , , ,  and .

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



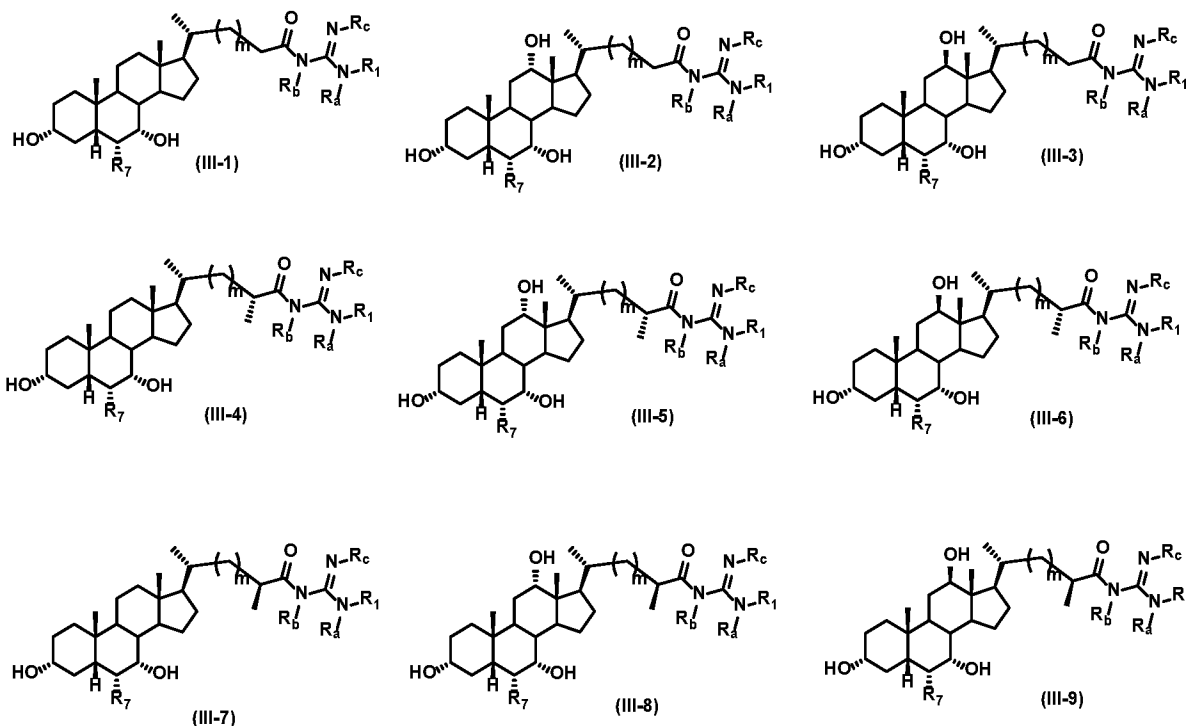
wherein, R_1 , R_a , R_b , R_c , R_2 , R_3 , R_4 , R_7 and m are as previously defined.

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

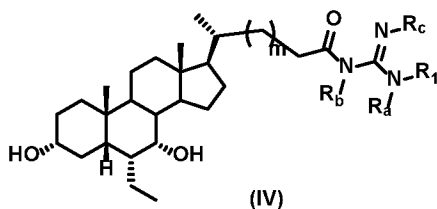


wherein R_1 , R_a , R_b , R_c , R_2 , R_3 , R_7 and m are as previously defined.

Illustrative structures of formula (III) can be represented, but not limited, by formulas III-1 to III-9, where R_1 , R_a , R_b , R_c , R_7 and m are as previously defined:

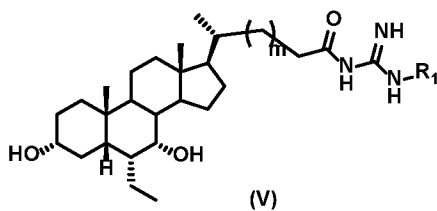


A fourth embodiment of the invention is a compound represented by Formula IV or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof:



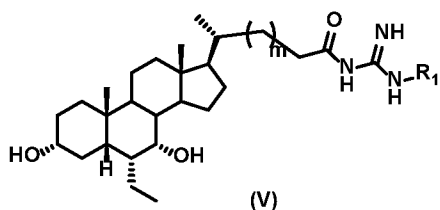
wherein R_1 , R_a , R_b , R_c , and m are as previously defined.

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



wherein, R_1 and m are as previously defined.

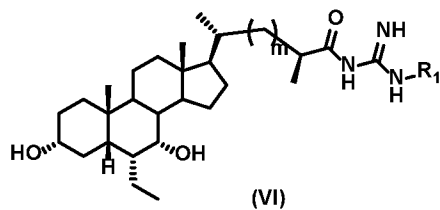
Representative compounds of the invention include, but are not limited to, the following compounds (example 1 to example 75 in Table 1) according to Formula V, wherein R_1 and m are delineated for each example in Table 1.

**Table 1**

Compound	m	R ₁	Compound	M	R ₁	Compound	m	R ₁
1	0	H	26	1	H	51	2	H
2	0	Methyl	27	1	Methyl	52	2	Methyl
3	0	Ethyl	28	1	Ethyl	53	2	Ethyl
4	0	Isopropyl	29	1	Isopropyl	54	2	Isopropyl
5	0	Butyl	30	1	Butyl	55	2	Butyl
6	0	t-Butyl	31	1	t-Butyl	56	2	t-Butyl
7	0	Propyl	32	1	Propyl	57	2	Propyl
8	0	Benzyl	33	1	Benzyl	58	2	Benzyl
9	0	Allyl	34	1	Allyl	59	2	Allyl
10	0	CF ₃	35	1	CF ₃	60	2	CF ₃
11	0		36	1		61	2	
12	0		37	1		62	2	
13	0		38	1		63	2	
14	0		39	1		64	2	
15	0		40	1		65	2	
16	0		41	1		66	2	
17	0		42	1		67	2	
18	0		43	1		68	2	
19	0		44	1		69	2	
20	0		45	1		70	2	
21	0		46	1		71	2	
22	0		47	1		72	2	

23	0		48	1		73	2	
24	0		49	1		74	2	
25	0		50	1		75	2	

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



5 wherein R_1 and m are as previously defined.

Representative compounds of the invention include, but are not limited to, the following compounds (example 76 to example 150 in Table 2) according to Formula VI, wherein R_1 and m are delineated for each example in Table 2.

