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(54) Title: COMPOUNDS MODULATING ACTIVITY OF FARNESOID X RECEPTOR AND METHODS FOR THE USE THEREOF

(57) Abstract: Compounds such as vidofludimus and its analogues useful in modulating the activity of nuclear receptor FXR. Also disclosed are the methods for treating FXR-mediated disease or process in a mammal, comprising administering to the mammal a therapeutically effective amount of a compound claimed, wherein the FXR-mediated disease or condition linked to chronic liver diseases such as nonalcoholic fatty liver disease and nonalcoholic steatohepatitis, inflammatory disease such as rheumatoid arthritis and inflammatory bowel disease, cardiovascular diseases, or metabolic diseases such as diabetes and obesity.

COMPOUNDS MODULATING ACTIVITY OF FARNESOID X RECEPTOR AND METHODS FOR THE USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

5 This application claims priority to, and the benefit of, China patent application 201710192430.1, filed on March 28, 2017, the disclosures of which are incorporated herein by reference.

FIELD OF THE INVENTION

10 The present invention relates to compounds, compositions and methods for treating diseases or conditions mediated by farnesoid X receptor (FXR), and methods for the compound design and applications.

BACKGROUND OF THE INVENTION

15 Nuclear receptors represent a type of ligand-regulated transcription factors involved in a variety of biological processes. For example, farnesoid X receptor (FXR), highly expressed in mammalian liver, intestine, kidney and adrenal gland, is one of the 48 known human nuclear receptors. Nuclear receptors, such as FXR, play an important role in regulating virtually all aspects of human physiology including metabolism, inflammation, hepatic
20 protection and regeneration, bile salt, fat and glucose homeostasis and other related physiological functions. As such, FXR has become an excellent drug target for the treatment of many FXR-mediated diseases like cancer, aging, metabolic diseases such as high blood glucose, insulin resistance, hypertriglyceridemia, hypercholesterolemia, diabetes, obesity, biliary obstruction, gallstones, nonalcoholic fatty liver, atherosclerosis and other diseases
25 (see, e.g., Fiorucci et al., (2010) Current Medicinal Chemistry, 17, 139-159 and Carotti et al., (2014) Current Topics in Medicinal Chemistry, 14, 2129-2142).

Small molecules known as ligands play important roles in modulating the activity of nuclear receptors, since the binding of ligands can determine the recruitment of coregulators. The coregulators include coactivators like the p160 factors that also referred to as the steroid
30 receptor coactivators (SRC) family. Given the critical roles in human diseases, the ligands have been studied intensively in pharmaceutical development.

Cholestasis is composed of a variety of human liver diseases such as primary biliary cirrhosis, primary sclerosing cholangitis, cystic fibrosis, and intrahepatic cholestasis of pregnancy (see, e.g., Pellicciari et al., (2002) Journal of medicinal chemistry 45, 3569-3572).
35 In the liver, activation of FXR induces transcription of transporter genes involved in promoting bile acid clearance and represses genes involved in bile acid synthesis. The enterohepatic circulation of bile acids enables the absorption of fats and fat-soluble vitamins from the intestine and allows the elimination of cholesterol, toxins, and metabolic by-products such as bilirubin from the liver. FXR-null mice exhibit cholestatic liver disorder. In the bile duct-ligation and α -naphthylisothiocyanate models of cholestasis, the
40 FXR activation resulted in reductions in inflammation, other markers of liver damage and increased expression of genes involved in bile acid transport (see e.g., Liu et al., (2003) Journal of Clinical Investigation 112, 1678-1687). These suggest therapeutic applications of FXR ligands can be used to treat liver disorders associated with cholestasis. FXR agonists
45 may be useful in the treatment of cholestatic liver diseases, such as cholestasis, liver inflammation, liver damage, primary sclerosing cholangitis, cystic fibrosis, and intrahepatic cholestasis of pregnancy.

FXR modulators impact both bile acid synthesis and lipid metabolism, and are effective pharmaceutical agents in preventing and treating liver diseases associated with bile acid mediated cellular injury, fatty liver disease, liver cancer as well as atherosclerosis and cardiovascular disease. Moreover, studies on wide type and FXR^{-/-} mice have determined that FXR plays pleiotropic roles in regulating triglyceride, lipid, cholesterol, glucose metabolism in addition to bile acids homeostasis (see e.g., Sinal et al., (2000) Cell 102, 731-44). Hypertriglycerides is a predictor of coronary heart disease risk factor, strategies targeting the hypertriglyceride are well prevention and treatment for coronary heart disease risk (see e.g., Cullen (2000) The American Journal of Cardiology 86, 943-949). This is mainly attributed to the inverse relationship between serum triglycerides (TGs) and HDL cholesterol, since low levels of HDL increase the risk of vascular diseases. Bile acid lowers serum TGs, reduces SREBP-1c and lipogenic genes dependent on activating FXR and inducing the expression of SHP (see e.g., Lambert et al., (2003) The Journal of biological chemistry 278, 2563-2570 and Watanabe et al., (2004) The Journal of clinical investigation 113, 1408-1418). As such, FXR modulators can be used to treat or prevent the hypertriglyceride and the related coronary heart disease.

FXR-null mice show features in glucose tolerance and insulin resistance (see e.g., Zhang et al., (2006) Proceedings of the National Academy of Sciences of the United States of America 103, 1006-1011). In addition to GW4064, FXR ligand Ivermectin was recently found to be specifically regulating glucose and cholesterol homeostasis dependent on FXR (see e.g., Jin et al., (2013) Nature communications 4, 1937). As such, FXR is a drug target in treating or preventing insulin resistance, hyperglycemia, hypercholesterol, obesity, diabetes as well as disorders related to glucose and cholesterol metabolism.

FXR is an ideal target for nonalcoholic fatty liver disease (NAFLD) drug development due to its crucial roles in lipid metabolism (see e.g., Carr and Reid, (2015) Curr Atheroscler Rep 17, 500). Activation of FXR reduced liver expression of genes involved in fatty acid synthesis, lipogenesis, and gluconeogenesis, as well as reducing the steatosis of obese rat (see e.g., Cipriani et al., (2010) J Lipid Res 51, 771-784). FXR ligands Avermectin analogues are effective in regulating metabolic parameters tested, including reducing hepatic lipid accumulation, lowering serum cholesterol and glucose levels, and improving NAFLD in an FXR dependent manner (see e.g., Jin et al., (2015) Scientific reports 5, 17288). Taken together, FXR has been proposed as a target for improving non-alcoholic steatohepatitis (NASH), or non-alcoholic fatty liver disease (NAFLD) from steatosis to cirrhosis, and even liver cancer.