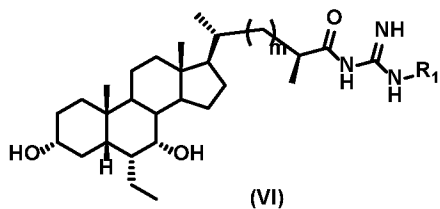
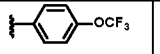
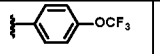
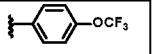
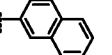
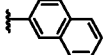
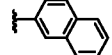
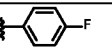
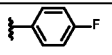
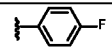
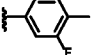
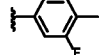
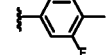
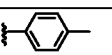
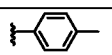
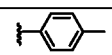
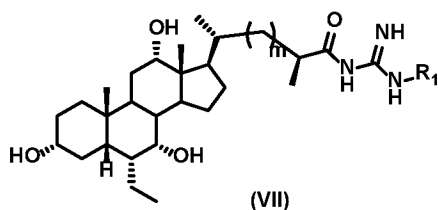


Table 2

Compound	m	R ₁	Compound	M	R ₁	Compound	m	R ₁
76	0	H	101	1	H	126	2	H
77	0	Methyl	102	1	Methyl	127	2	Methyl
78	0	Ethyl	103	1	Ethyl	128	2	Ethyl
79	0	Isopropyl	104	1	Isopropyl	129	2	Isopropyl
80	0	Butyl	105	1	Butyl	130	2	Butyl
81	0	t-Butyl	106	1	t-Butyl	131	2	t-Butyl
82	0	Propyl	107	1	Propyl	132	2	Propyl
83	0	Benzyl	108	1	Benzyl	133	2	Benzyl
84	0	Allyl	109	1	Allyl	134	2	Allyl
85	0	CF ₃	110	1	CF ₃	135	2	CF ₃
86	0		111	1		136	2	
87	0		112	1		137	2	
88	0		113	1		138	2	
89	0		114	1		139	2	
90	0		115	1		140	2	
91	0		116	1		141	2	
92	0		117	1		142	2	
93	0		118	1		143	2	
94	0		119	1		144	2	
95	0		120	1		145	2	
96	0		121	1		146	2	
97	0		122	1		147	2	
98	0		123	1		148	2	
99	0		124	1		149	2	
100	0		125	1		150	2	

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

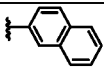
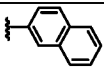
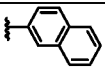
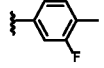
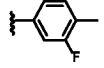
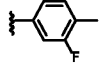
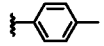
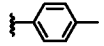
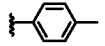
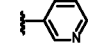
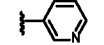
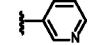
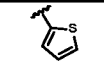
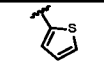
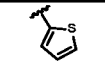
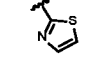
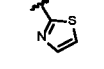
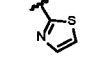


wherein R_1 and m are as previously defined.

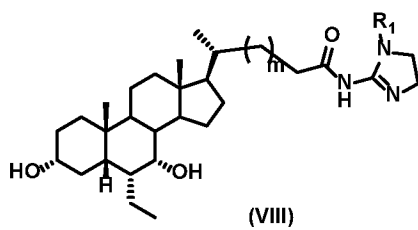
- 5 Representative compounds of the invention include, but are not limited to, the following compounds (example 151 to example 225 in Table 3) according to Formula VII, wherein, R_1 and m are delineated for each example in Table 3.

Table 3

Compound	m	R_1	Compound	m	R_1	Compound	m	R_1
151	0	H	176	1	H	201	2	H
152	0	Methyl	177	1	Methyl	202	2	Methyl
153	0	Ethyl	178	1	Ethyl	203	2	Ethyl
154	0	Isopropyl	179	1	Isopropyl	204	2	Isopropyl
155	0	Butyl	180	1	Butyl	205	2	Butyl
156	0	t-Butyl	181	1	t-Butyl	206	2	t-Butyl
157	0	Propyl	182	1	Propyl	207	2	Propyl
158	0	Benzyl	183	1	Benzyl	208	2	Benzyl
159	0	Allyl	184	1	Allyl	209	2	Allyl
160	0	CF_3	185	1	CF_3	210	2	CF_3
161	0		186	1		211	2	
162	0		187	1		212	2	
163	0		188	1		213	2	
164	0		189	1		214	2	
165	0		190	1		215	2	
166	0		191	1		216	2	
167	0		192	1		217	2	
168	0		193	1		218	2	

169	0		194	1		219	2	
170	0		195	1		220	2	
171	0		196	1		221	2	
172	0		197	1		222	2	
173	0		198	1		223	2	
174	0		199	1		224	2	
175	0		200	1		225	2	

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



5 wherein R_1 and m are as previously defined.

Representative compounds of the invention include, but are not limited to, the following compounds (example 226 to example 300 in Table 4) according to Formula VIII, wherein, R_1 and m are delineated for each example in Table 4.

Table 4

Compound	m	R_1	Compound	m	R_1	Compound	m	R_1
226	0	H	251	1	H	276	2	H
227	0	Methyl	252	1	Methyl	277	2	Methyl
228	0	Ethyl	253	1	Ethyl	278	2	Ethyl
229	0	Isopropyl	254	1	Isopropyl	279	2	Isopropyl
230	0	Butyl	255	1	Butyl	280	2	Butyl
231	0	t-Butyl	256	1	t-Butyl	281	2	t-Butyl
232	0	Propyl	257	1	Propyl	282	2	Propyl
233	0	Benzyl	258	1	Benzyl	283	2	Benzyl
234	0	Allyl	259	1	Allyl	284	2	Allyl

235	0	CF ₃	260	1	CF ₃	285	2	CF ₃
236	0		261	1		286	2	
237	0		262	1		287	2	
238	0		263	1		288	2	
239	0		264	1		289	2	
240	0		265	1		290	2	
241	0		266	1		291	2	
242	0		267	1		292	2	
243	0		268	1		293	2	
244	0		269	1		294	2	
245	0		270	1		295	2	
246	0		271	1		296	2	
247	0		272	1		297	2	
248	0		273	1		298	2	
249	0		274	1		299	2	
250	0		275	1		300	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 insulinoma. 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 insulinoma.