Hypercholesterolemia and dyslipidemia are important risk factors for cardiovascular disease (CVD) and atherosclerosis, characterized by elevated plasma triglycerides (TGs) and lowered HDL-cholesterol (HDL-C), in combination with obesity, elevated blood glucose levels, and/or hypertension termed the metabolic syndrome (see e.g., Porez et al., (2012) J Lipid Res 53, 1723-1737). FXR activation protects against atherosclerosis development as well as hyperlipidemia in ApoE^{-/-} mice (see e.g., Hartman et al., (2009) J Lipid Res 50, 1090-1100 and Mencarelli et al., (2009) Am J Physiol Heart Circ Physiol 296, H272-281). Thus, FXR ligands might be used in prevention and treatment of atherosclerosis and cardiovascular disease.

FXR and its ligands are also involved in cellular inflammatory and immune responses (See e.g., Shaik F.B., et al., (2015) Inflamm Res 64(1):9-20). The regulation of FXR can negatively regulate various inflammatory factors such as tumor necrosis factor TNF α , IL-6, cyclooxygenase (COX)-1, COX-2 etc., thereby inhibiting the occurrence and development of inflammation (see e.g., Gadaleta R.M., et al., Gut. 60 (4): 463-72, 2011]. Meanwhile, FXR modulators can inhibit the occurrence and development of inflammation by inducing cytokine signaling inhibitor repressors like SOCS3 (see e.g., Xu Z.Z., et al., Cellular Signaling 24: 1658-1664, 2012). FXR deficiency is susceptible to gallbladder inflammation

and cholesterol gallstone disease (CGD), indicating that FXR is a potential target in treating CGD (see e.g., Moschetta et al., (2004) *Nature medicine* 10, 1352-1358). Emerging roles for FXR in the gut include protection against bacterial overgrowth and maintenance of intestinal barrier function. FXR activation protects against murine models of induced colitis (see e.g., Gadaleta et al., (2011) *Gut* 60, 463-472 and Vavassori et al., (2009) *J Immunol* 183, 6251-6261). These suggest that FXR modulators can extenuate the damage of inflammation to various disorders, which can be useful as a therapeutic strategy for various inflammatory diseases. The inflammatory diseases include, but not limited to, rheumatoid arthritis (RA), tendinitis or bursitis, fibromyalgia, muscular low back pain, chronic obstructive pulmonary disease (COPD), psoriasis, pelvic inflammatory disease (PID), asthma, pneumonia, polymyalgia rheumatica and gout.

For example, rheumatoid arthritis (RA) is an autoimmune disease affecting approximately 1% of the population worldwide. The monocytes/macrophages, which are inflammatory cells, have been implicated in RA, leading to bone and cartilage destruction (see e.g., Wang Y., et al., *Int Immunopharmacol.* 2017;50:345-352). Among the released cytokines, TNF α is of major importance in rheumatoid arthritis (see e.g., Feldman M, et al., *Annu Rev Immunol.* 1996;14:397-440). It has been reported that the levels of TNF α in serum and synovial fluid were significantly higher in RA and osteoarthritis arthritis (OA) patients than that in the normal group (see e.g., Guan X., et al., *Qingdao Med J* 2008, 40(1):9-12; Sun B., et al., *Journal of Radioimmunology* 2000, 13(3):169). Also, the serum level of TNF α is reported significantly higher in patients with active Juvenile Idiopathic Arthritis (JIA) (see e.g., Shen H., et al., *Chinese J Trad Med T raum & Orthop* 2007, 15(9):3-5). These results suggest that TNF α may serve as an indicator of activity in arthritis, including RA, OA and JIA. Overproduction of TNF α leads to autoimmune reactions, which may enhance the inflammatory and destructive process (see e.g., Hayer S, et al., *J Immunol.* 2005, 175(12):8327-36). Several groups have shown that collagen-induced arthritis (CIA) in mice may be treated effectively with anti-TNF α antibody or other TNF α inhibitors (see e.g., MaY and Pope RM. *Curr Pharm Des.* 2005, 11(5):5695-80). These reports suggest that FXR may be a target for treating arthritis by inhibiting TNF α .

FXR activation increased bile acid flux, which is a signal for liver regeneration in mice. FXR may promote homeostasis not only by regulating expression of appropriate metabolic target genes but also by driving homeotrophic liver growth (see e.g., Huang et al., (2006) *Science* 312, 233-6). However, irregular regeneration of hepatocytes with cells over proliferation has been reported as an important factor in carcinogenesis (see e.g., Ueno et al., (2001) *Hepatology* 33, 357-362 and Wang et al., (2008) *Hepatology* 48, 1632-1643). FXR^{-/-} mice spontaneously developed liver tumors, while intestinal-selective FXR modulators activation is sufficient to prevent hepatic malignancy (see, e.g., Yang et al., (2007) *Cancer Res* 67, 863-867 and Degirolamo et al., (2015) *Hepatology* 61:161-70). FXR deficiency in the intestine promotes Wnt signaling with expansion of the basal proliferative compartment, while FXR activation can induce the apoptosis of colon cancer cells (see e.g., Modica et al., (2008) *Cancer Res* 68, 9589-9594). Taken together, FXR can be a target to protect against carcinogenesis such as liver and intestinal cancer.

FXR also plays a critical role in aging-induced fatty liver (see e.g., Xiong et al., (2014) *J Hepatol.* 60(4):847-54), and expression and activity of FXR are increased in the livers of the long-lived Little mice, both suggesting an association between FXR and aging (see e.g., Jiang et al., (2013) *Mech Ageing Dev.* 134(9): 407-15). Activation of FXR is able to alleviate age-related liver regeneration defects (see e.g., Chen et al., (2010) *Hepatology* 51(3):953-62). These findings highlight FXR as a potential target of drug design for disorders related to aging such as liver regeneration and extension of chronological lifespan.

The regulation of FXR by ligands has beneficial effects on bone metabolism through modulating bone formation, differentiation and resorption, resulting in preventing bone loss

150 and enhancing bone mass gain (see e.g., Cho et al., (2013) J Bone Miner Res. 28(10):2109-21), suggesting therapeutic roles of FXR ligands in treating disorders related to bone formation such as osteoporosis, bone hyperplasia and osteoarthritis.