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 C₁-C₆ alkyl and C₁-C₈ alkyl radicals. Examples of C₁-C₆ alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, *n*-butyl, *tert*-butyl, neopentyl, *n*-hexyl groups; and examples of C₁-C₈ 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 C₂-C₆ alkenyl and C₂-C₈ 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 C₂-C₆ alkynyl and C₂-C₈ alkynyl groups. Representative alkynyl groups include, but are not limited to, for example, ethynyl, 1-propynyl, 1-butyne, 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 atoms. "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, the carbocycle group can be attached to the two other elements through any two substitutable ring atoms. A C₄-C₆ 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 C₃-C₈ cycloalkyl and C₃-C₁₂ cycloalkyl groups. Examples of C₃-C₈-cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl; and examples of C₃-C₁₂-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 C₃-C₈ cycloalkenyl and C₃-C₁₂ cycloalkenyl groups. Examples of C₃-C₈-cycloalkenyl include, but are not limited to, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl, and the like; and examples of C₃-C₁₂-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 C₁-C₃ alkyl or C₁-C₆ 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 C₁-C₃ alkyl or C₁-C₆ alkyl 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, -NO₂, -CN, -NH₂, N₃, protected amino, alkoxy, thioalkoxy, oxo, C₁-C₁₂-alkyl, C₂-C₁₂-alkenyl, C₂-C₁₂-alkynyl, C₃-C₁₂-cycloalkyl, -halo- C₁-C₁₂-alkyl, -halo- C₂-C₁₂-alkenyl, -halo- C₂-C₁₂-alkynyl, -halo-C₃-C₁₂-cycloalkyl, -NH -C₁-C₁₂-alkyl, -NH -C₂-C₁₂-alkenyl, -NH -C₂-C₁₂-alkynyl, -NH -C₃-C₁₂-cycloalkyl, -NH -aryl, -NH -heteroaryl, -NH -heterocycloalkyl, -dialkylamino, -diaryl amino, -diheteroaryl amino, -O-C₁-C₁₂-alkyl, -O-C₂-C₁₂-alkenyl, -O-C₂-C₁₂-alkynyl, -O-C₃-C₁₂-cycloalkyl, -O-aryl, -O-heteroaryl, -O-heterocycloalkyl, -C(O)- C₁-C₁₂-alkyl, -C(O)- C₂-C₁₂-alkenyl, -C(O)- C₂-C₁₂-alkynyl, -C(O)-C₃-C₁₂-cycloalkyl, -C(O)-aryl, -C(O)-heteroaryl, -C(O)-heterocycloalkyl, -CONH₂, -CONH- C₁-C₁₂-alkyl, -CONH- C₂-C₁₂-alkenyl, -CONH- C₂-C₁₂-alkynyl, -CONH-C₃-C₁₂-cycloalkyl, -CONH-aryl, -CONH-heteroaryl, -CONH-

- heterocycloalkyl, -OCO₂- C₁-C₁₂-alkyl, -OCO₂- C₂-C₁₂-alkenyl, -OCO₂- C₂-C₁₂-alkynyl, -
OCO₂-C₃-C₁₂-cycloalkyl, -OCO₂-aryl, -OCO₂-heteroaryl, -OCO₂-heterocycloalkyl, -
OCONH₂, -OCONH- C₁-C₁₂-alkyl, -OCONH- C₂-C₁₂-alkenyl, -OCONH- C₂-C₁₂-alkynyl, -
OCONH- C₃-C₁₂-cycloalkyl, -OCONH- aryl, -OCONH- heteroaryl, -OCONH-
- 5 heterocycloalkyl, -NHC(O)- C₁-C₁₂-alkyl, -NHC(O)-C₂-C₁₂-alkenyl, -NHC(O)-C₂-C₁₂-
alkynyl, -NHC(O)-C₃-C₁₂-cycloalkyl, -NHC(O)-aryl, -NHC(O)-heteroaryl, -NHC(O)-
heterocycloalkyl, -NHCO₂- C₁-C₁₂-alkyl, -NHCO₂- C₂-C₁₂-alkenyl, -NHCO₂- C₂-C₁₂-
alkynyl, -NHCO₂- C₃-C₁₂-cycloalkyl, -NHCO₂- aryl, -NHCO₂- heteroaryl, -NHCO₂-
heterocycloalkyl, -NHC(O)NH₂, -NHC(O)NH- C₁-C₁₂-alkyl, -NHC(O)NH-C₂-C₁₂-alkenyl, -
- 10 NHC(O)NH-C₂-C₁₂-alkynyl, -NHC(O)NH-C₃-C₁₂-cycloalkyl, -NHC(O)NH-aryl, -
NHC(O)NH-heteroaryl, -NHC(O)NH-heterocycloalkyl, NHC(S)NH₂, -NHC(S)NH- C₁-C₁₂-
alkyl, -NHC(S)NH-C₂-C₁₂-alkenyl, -NHC(S)NH-C₂-C₁₂-alkynyl, -NHC(S)NH-C₃-C₁₂-
cycloalkyl, -NHC(S)NH-aryl, -NHC(S)NH-heteroaryl, -NHC(S)NH-heterocycloalkyl, -
NHC(NH)NH₂, -NHC(NH)NH- C₁-C₁₂-alkyl, -NHC(NH)NH-C₂-C₁₂-alkenyl, -
- 15 NHC(NH)NH-C₂-C₁₂-alkynyl, -NHC(NH)NH-C₃-C₁₂-cycloalkyl, -NHC(NH)NH-aryl, -
NHC(NH)NH-heteroaryl, -NHC(NH)NH-heterocycloalkyl, -NHC(NH)-C₁-C₁₂-alkyl, -
NHC(NH)-C₂-C₁₂-alkenyl, -NHC(NH)-C₂-C₁₂-alkynyl, -NHC(NH)-C₃-C₁₂-cycloalkyl, -
NHC(NH)-aryl, -NHC(NH)-heteroaryl, -NHC(NH)-heterocycloalkyl, -C(NH)NH-C₁-C₁₂-
alkyl, -C(NH)NH-C₂-C₁₂-alkenyl, -C(NH)NH-C₂-C₁₂-alkynyl, -C(NH)NH-C₃-C₁₂-cycloalkyl,
- 20 -C(NH)NH-aryl, -C(NH)NH-heteroaryl, -C(NH)NH-heterocycloalkyl, -S(O)-C₁-C₁₂-alkyl, -
S(O)-C₂-C₁₂-alkenyl, - S(O)-C₂-C₁₂-alkynyl, - S(O)-C₃-C₁₂-cycloalkyl, - S(O)-aryl, - S(O)-
heteroaryl, - S(O)-heterocycloalkyl -SO₂NH₂, -SO₂NH- C₁-C₁₂-alkyl, -SO₂NH- C₂-C₁₂-
alkenyl, -SO₂NH- C₂-C₁₂-alkynyl, -SO₂NH- C₃-C₁₂-cycloalkyl, -SO₂NH- aryl, -SO₂NH-
heteroaryl, -SO₂NH- heterocycloalkyl, -NHSO₂-C₁-C₁₂-alkyl, -NHSO₂-C₂-C₁₂-alkenyl, -
- 25 NHSO₂-C₂-C₁₂-alkynyl, -NHSO₂-C₃-C₁₂-cycloalkyl, -NHSO₂-aryl, -NHSO₂-heteroaryl, -
NHSO₂-heterocycloalkyl, -CH₂NH₂, -CH₂SO₂CH₃, -aryl, -arylalkyl, -heteroaryl, -
heteroarylalkyl, -heterocycloalkyl, -C₃-C₁₂-cycloalkyl, polyalkoxyalkyl, polyalkoxy, -
methoxymethoxy, -methoxyethoxy, -SH, -S-C₁-C₁₂-alkyl, -S-C₂-C₁₂-alkenyl, -S-C₂-C₁₂-
alkynyl, -S-C₃-C₁₂-cycloalkyl, -S-aryl, -S-heteroaryl, -S-heterocycloalkyl, methylthiomethyl,
- 30 or -L'-R', wherein L' is C₁-C₆alkylene, C₂-C₆alkenylene or C₂-C₆alkynylene, and R' is aryl,
heteroaryl, heterocyclic, C₃-C₁₂cycloalkyl or C₃-C₁₂cycloalkenyl. 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, -NO₂, -CN, or -NH₂.

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 terms "heterocyclic" or "heterocycloalkyl" can be used interchangeably and refer to a non-aromatic ring or a bi- or tri-cyclic group fused, bridged or spiro system, where (i) each ring system contains at least one heteroatom independently selected from oxygen, sulfur and nitrogen, (ii) each ring system can be saturated or unsaturated (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 an aromatic 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, 2-azabicyclo[2.2.1]heptyl, 8-azabicyclo[3.2.1]octyl, 5-

azaspiro[2.5]octyl, 1-oxa-7-azaspiro[4.4]nonanyl, and tetrahydrofuryl. Such heterocyclic groups may be further substituted. Heteroaryl or heterocyclic groups can be C-attached or N-attached (where possible).

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, cycloalkylene, cycloalkenylene, cycloalkynylene, arylalkylene, heteroarylalkylene 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, methoxymethyl groups, for example.

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 art 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-methoxybenzyl, diphenylmethyl, triphenylmethyl

(trityl), tetrahydrofuryl, methoxymethyl, methylthiomethyl, benzyloxymethyl, 2,2,2-trichloroethoxymethyl, 2-(trimethylsilyl)ethoxymethyl, methanesulfonyl, para-toluenesulfonyl, trimethylsilyl, triethylsilyl, triisopropylsilyl, and the like. Preferred hydroxy

5 C(O)C₆H₅), and trimethylsilyl (TMS or -Si(CH₃)₃).

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

In certain embodiments, the invention provides compounds of each formula herein which are isotopically labelled compounds. An "isotopically labelled compound" is a
10 compound in which at least one atomic position is enriched in a specific isotope of the designated element to a level which is significantly greater than the natural abundance of that isotope. For example, one or more hydrogen atom positions in a compound can be enriched with deuterium to a level which is significantly greater than the natural abundance of deuterium, for example, enrichment to a level of at least 1%, preferably at least 20% or at
15 least 50%. Such a deuterated compound may, for example, be metabolized more slowly than its non-deuterated analog, and therefore exhibit a longer half-life when administered to a subject. Such compounds can be synthesized using methods known in the art, for example by employing deuterated starting materials. Unless stated to the contrary, isotopically labelled compounds are pharmaceutically acceptable.

20 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
25 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
30 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.

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 art 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
5 can also include inhibiting, i.e. arresting the development, of an 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
10 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,
15 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
20 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 H₂O, 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
25 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
30 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, polyvinylpyrrolidone, 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.

5

Abbreviations

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

ACN for acetonitrile;

10

BME for 2-mercaptoethanol;

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

BzCl for benzoyl chloride;

CDI for carbonyldiimidazole;

15

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)- aminoethyl-
1-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite;

20

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

DCC for N, N'-dicyclohexylcarbodiimide;

DCM for dichloromethane;

DIAD for diisopropyl azodicarboxylate;

DIBAL-H for diisobutylaluminum hydride;

25

DIPEA for diisopropyl ethylamine;

DMAP for N,N-dimethylaminopyridine;

DME for ethylene glycol dimethyl ether;

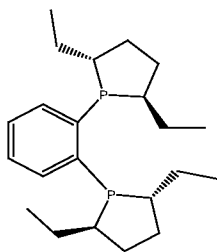
DMEM for Dulbecco's Modified Eagles Media;

DMF for N,N-dimethyl formamide;

30

DMSO for dimethylsulfoxide;

DSC for N, N'-disuccinimidyl carbonate;



DUPHOS for

;

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

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

EtOAc for ethyl acetate;

5 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)

10 (tricyclohexylphosphine)ruthenium(II);

In for indium;

KHMDS is potassium bis(trimethylsilyl) amide;

Ms for mesyl;

NMM for N-4-methylmorpholine;

15 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;

20 RT for reverse transcription;

RT-PCR for reverse transcription-polymerase chain reaction;

TBME for tert-butyl methyl ether;

TEA for triethyl amine;

Tf₂O for trifluoromethanesulfonic anhydride;

25 TFA for trifluoroacetic acid;

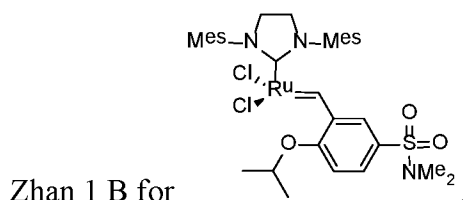
THF for tetrahydrofuran;

TLC for thin layer chromatography;

(TMS)₂NH for hexamethyldisilazane;

TMSOTf for trimethylsilyl trifluoromethanesulfonate;

TBS for t-Butyldimethylsilyl;
 TMS for trimethylsilyl;
 TPAP tetrapropylammonium perruthenate;
 TPP or PPh₃ for triphenylphosphine;
 5 TrCl for trityl chloride;
 DMTrCl for 4,4'-dimethoxytrityl chloride;
 tBOC or Boc for tert-butyloxy carbonyl;
 Xantphos for 4,5-Bis-diphenylphosphanyl-9,9-dimethyl-9H-xanthene; and

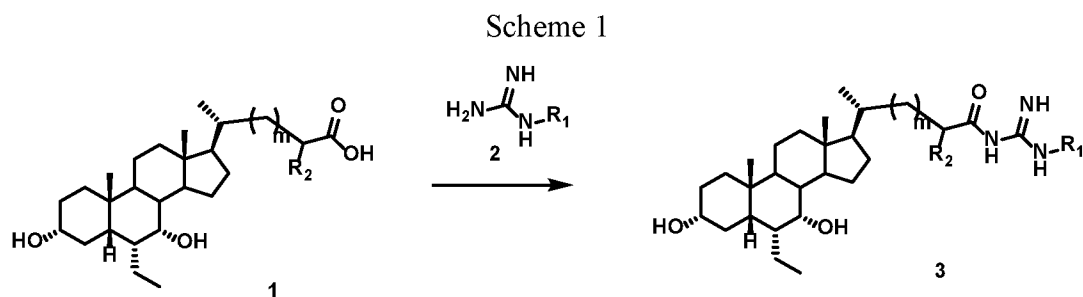


Synthetic Methods

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 compound of formula (3) are prepared from the bile acid compound of formula (1) and guanidine compound of formula (2), wherein R₂, m and R₁ are defined as previously. Thus, the compound of formula (1) is coupled with guanidine compound of formula (2) using suitable coupling condition to give the compound of formula (3). 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, CH₂Cl₂, DMF or THF. The reaction temperature can vary from 0°C to about 50°C.