Many FXR ligands have been described, but have limitations owing to side effects and uncertain bioavailabilities (see e.g., Watanabe et al., (2011) The Journal of biological
155 chemistry 286, 26913-26920). Accordingly, there is a need for compositions, compounds, and systems to treat FXR-mediated diseases.

SUMMARY OF THE INVENTION

The present application relates to compounds, or pharmaceutically acceptable salt, isomers, or prodrugs thereof, that bind to the farnesoid X receptor (FXR), for the treatment
160 of FXR-mediated diseases or conditions, including but not limited to inflammation, analgesia, cholestasis, colitis, chronic liver diseases, gastrointestinal diseases, renal diseases, cardiovascular disease, kidney disease, inflammatory disorder, metabolic diseases and various cancers.

165 Another aspect of this invention is directed to methods of treating, preventing, inhibiting, or ameliorating the symptoms of a disease or disorder or a condition that is modulated by FXR activity, by administering to the mammal a therapeutically effective amount of at least one compound or combinations of compounds disclosed herein.

As used herein, the term "FXR ligand" refers to any compounds that regulate FXR
170 activity as full agonists, partial agonists, antagonists, inverse agonists, or selective nuclear receptor modulators, due to their diverse characteristics in FXR binding mode, regulating transcription and post-translational modification and their ability in inducing FXR to recruit various co-regulators. Post-translational modifications, such as SUMOylation and phosphorylation, are also differentially associated with transactivation or transrepression,
175 respectively.

As used herein, the term "FXR activity" refers any FXR activities relating to therapeutic effects on human disease. For example, FXR activity regulated by compounds for use in accordance with the present invention include, but is not limited to, transcriptional activity, phosphorylation, acetylation, methylation, ubiquitination, sumoylation, any other
180 postranscriptional activity, any other protein modification, and protein-protein interactions relating to signal transduction.

In some embodiments, the compounds for use in the methods described herein may be formulated as a therapeutically effective amount of pharmaceutical compositions. Pharmaceutical compositions of this invention may comprise the compounds described
185 herein or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier. Such compositions may optionally comprise an additional therapeutic agent.

As used herein, "EC50" refers to a dosage, concentration or amount of a said compound which induces a response halfway between the baseline and maximum after a specified exposure time, commonly used as a measure of drug's potency.

190 The technology used herein, is also described in Jin et al., (2013) Nature communications 4, 1937 and Jin et al., (2015) Scientific reports 5, 17288.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1. The structure of vidofludimus bound with FXR LBD in cartoon representation
195 (A). 2Fo-Fc electron density map (1.0σ) showing the bound vidofludimus and the surrounding FXR residues (B).

Figure 2. H&E stained liver sections from compounds pretreated mice with APAP-induced liver injury.

Figure 3. The H&E staining (A) and Oil Red O staining (B) of liver sections from ob/ob
200 mice treated with 10 mg/kg of compounds for 10 days.

Figure 4. The H&E staining (A) and Oil Red O staining (B) of liver sections from db/db mice treated with 10 mg/kg of compounds for 10 days.

Figure 5. H&E stained colonic sections from compounds pretreated mice with DSS-induced colitis.

EXAMPLES

The following specific examples:

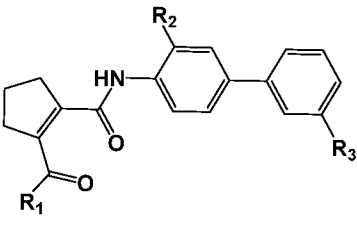
Example 1. Vidofludimus is a FXR ligand.

In search of novel ligands for FXR, we used FXR ligand binding domain (LBD) as a bait to screen chemical libraries based on AlphaScreen biochemical assay, which determines the efficacy of small molecules in influencing binding affinity of FXR with coregulator peptides (see e.g., Jin et al., (2013) Nature communications 4, 1937). Results from a clinical compounds library revealed vidofludimus (synonyms: 4sc-101; SC12267) potently promoted the interaction of FXR with coactivator LXXLL motifs from SRC1-2 and SRC2-3 (Table 1), indicating this compound is able to regulate FXR activity. To unravel the molecular basis for the recognition of FXR by vidofludimus, we performed structural studies on the FXR LBD complexed with vidofludimus. The AlphaScreen and crystallization of FXR LBD protein were performed as described previously (see e.g., Jin et al., (2013) Nature communications 4, 1937). The structure reveals that the vidofludimus-bound FXR LBD resembles most agonist-bound nuclear receptor structures (Figure 1A). The existence of vidofludimus was apparent from the highly revealing electron density map shown in Figure 1B.

Example 2. Compounds modulate FXR activity.

Based on the structure-activity-relationship of vidofludimus and FXR, we have designed and synthesized series of vidofludimus analogues targeting FXR (Table 2). The compounds that modulate FXR activity are shown in Table 1. The in vivo upregulated FXR target genes, such as OST α , GCLM and UGT1A1 (Lee et al., Mol Endocrinol 2010, 24(8):1626–1636) in liver (Table 3), and FGF15 (Inagaki et al., Cell Metab. 2005 Oct;2(4):217-25) in intestine (Table 9 and Table 20) further confirmed that these compounds are physiologically functional FXR ligands.

Table 1. Potency of compounds in regulating FXR activity as determined by the ability in inducing FXR to recruit coactivator motifs by AlphaScreen assay. The indicated values are fold changes by 5 μ M compounds.

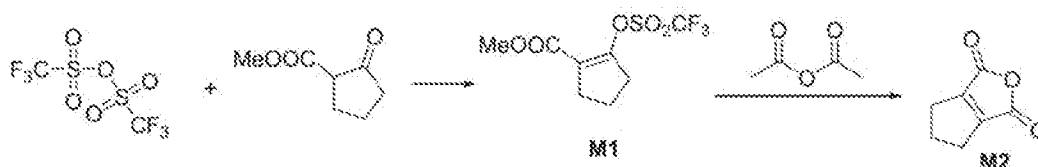
compounds				Fold induction in recruiting coactivator motif SRC1-2	Fold induction in recruiting coactivator motif SRC2-3
	R1	R2	R3		
Vidofludimus	OH	F	OCH ₃	>10	>10
FD1	NH ₂	F	OCH ₃	>10	>10
FD2	NHCH ₃	F	OCH ₃	>10	>10
FD3	OCH ₂ CH ₃	F	OCH ₃	>10	>10
FD5	OCH ₃	F	OCH ₃	>5	>5
FD6	OH	F	OCH ₂ CH ₃	>10	>10

FD9	OH	F	CH ₃	>10	>10
FD10	OH	F	CH ₂ CH ₃	>10	>10
FD11	OH	F	CH(CH ₃) ₂	>10	>10
FD12	OH	CH ₃	OCH ₃	>10	>10
FD13	OH	Cl	OCH ₃	>5	>5
FD20	OH	CH ₃	CH ₂ CH ₃	>10	>10
FD29	OH	CH ₃	CH ₃	>10	>10
FD31	OH	CH ₃	CH(CH ₃) ₂	>10	>10

Example 2. Preparation of compounds.

The following examples illustrate synthetic routes of compounds listed in Table 3.

1. Synthesis of Intermediates **M1** and **M2**



1) Synthesis of **M1**

80 mL of dichloromethane was added into 2.85 g (20 mmol) of methyl 2-oxocyclopentanecarboxylate, and the reaction was cooled to -78°C. Then, 17 mL of N, N-diisopropyl was added to the reaction, and continuously stirred for 15 min. After trifluoromethanesulfonic anhydride (2.5 mL, 24.0 mmol) was added dropwise for about 30 min, the reaction was warmed to room temperature and allowed to react overnight. Then, the reaction was cooled to 0°C, and was quenched with 30 mL of water. The liquid was then separated and the organic phase was washed with 50 mL of 5% citric acid aqueous solution twice, and dried over sodium sulfate. After being filtered, the organic phase was concentrated and purified by column chromatography to give 5.17 g of a pale yellow oil, **M1**, with yield of 94%. (Reference: Org Lett. 2012 Jun 15;14(12):2940-3.)

2) Synthesis of **M2**

100 mL of dry DMF was added into 5 g (18.2 mmol) of **M1**, then 3A molecular sieve and sodium formate (3.7 g, 54 mmol) were added, with nitrogen replacement for three times. After 2.3 g (54 mmol) of lithium chloride added into the reaction, the reaction was put into ice bath, and acetic anhydride (3.7 g, 36 mmol) and diisopropylamine (3.6 g, 36 mmol) were added dropwise. The reaction was warmed up to room temperature, and palladium acetate (0.4 g, 1.8 mmol) was added. After continuous stirring for 24 h, the reaction was cooled to temperature below 5°C, and 50 mL of 2N HCl was added dropwise within 5 min, continuously stirred for 10 min. The solution was extracted with 50 mL of ethyl acetate for three times, and then washed with 50 mL of saturated sodium chloride solution twice. The product was dried with anhydrous magnesium sulfate, filtered, concentrated, and purified by column chromatography. 1.17 g of **M2** was achieved, with yield of 47%. (Reference: Angew Chem Int Ed Engl. 2015 Jan 26;54(5):1527-31)

2. Synthesis of **FD1**

1 mL of concentrated ammonia and 3 g of anhydrous sodium sulfate were added into 5 mL of dichloromethane, stirred in ice bath for 30 min. This reaction gave the solution of ammonia in methylene chloride. 5 mL of dichloromethane and 1 g of thionyl chloride was added into 150 mg (0.42 mmol) of vidofludimus, reflux reaction for 4 h. The organic solvent was removed by concentration under reduced pressure. 2 mL of dichloromethane was added with stirring in ice bath, then 2.5 mL of the solution of ammonia in methylene chloride was added into the reaction dropwise. After that, the

reaction was warmed up to room temperature, stirred continuously for 5 h. Then, 10 mL of dichloromethane and 5 mL of water were added. After stirring for 5 min, the liquid was separated. The organic phase was washed with 5 mL of water twice, dried with anhydrous sodium sulfate, concentrated and purified by column chromatography. 108 mg of **FD1** was achieved, with yield of 73%.

3. Synthesis of **FD2**

40% methylamine aqueous solution and 3 g of anhydrous sodium sulfate were added into 5 mL of dichloromethane, stirred in ice bath for 30 min. This reaction gave the solution of methylamine in methylene chloride. 5 mL of dichloromethane and 1 g of thionyl chloride were added into 150 mg (0.42 mmol) of vidofludimus, reflux reaction for 4 h. The organic solvent was removed by concentration under reduced pressure. 4 mL of dichloromethane was added with stirring in ice bath, then 1 mL of the solution of methylamine in methylene chloride was added into the reaction dropwise. After that, the reaction was warmed up to room temperature, stirred continuously for 5 h. Then, 5 mL of dichloromethane and 5 mL of water were added. After stirring for 5 min, the liquid was separated. The organic phase was washed with 5 mL of water for three times, dried with anhydrous sodium sulfate, concentrated and purified by column chromatography. 121 mg of **FD2** was achieved, with yield of 78%.

4. Synthesis of **FD3**

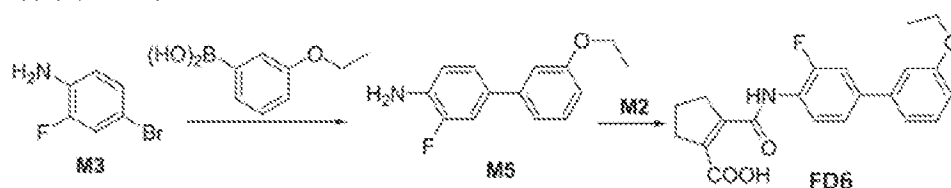
5 mL of ethanol was added into 380 mg of thionyl chloride, which gave the solution of thionyl chloride in ethanol.

5 mL of ethanol was added into 150 mg (0.42 mmol) of vidofludimus, and then 1 mL of the solution of thionyl chloride in ethanol was added into the reaction. After warming up for reflux reaction for 3 h, the solvent was removed under reduced pressure. 5 mL of ethanol was added and the thionyl chloride was removed by concentration under reduced pressure twice. Then 10 mL of dichloromethane and 5 mL of water were added. After stirring for 10 min, the liquid was separated. The organic phase was washed with 5 mL of water for three times, dried with anhydrous sodium sulfate, filtered, concentrated and purified by column chromatography. 128 mg of **FD3** was achieved, with yield of 79.5%.

5. Synthesis of **FD5**

5 mL of anhydrous methanol was added into 380 mg of thionyl chloride, which gave the solution of dichlorosulfoxide in methanol. 5 mL of ethanol was added into 150 mg (0.42 mmol) of vidofludimus, and then 1 mL of the solution of dichlorosulfoxide in methanol was added into the reaction. After warming up for reflux reaction for 3 h, the solvent was removed under reduced pressure. 5 mL of ethanol was added and the thionyl chloride was removed by concentration under reduced pressure twice. Then 10 mL of dichloromethane and 5 mL of water were added. After stirring for 10 min, the liquid was separated. The organic phase was washed with 5 mL of water for three times, dried with anhydrous sodium sulfate, filtered, concentrated and purified by column chromatography. 134 mg of **FD5** was achieved, with yield of 86.5%.