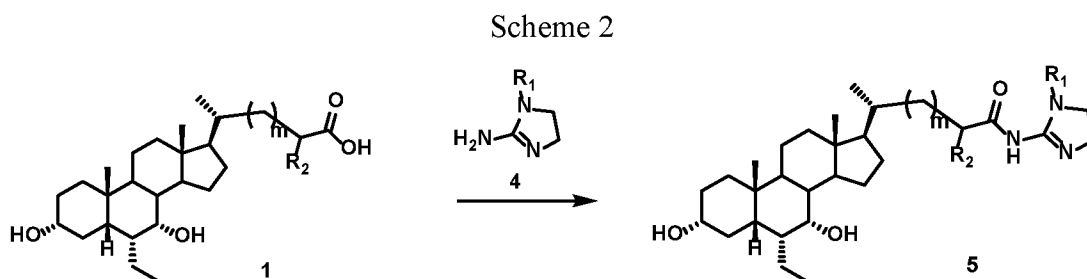
5



10 Similarly novel bile acid analogs of the compound of formula (5) as shown in Scheme 2 are prepared from the bile acid compound of formula (1) and guanidine compound of formula (4), wherein R₂, m and R₁ are defined as previously. Thus, the compound of formula (1) is coupled with guanidine compound of formula (4) using suitable coupling condition to give the compound of formula (5). The coupling reagent can be selected from, but not limited to, DCC, EDC, CDI, di-isopropyl carbodiimide, BOP-Cl, PyBOP, PyAOP, TFFH and HATU.

15 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, CH₂Cl₂, DMF or THF. The reaction temperature can vary from 0°C to about 50°C.

20

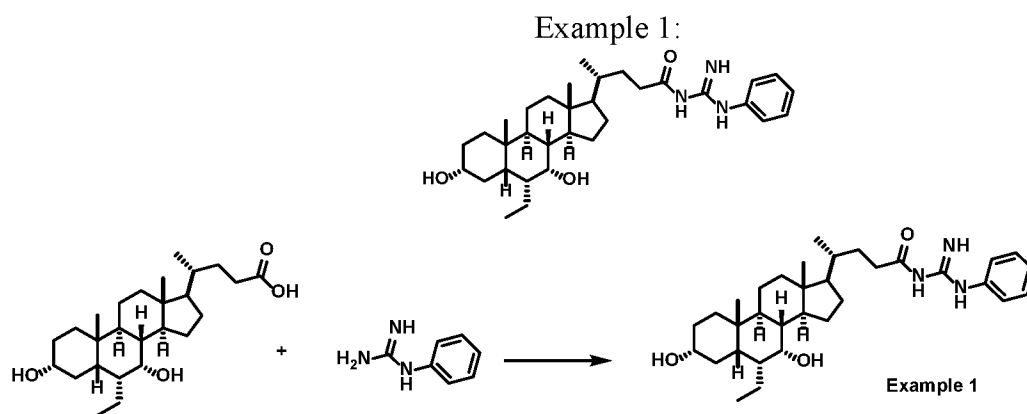


Examples

25 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.

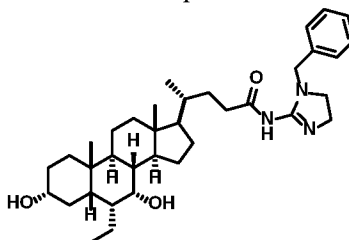
5

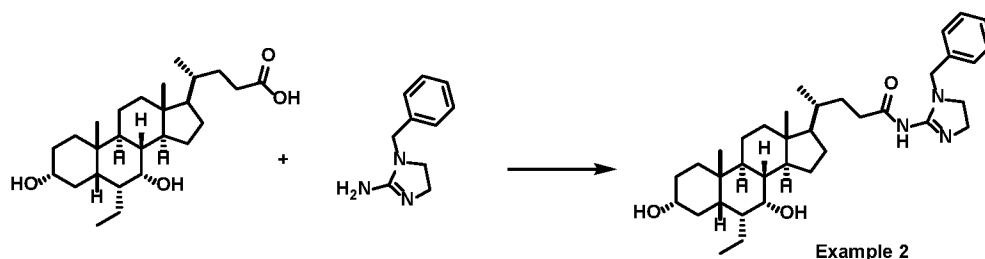


- 10 The bile acid analog 6-ECDCA (J. Med. Chem. 2014, 57, 937-954) (210 mg, 0.50 mmol) was dissolved in DMF (3 mL). To the solution was added N''-phenylguanidine nitrate (198 mg, 1.0 mmol), DIPEA (435 μ L, 2.5 mmol), and HATU (380 mg, 1.0 mmol). The resulting mixture was stirred at 23°C for 4 h, diluted with EtOAc, and washed with sat. NaCl (3 $\times$). The collected organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*.
- 15 Purification of the residue by SiO₂ chromatography provided Example 1 (43 mg). ¹HNMR (500 MHz, CDCl₃) 7.54 (2H, m), 7.50 (1H, m), 7.31 (2H, d, J = 7.5 Hz), 3.70 (1H, s), 3.41 (1H, br s), 2.70 (1H, br s), 2.58 (1H, br s), 0.98 (3H, d, J = 5.0 Hz), 0.90 (6H, br s), 0.67 (3H, s). LC/MS observed [M+HCOOH-1]⁻, 582.33.

20

Example 2:





The bile acid analog 6-ECDC (J. Med. Chem. 2014, 57, 937-954) (210 mg, 0.50 mmol) is dissolved in DMF (3 mL). To the solution is added 1-Benzyl-4,5-dihydro-1H-imidazol-2-ylamine (Chem. Pharm. Bull., 1978, 26(12), 3658-65) (1.0 mmol), DIPEA (435 μ L, 2.5 mmol), and HATU (380 mg, 1.0 mmol). The resulting mixture is stirred at 23°C for 4 h, diluted with EtOAc, and washed with sat. NaCl 3 times. The collected organic layer is dried over Na₂SO₄, filtered and concentrated *in vacuo*. Purification of the residue by SiO₂ chromatography provides Example 2.