6. Synthesis of **FD6**



1) Synthesis of **M5**

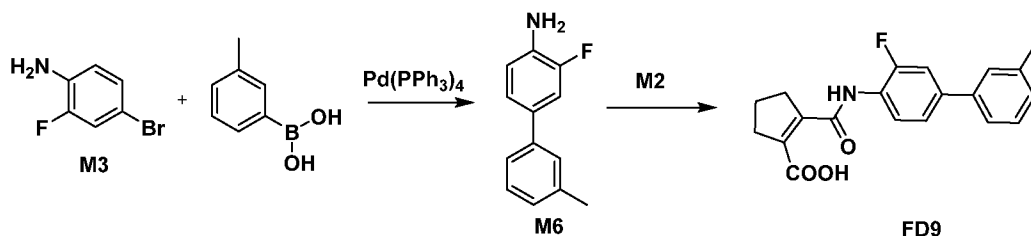
Toluene (9 mL), ethanol (6 mL) and water (3 mL) were added into the mixture of **M3** (0.95 g, 5 mmol), 3-Methoxyboronic acid (0.83 g, 5 mmol) and potassium carbonate (1.73 g, 12.5 mmol), stirred with nitrogen replacement for three times. Additional replacement with nitrogen was performed for three times after 0.3 g of

tetraphenylphenylphosphine palladium was added. Then the reaction was warmed up for reflux reaction overnight. The product was filtered and concentrated, then 20 mL of dichloromethane and 10 mL of water were added and stirred for 10 min. The liquid was separated and washed with 10 mL of water twice, dried with anhydrous sodium sulfate, filtered, concentrated and purified by column chromatography. With 0.73 g of **M5** achieved.

2) Synthesis of **FD6**

5 mL of DCM was added into 138 mg (1.0 mmol) of **M2** and stirred. Then **M5** (231 mg, 1.0 mmol) was added into the reaction, and stirred for 24 h. After that, 5 mL of water was added and stirred for 5 min, the liquid was separated and dried with anhydrous sodium sulfate, concentrated and purified by column chromatography. 210 mg of **FD6** was achieved, with yield of 57%.

7. Synthesis of **FD9**



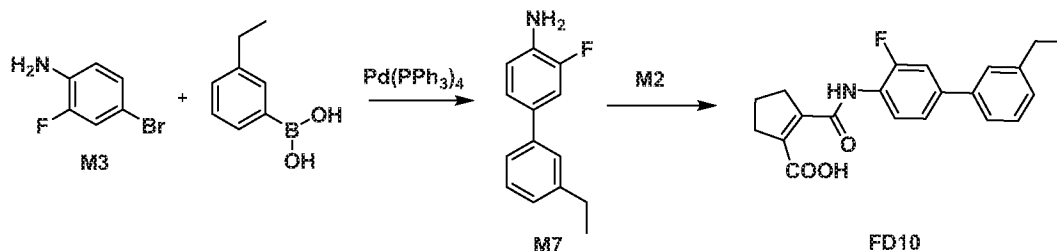
1) Synthesis of **M6**

Toluene (9 mL), ethanol (6 mL) and water (3 mL) were added into the mixture of **M3** (0.95 g, 5 mmol), 3-Methylboronic acid (0.68 g, 5 mmol) and potassium carbonate (1.73 g, 12.5 mmol), stirred with nitrogen replacement for three times. Additional replacement with nitrogen was performed for three times after 0.3 g of tetraphenylphenylphosphine palladium was added. Then the reaction was warmed up for reflux reaction overnight. The product was filtered and concentrated, then 20 mL of dichloromethane and 10 mL of water were added and stirred for 10 min. The liquid was separated and washed with 10 mL of water twice, dried with anhydrous sodium sulfate, filtered, concentrated and purified by column chromatography. 0.73 g of **M6** was achieved.

2) Synthesis of **FD9**

5 mL of DCM was added into 138 mg (1.0 mmol) of **M2** and stirred. Then **M5** (231 mg, 1.0 mmol) was added into the reaction, and stirred for 24 h. After that, 5 mL of water was added and stirred for 5 min, the liquid was separated and dried with anhydrous sodium sulfate, concentrated and purified by column chromatography. 210 mg of **FD9** was achieved, with yield of 57%.

8. Synthesis of **FD10**



1) Synthesis of **M7**

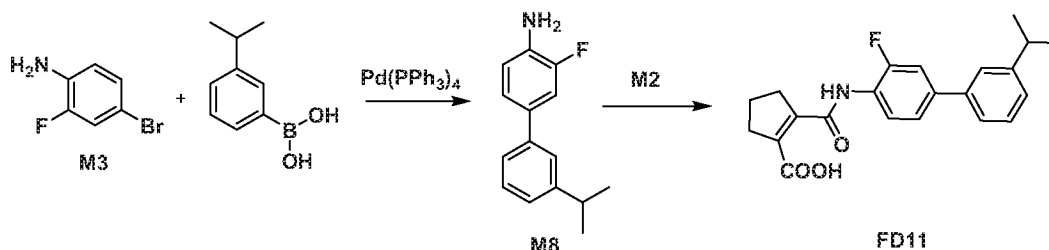
Toluene (9 mL), ethanol (6 mL) and water (3 mL) were added into the mixture of **M3** (0.95 g, 5 mmol), 3-Methylboronic acid (0.75 g, 5 mmol) and potassium carbonate (1.73 g, 12.5 mmol), stirred with nitrogen replacement for three times. Additional replacement with nitrogen was performed for three times after 0.3 g of tetraphenylphenylphosphine palladium was added. Then the reaction was warmed up for reflux reaction overnight. The product was filtered and concentrated, then 20 mL of dichloromethane and 10 mL of

water were added and stirred for 10 min. The liquid was separated and washed with 10 mL of water twice, dried with anhydrous sodium sulfate, filtered, concentrated and purified by column chromatography. 0.73 g of **M7** was achieved.

2) Synthesis of **FD10**

5 mL of DCM was added into 138 mg (1.0 mmol) of **M2** and stirred. Then **M7** (211 mg, 1.0 mmol) was added into the reaction, and stirred for 24 h. After that, 5 mL of water was added and stirred for 5 min, the liquid was separated and dried with anhydrous sodium sulfate, concentrated and purified by column chromatography. 208 mg of **FD9** was achieved, with yield of 56%.

9. Synthesis of **FD11**



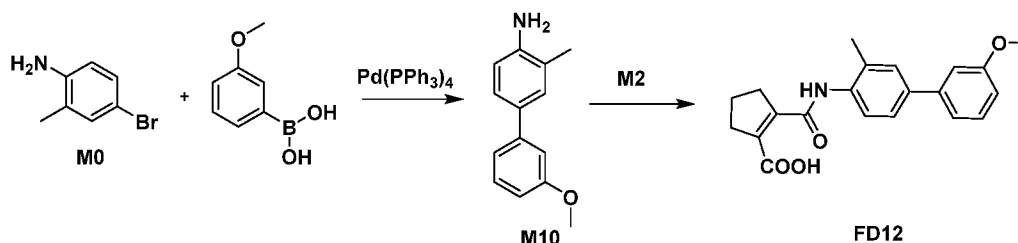
1) Synthesis of **M8**

Toluene (9 mL), ethanol (6 mL) and water (3 mL) were added into the mixture of **M3** (0.95 g, 5 mmol), 3-Methylboronic acid (0.82 g, 5 mmol) and potassium carbonate (1.73 g, 12.5 mmol), stirred with nitrogen replacement for three times. Additional replacement with nitrogen was performed for three times after 0.3 g of tetraphenylphenylphosphine palladium was added. Then the reaction was warmed up for reflux reaction overnight. The product was filtered and concentrated, then 20 mL of dichloromethane and 10 mL of water were added and stirred for 10 min. The liquid was separated and washed with 10 mL of water twice, dried with anhydrous sodium sulfate, filtered, concentrated and purified by column chromatography. 0.77 g of **M8** was achieved.

2) Synthesis of **FD11**

5 mL of DCM was added into 138 mg (1.0 mmol) of **M2** and stirred. Then **M8** (241 mg, 1.0 mmol) was added into the reaction, and stirred for 24 h. After that, 5 mL of water was added and stirred for 5 min, the liquid was separated and dried with anhydrous sodium sulfate, concentrated and purified by column chromatography. 230 mg of **FD11** was achieved, with yield of 67%.

10. Synthesis of **FD12**



1) Synthesis of **M10**

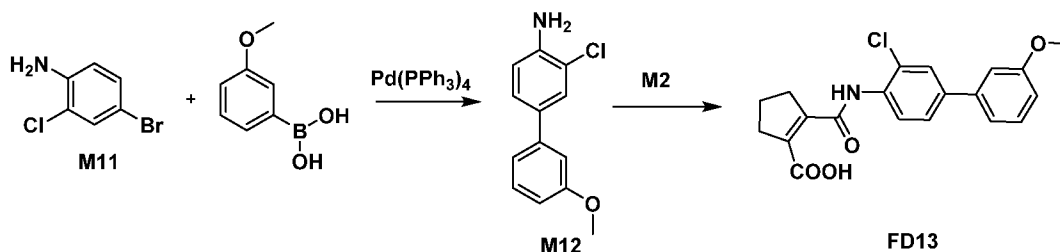
Toluene (9 mL), ethanol (6 mL) and water (3 mL) were added into the mixture of **M0** (0.95 g, 5 mmol), (3-methoxyphenyl) boronic acid (0.76 g, 5 mmol) and potassium carbonate (1.73 g, 12.5 mmol), stirred with nitrogen replacement for three times. Additional replacement with nitrogen was performed for three times after 0.3 g of tetraphenylphenylphosphine palladium was added. Then the reaction was warmed up for reflux reaction overnight. The product was filtered and concentrated, then 20 mL of dichloromethane and 10 mL of water were added and stirred for 10 min. The liquid was separated and washed with 10 mL of water twice, dried with anhydrous sodium sulfate, filtered, concentrated and purified by column chromatography. 0.713 g of **M10** was

achieved.

2) Synthesis of **FD12**

5 mL of DCM was added into 138 mg of **M2** and stirred. Then **M10** (221 mg, 1.0 mmol) was added into the reaction, and stirred for 24 h. After that, 5 mL of water was added and stirred for 5 min, the liquid was separated and dried with anhydrous sodium sulfate, concentrated and purified by column chromatography. 205 mg of **FD12** was achieved, with yield of 58%.

11. Synthesis of **FD13**



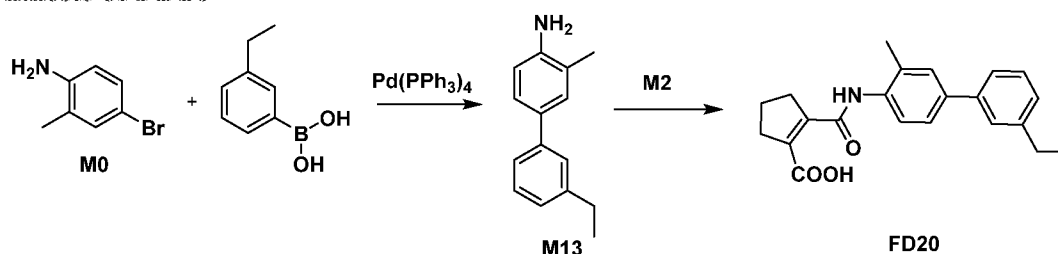
1) Synthesis of **M6**

Toluene (9 mL), ethanol (6 mL) and water (3 mL) were added into the mixture of **M11** (1.02 g, 5 mmol) 4-bromo-2-chloroaniline, (3-methoxyphenyl) boronic acid (0.76 g, 5 mmol) and potassium carbonate (1.73 g, 12.5 mmol), stirred with nitrogen replacement for three times. Additional replacement with nitrogen was performed for three times after 0.3 g of tetraphenylphenylphosphine palladium was added. Then the reaction was warmed up for reflux reaction overnight. The product was filtered and concentrated, then 20 mL of dichloromethane and 10 mL of water were added and stirred for 10 min. The liquid was separated and washed with 10 mL of water twice, dried with anhydrous sodium sulfate, filtered, concentrated and purified by column chromatography. 0.83 g of **M12** was achieved, with yield of 63%.

2) Synthesis of **FD13**

5 mL of DCM was added into 138 mg (1.0 mmol) of **M2** and stirred. Then **M12** (231 mg, 1.0 mmol) was added into the reaction, and stirred for 24 h. After that, 5 mL of water was added and stirred for 5 min, the liquid was separated and dried with anhydrous sodium sulfate, concentrated and purified by column chromatography. 230 mg of **FD13** was achieved, with yield of 67%.