10 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 principle 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. EC₅₀ 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 is 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 are incubated for additional 22 hours before luciferase activities are 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. EC₅₀ 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 are 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 brief, 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 μ L compound + 45 μ L Cell Assay Buffer/cAMP Antibody Reagent). For each dilution, the final concentration of solvent should remain constant. Transfer 15 μ L diluted compound to the assay plate and incubate the plate for 30 minutes at 37°C. Following agonist incubation, add 60 μ L 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 μ L 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 average EC₅₀ 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 5. The efficacy was normalized to CDCA set as 100%. (A: EC₅₀ < 0.1 μ M; B: 0.1 μ M < EC₅₀ < 1.0 μ M; C: 1.0 μ M < EC₅₀ < 10 μ M; D: EC₅₀ > 10 μ M).

Table 5

Example	EC ₅₀ (μ M)	Efficacy (%)
CDCA	D	100
6-ECDCA	B	288
1	B	248

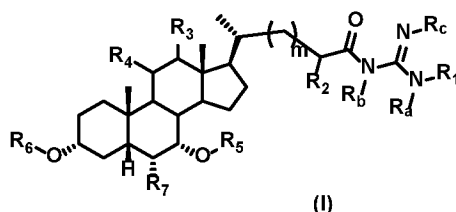
CDCA = chenodeoxycholic acid; 6-ECDCA = 6- α -ethylchenodeoxycholic acid

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.

CLAIMS

WHAT IS CLAIMED IS:

1. A compound represented by Formula I or pharmaceutically acceptable salt, solvate,
 5 hydrate, ester or prodrug thereof:



wherein:

R₁ is selected from the group consisting of:

- 1) Hydrogen;
- 10 2) Substituted or unsubstituted –C₁–C₈ alkyl;
- 3) Substituted or unsubstituted –C₂–C₈ alkenyl;
- 4) Substituted or unsubstituted –C₂–C₈ alkynyl;
- 5) Substituted or unsubstituted –C₃–C₈ cycloalkyl;
- 6) Substituted or unsubstituted aryl;
- 15 7) Substituted or unsubstituted arylalkyl;
- 8) Substituted or unsubstituted heterocycloalkyl;
- 9) Substituted or unsubstituted heteroaryl; and
- 10) Substituted or unsubstituted heteroarylalkyl;

R_a, R_b, and R_c are each independently selected from R₁; or R_a and R_b, or R_a and R_c, or R_b and

- 20 R_c are taken together with the two nitrogen atoms to which they are attached and the intervening carbon atom to form a heterocyclic, heteroaryl or heterocycloalkenyl ring;

R₂ is selected from the group consisting of:

- 1) Hydrogen;
- 2) Halogen;
- 25 3) Substituted or unsubstituted –C₁–C₈ alkyl;
- 4) Substituted or unsubstituted –C₂–C₈ alkenyl;
- 5) Substituted or unsubstituted –C₂–C₈ alkynyl;
- 6) Substituted or unsubstituted arylalkyl; and
- 7) Substituted or unsubstituted aryl;

m is 0, 1, 2 or 3;

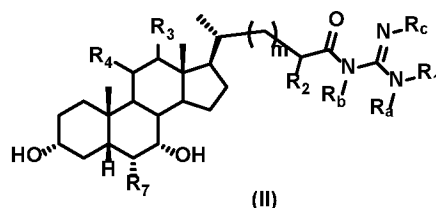
R₃ is hydrogen, hydroxyl, -OSO₃H, -OSO₃⁻, -OAc, -OPO₃H₂ or -OPO₃²⁻;

R₄ is hydrogen, halogen, CN, N₃, hydroxyl, -OSO₃H, -OSO₃⁻, -OAc, -OPO₃H₂, -OPO₃²⁻, -SR₂ or -NHR₂, wherein, R₂ is as defined previously; or R₃ and R₄ are taken together with the carbons to which they are attached to form -CH=CH-, a cycloalkyl ring or a heterocycloalkyl ring;

R₅ and R₆ are each independently selected from hydrogen or hydroxyl protecting group; and R₇ is selected from the group consisting of:

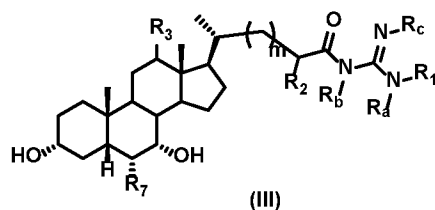
- 1) Hydrogen;
- 2) Halogen;
- 3) Substituted or unsubstituted -C₁-C₈ alkyl;
- 4) Substituted or unsubstituted -C₂-C₈ alkenyl;
- 5) Substituted or unsubstituted -C₂-C₈ alkynyl; and
- 6) Substituted or unsubstituted -C₃-C₈ cycloalkyl.

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



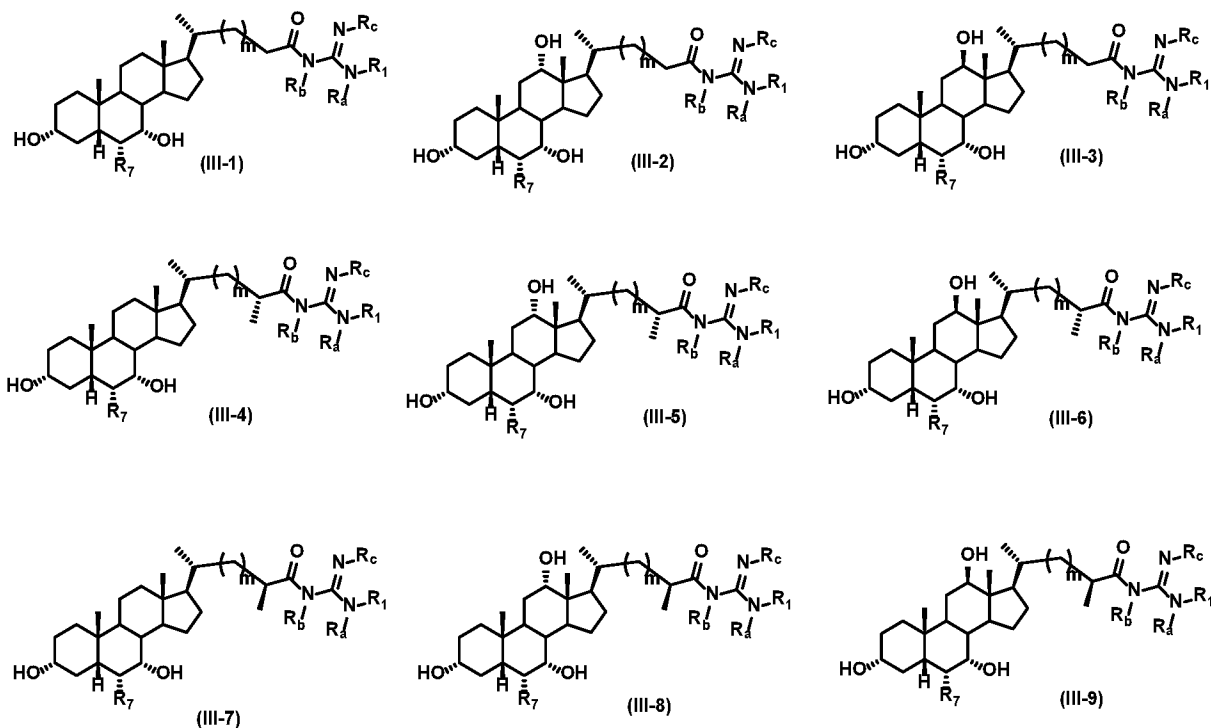
wherein R₁, R_a, R_b, R_c, R₂, R₃, R₄, R₇ and m are as defined in claim 1.