12. Synthesis of **FD20**

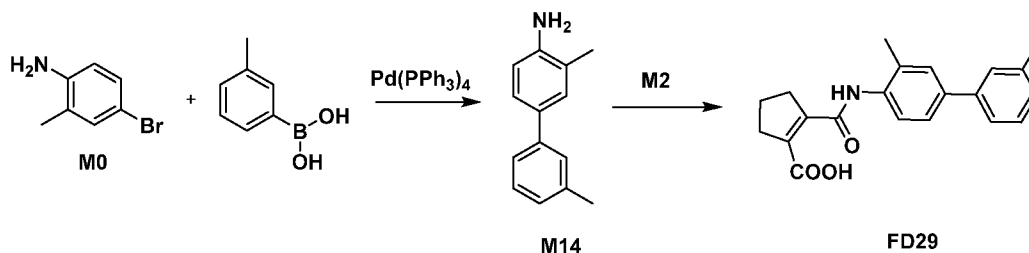


1) Synthesis of **M13**

Toluene (9 mL), ethanol (6 mL) and water (3 mL) were added into the mixture of **M0** (0.95 g, 5 mmol) 4-bromo-2-chloroaniline, (3-ethylphenyl) boronic acid (0.75 g, 5 mmol) and potassium carbonate (1.73 g, 12.5 mmol), stirred with nitrogen replacement for three times. Additional replacement with nitrogen was performed for three times after 0.3 g of tetraphenylphenylphosphine palladium was added. Then the reaction was warmed up for reflux reaction overnight. The product was filtered and concentrated, then 20 mL of dichloromethane and 10 mL of water were added and stirred for 10 min. The liquid was separated and washed with 10 mL of water twice, dried with anhydrous sodium sulfate, filtered, concentrated and purified by column chromatography. 0.67 g of **M13** was achieved, with yield of 64%.

2) Synthesis of **FD20**

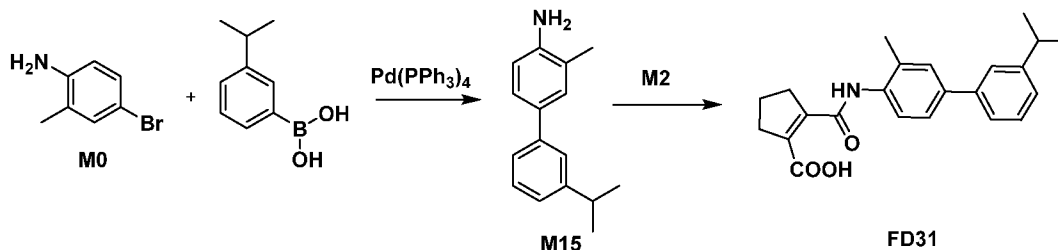
5 mL of DCM was added into 138 mg (1.0 mmol) of **M2** and stirred. Then **M13** (211 mg, 1.0 mmol) was added into the reaction, and stirred for 24 h. After that, 5 mL of water was added and stirred for 5 min, the liquid was separated and dried with anhydrous sodium sulfate, concentrated and purified by column chromatography. 244 mg of **FD20** was achieved, with yield of 70%.

13. Synthesis of **FD29**1) Synthesis of **M14**

Toluene (9 mL), ethanol (6 mL) and water (3 mL) were added into the mixture of **M0** (0.95 g, 5 mmol) 4-bromo-2-chloroaniline, m-tolylboronic acid (0.68 g, 5 mmol) and potassium carbonate (1.73 g, 12.5 mmol), stirred with nitrogen replacement for three times. Additional replacement with nitrogen was performed for three times after 0.3 g of tetraphenylphenylphosphine palladium was added. Then the reaction was warmed up for reflux reaction overnight. The product was filtered and concentrated, then 20 mL of dichloromethane and 10 mL of water were added and stirred for 10 min. The liquid was separated and washed with 10 mL of water twice, dried with anhydrous sodium sulfate, filtered, concentrated and purified by column chromatography. 0.72 g of **M14** was achieved, with yield of 72%.

2) Synthesis of **FD29**

5 mL of DCM was added into 138 mg (1.0 mmol) of **M2** and stirred. Then **M14** (197 mg, 1.0 mmol) was added into the reaction, and stirred for 24 h. After that, 5 mL of water was added and stirred for 5 min, the liquid was separated and dried with anhydrous sodium sulfate, concentrated and purified by column chromatography. 227 mg of **FD13** was achieved, with yield of 68%.

14. Synthesis of **FD31**1) Synthesis of **M15**

Toluene (9 mL), ethanol (6 mL) and water (3 mL) were added into the mixture of **M0** (0.95 g, 5 mmol) 4-bromo-2-chloroaniline, (3-isopropylphenyl) boronic acid (0.82 g, 5 mmol) and potassium carbonate (1.73 g, 12.5 mmol), stirred with nitrogen replacement for three times. Additional replacement with nitrogen was performed for three times after 0.3 g of tetraphenylphenylphosphine palladium was added. Then the reaction was warmed up for reflux reaction overnight. The product was filtered and concentrated, then 20 mL of dichloromethane and 10 mL of water were added and stirred for 10 min. The liquid was separated and washed with 10 mL of water twice, dried with anhydrous sodium sulfate, filtered, concentrated and purified by column chromatography. 0.83 g of **M15** was achieved, with yield of 73%.

2) Synthesis of **FD31**

5 mL of DCM was added into 138 mg (1.0 mmol) of **M2** and stirred. Then **M15** (225 mg, 1.0 mmol) was added into the reaction, and stirred for 24 h. After that, 5 mL of water was added and stirred for 5 min, the liquid was separated and dried with anhydrous sodium sulfate, concentrated and purified by column chromatography. 254 mg of **FD31** was achieved, with yield of 69%

Table 2. Chemical No., names, structures and ^1H -NMR data for compounds disclosed herein.