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



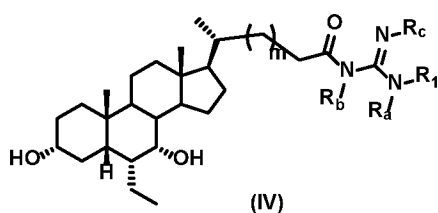
wherein, R₁, R_a, R_b, R_c, R₂, R₃, R₇ and m are as defined in claim 1.

4. The compound of claim 3, represented by one of formulas (III-1) to (III-9), or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof:



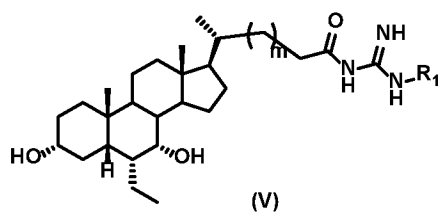
5 wherein, R_1 , R_a , R_b , R_c , R_2 , R_3 , R_7 and m are as defined in claim 3.

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



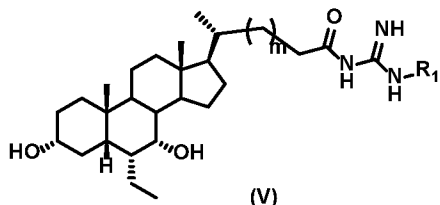
10 wherein, R_1 , R_a , R_b , R_c , and m are as defined in claim 1.

6. A compound of claim 1, represented by Formula V or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof:



15 wherein R_1 and m are as defined in claim 1

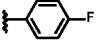
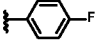
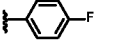
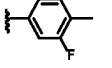
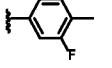
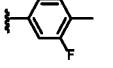
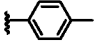
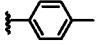
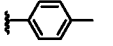
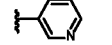
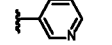
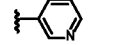
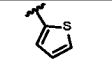
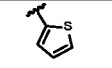
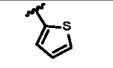
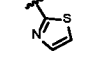
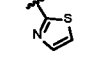
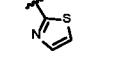
7. A compound represented by Formula V, or a or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof, wherein R₁ and m are delineated for each example in Table 1:



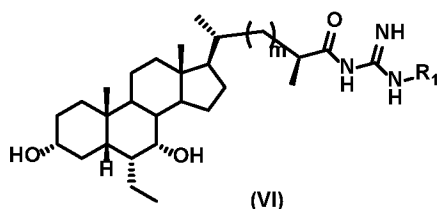
5

Table 1

Compound	m	R ₁	Compound	M	R ₁	Compound	m	R ₁
1	0	H	26	1	H	51	2	H
2	0	Methyl	27	1	Methyl	52	2	Methyl
3	0	Ethyl	28	1	Ethyl	53	2	Ethyl
4	0	Isopropyl	29	1	Isopropyl	54	2	Isopropyl
5	0	Butyl	30	1	Butyl	55	2	Butyl
6	0	t-Butyl	31	1	t-Butyl	56	2	t-Butyl
7	0	Propyl	32	1	Propyl	57	2	Propyl
8	0	Benzyl	33	1	Benzyl	58	2	Benzyl
9	0	Allyl	34	1	Allyl	59	2	Allyl
10	0	CF ₃	35	1	CF ₃	60	2	CF ₃
11	0		36	1		61	2	
12	0		37	1		62	2	
13	0		38	1		63	2	
14	0		39	1		64	2	
15	0		40	1		65	2	
16	0		41	1		66	2	
17	0		42	1		67	2	
18	0		43	1		68	2	
19	0		44	1		69	2	

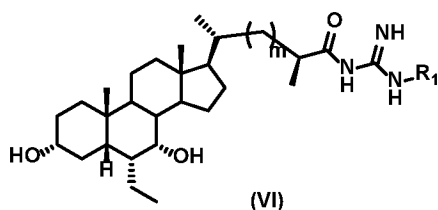
20	0		45	1		70	2	
21	0		46	1		71	2	
22	0		47	1		72	2	
23	0		48	1		73	2	
24	0		49	1		74	2	
25	0		50	1		75	2	

8. The compound of claim 1, represented by Formula VI or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof:



5 wherein, R₁ and m are as defined in claim 1.

9. A compound represented by Formula VI, or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof, wherein R₁ and m are delineated for each example in Table 2:



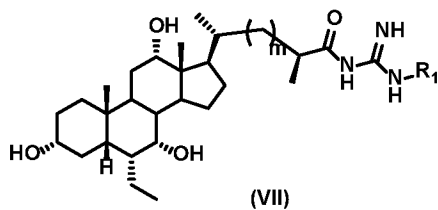
10

Table 2

Compound	m	R ₁	Compound	m	R ₁	Compound	m	R ₁
76	0	H	101	1	H	126	2	H
77	0	Methyl	102	1	Methyl	127	2	Methyl
78	0	Ethyl	103	1	Ethyl	128	2	Ethyl
79	0	Isopropyl	104	1	Isopropyl	129	2	Isopropyl
80	0	Butyl	105	1	Butyl	130	2	Butyl

81	0	t-Butyl	106	1	t-Butyl	131	2	t-Butyl
82	0	Propyl	107	1	Propyl	132	2	Propyl
83	0	Benzyl	108	1	Benzyl	133	2	Benzyl
84	0	Allyl	109	1	Allyl	134	2	Allyl
85	0	CF ₃	110	1	CF ₃	135	2	CF ₃
86	0		111	1		136	2	
87	0		112	1		137	2	
88	0		113	1		138	2	
89	0		114	1		139	2	
90	0		115	1		140	2	
91	0		116	1		141	2	
92	0		117	1		142	2	
93	0		118	1		143	2	
94	0		119	1		144	2	
95	0		120	1		145	2	
96	0		121	1		146	2	
97	0		122	1		147	2	
98	0		123	1		148	2	
99	0		124	1		149	2	
100	0		125	1		150	2	

10. The compound of claim 1, represented by Formula VII or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof:



wherein R₁ and m are as defined in claim 1.

11. A compound represented by Formula VII, or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof, wherein, R₁ and m are delineated for each example

5 in Table 3:

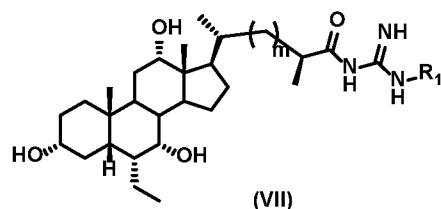
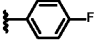
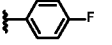
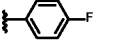
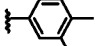
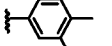
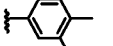
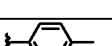
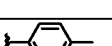
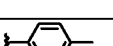
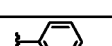
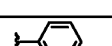
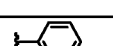
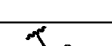
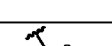
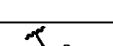
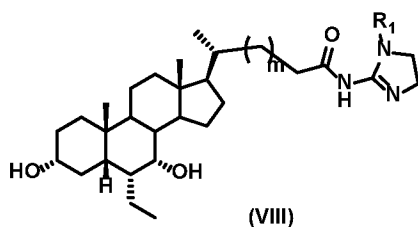


Table 3

Compound	m	R ₁	Compound	m	R ₁	Compound	m	R ₁
151	0	H	176	1	H	201	2	H
152	0	Methyl	177	1	Methyl	202	2	Methyl
153	0	Ethyl	178	1	Ethyl	203	2	Ethyl
154	0	Isopropyl	179	1	Isopropyl	204	2	Isopropyl
155	0	Butyl	180	1	Butyl	205	2	Butyl
156	0	t-Butyl	181	1	t-Butyl	206	2	t-Butyl
157	0	Propyl	182	1	Propyl	207	2	Propyl
158	0	Benzyl	183	1	Benzyl	208	2	Benzyl
159	0	Allyl	184	1	Allyl	209	2	Allyl
160	0	CF ₃	185	1	CF ₃	210	2	CF ₃
161	0		186	1		211	2	
162	0		187	1		212	2	
163	0		188	1		213	2	
164	0		189	1		214	2	
165	0		190	1		215	2	
166	0		191	1		216	2	
167	0		192	1		217	2	
168	0		193	1		218	2	
169	0		194	1		219	2	

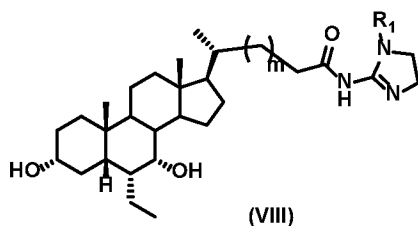
170	0		195	1		220	2	
171	0		196	1		221	2	
172	0		197	1		222	2	
173	0		198	1		223	2	
174	0		199	1		224	2	
175	0		200	1		225	2	

12. The compound of claim 1, represented by Formula VIII or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof:



5 wherein R₁ and m are as defined in claim 1.

13. A compound represented by Formula VIII, or a pharmaceutically acceptable salt, solvate, hydrate, ester or prodrug thereof, wherein, R₁ and m are delineated for each example in Table 4:



10

Table 4

Compound	m	R ₁	Compound	m	R ₁	Compound	m	R ₁
226	0	H	251	1	H	276	2	H
227	0	Methyl	252	1	Methyl	277	2	Methyl
228	0	Ethyl	253	1	Ethyl	278	2	Ethyl
229	0	Isopropyl	254	1	Isopropyl	279	2	Isopropyl
230	0	Butyl	255	1	Butyl	280	2	Butyl

231	0	t-Butyl	256	1	t-Butyl	281	2	t-Butyl
232	0	Propyl	257	1	Propyl	282	2	Propyl
233	0	Benzyl	258	1	Benzyl	283	2	Benzyl
234	0	Allyl	259	1	Allyl	284	2	Allyl
235	0	CF ₃	260	1	CF ₃	285	2	CF ₃
236	0		261	1		286	2	
237	0		262	1		287	2	
238	0		263	1		288	2	
239	0		264	1		289	2	
240	0		265	1		290	2	
241	0		266	1		291	2	
242	0		267	1		292	2	
243	0		268	1		293	2	
244	0		269	1		294	2	
245	0		270	1		295	2	
246	0		271	1		296	2	
247	0		272	1		297	2	
248	0		273	1		298	2	
249	0		274	1		299	2	
250	0		275	1		300	2	

14. 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 1-13.

5

15. The method according to claim 14, 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.

16. The method according to claim 15, 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.

17. The method according to claim 15, 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.

18. The method according to claim 15, wherein the cardiovascular disease is selected from the group consisting of atherosclerosis, arteriosclerosis, dyslipidemia, hypercholesterolemia, and hypertriglyceridemia.

19. The method according to claim 15, wherein the metabolic disease is selected from the group consisting of insulin resistance, Type I and Type II diabetes, and obesity.

20. The 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 1-13.

21. The pharmaceutical composition comprising a compound of formula (I) according to any one of claims 1-13, and a pharmaceutically acceptable carrier.
22. Use of a compound of formula (I) as defined in any one of claims 1-13, for the
5 preparation of a pharmaceutical composition for the prevention or treatment of FXR-mediated or TGR5-mediated diseases or conditions.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/017554

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/57 (2016.01)

CPC - A61K 31/57 (2016.02)

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 31/56, 31/57, 45/06 (2016.01)

CPC - A61K 31/56, 31/57, 45/06 (2016.02)

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

IPC(8) - A61K 31/56, 31/57, 45/06 (2016.01); CPC - A61K 31/56, 31/57, 45/06 (2016.02); USPC - 514/169, 171, 182 (keyword delimited)

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

PatBase, STN, PubChem, Google Patents, Google Scholar

Search terms used: bile acid derivatives urea FXR TGR5

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JPH 11-60594 A (SANKYO CO) 02 March 1999 (02.03.1999) entire translated document	1-4
---		-----
Y		14-22
Y	US 2010/0063018 A1 (PELLICCIARI et al) 11 March 2010 (11.03.2010) entire document	14-19, 21, 22
Y	US 2013/0116218 A1 (ETHICON-SURGERY INC) 09 May 2013 (09.05.2013) entire document	20

☐ 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

22 March 2016

Date of mailing of the international search report

22 APR 2016

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774