No.	Full Name	Structure	^1H -NMR data
FD1	N-(3-fluoro-3'-methoxy-[1,1'-biphenyl]-4-yl)cyclopent-1-ene-1,2-dicarboxamide		^1H -NMR (z, DMSO, ppm) δ 11.36(br, 2H), 8.47-8.50(m, 1H), 7.33-7.38(d, 3H), 7.27(m, 1H), 7.15-7.17(d, J=8.0, 1H), 7.10(m, 1H), 7.09(m, 1H), 3.87(m, 3H), 3.03-2.89(m, 4H), 1.95-1.98(m, 2H).
FD2	N ¹ -(3-fluoro-3'-methoxy-[1,1'-biphenyl]-4-yl)-N ² -methylcyclopent-1-ene-1,2-dicarboxamide		^1H -NMR (400MHz, DMSO, ppm) δ 11.59(m, 1H), 8.43-8.47(m, 1H), 7.35-7.36(d, 3H), 7.26(m, 1H), 7.15-7.16(d, J=8.0, 1H), 7.10(m, 1H), 6.89-6.90(m, 1H), 3.87(m, 3H), 3.03-2.87(m, 7H), 1.57(m, 2H).
FD3	ethyl 2-((3-fluoro-3'-methoxy-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylate		^1H -NMR (400MHz, DMSO, ppm) δ 11.09(m, 1H), 8.49-8.55(m, 1H), 7.28-7.39(m, 3H), 7.28(m, 1H), 7.16-7.19(d, J=8.0, 1H), 7.11(m, 1H), 6.89-6.90(m, 1H), 4.30-4.37(m, 2H), 3.89(m, 3H), 3.03-2.93(m, 4H), 1.90-1.95(m, 2H), 1.28-1.62(m, 3H).
FD5	methyl 2-((3-fluoro-3'-methoxy-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylate		^1H -NMR (400MHz, DMSO, ppm) δ 11.03(m, 1H), 8.48-8.52(m, 1H), 7.33-7.39(m, 3H), 7.27(m, 1H), 7.15-7.17(d, J=8.0, 1H), 7.10(m, 1H), 6.89-6.91(m, 1H), 3.87(m, 6H), 3.03-2.89(m, 4H), 1.89-1.93(m, 2H).
FD6	2-((3'-ethoxy-3-fluoro-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid		^1H -NMR (400MHz, DMSO, ppm) δ 11.09(m, 1H), 8.49-8.55(m, 1H), 7.28-7.39(m, 3H), 7.28(m, 1H), 7.16-7.19(d, J=8.0, 1H), 7.11(m, 1H), 6.89-6.90(m, 1H), 4.30-4.37(m, 2H), 3.89(m, 3H), 3.03-2.93(m, 4H), 1.90-1.95(m, 2H), 1.28-1.62(m, 3H).
FD9	2-((3-fluoro-3'-methyl-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid		^1H -NMR (400MHz, DMSO, ppm) δ 13.96(br, 1H), 10.574(m, 1H), 8.090-8.0413(m, 1H), 7.47-7.61(m, 3H), 7.32-7.37(m, 1H), 7.17-7.19(m, 1H), 2.67-2.82(m, 4H), 2.49-2.51(m, 3H), 1.87-1.94(m, 2H).

FD10	2-((3'-ethyl-3-fluoro-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid		¹ H-NMR (400MHz, DMSO, ppm) δ 13.06 (br, 1H), 10.57(m, 1H), 8.090-8.0412(m, 1H), 7.49-7.62 (m, 3H), 7.39-7.34(m, 1H), 7.23-7.20(m, 1H), 2.67-3.35(m, 6H), 1.89-1.94 (m, 2H), 1.84-1.20(m, 3H).
FD11	2-((3-fluoro-3'-isopropyl-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid		¹ H-NMR (400MHz, DMSO, ppm) δ 13.07(br, 1H), 10.58(m, 1H), 7.24-8.10 (m, 7H), 3.35(m, 1H), 2.78-2.99(m, 4H), 1.92(m, 2H), 1.24-1.27 (m, 6H).
FD12	2-((3'-methoxy-3-methyl-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid		¹ H-NMR (400MHz, DMSO, ppm) δ 13.11 (br, 1H), 9.92(m, 1H), 6.90-7.57(m, 7H), 3.82(m, 3H), 3.35-2.67 (m, 4H), 2.50(m, 3H), 1.96-1.86(m, 2H).
FD13	2-((3-chloro-3'-methoxy-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid		¹ H-NMR (400MHz, DMSO, ppm) δ 13.16 (br, 1H), 10.34(m, 1H), 8.090-8.0412(m, 1H), 7.49-6.94 (m, 6H), 3.83(m, 1H), 2.51-2.68(m, 2H), 2.50-2.51 (m, 3H), 1.95-1.87(m, 2H).
FD20	2-((3'-ethyl-3-methyl-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid		¹ H-NMR (400MHz, DMSO, ppm) δ 13.165 (br, 1H), 9.917(m, 1H), 7.448-7.5622(m, 5H), 7.342-7.380 (m, 1H), 7.187-7.205 (m, 1H), 2.850-2.813 (m, 2H), 2.665-2.713(m, 4H), 2.506-2.510(m, 3H), 1.913-2.300 (m, 2H), 1.918-1.956 (m, 3H).
FD29	2-((3,3'-dimethyl-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid		¹ H-NMR (400MHz, DMSO, ppm) δ 13.149 (br, 1H), 9.911(m, 1H), 7.433-7.562 (m, 5H), 7.318-7.356 (m, 1H), 7.154-7.172(m, 1H), 2.84-2.713 (m, 2H), 2.676-2.694 (m, 2H), 2.511-2.502 (m, 3H), 2.270-2.377 (m, 6H), 1.956-2.028 (m, 1H).
FD31	2-((3'-isopropyl-3-methyl-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid		¹ H-NMR (400MHz, DMSO, ppm) δ 13.06 (br, 1H), 9.91(m, 1H), 7.56-7.38(m, 6H), 7.36-7.22 (m, 1H), 2.84-2.30(m, 6H), 1.90-1.93 (m, 2H), 1.25-1.26(m, 6H).

Example 3. Therapeutic effects of compounds on liver injury.

Methods: Acetaminophen (APAP)-induced liver injury in mouse is a commonly used model to study drugs in protecting liver. Overdose of APAP causes liver injury by inducing the production of reactive oxygen species and reactive nitrogen species, and excessive consumption of reductive substances such as antioxidant glutathione (GSH), leading to the

reduction of GSH in vivo, and the following upregulation of the activities of the aspartate aminotransferase (AST) the alanine aminotransferase (ALT), the alkaline phosphatase (ALP) and the lactate dehydrogenase (LDH), which will result in liver inflammation and necrosis. In this example, the APAP-induced liver injury was used to detect the protective and repairing functions of our compounds in liver injury.

17-week-old male C57BL/6J mice were maintained under environmentally controlled conditions with free access to standard chow diet and water. Animal experiments were conducted in the barrier facility of the Laboratory Animal Center, Xiamen University, approved by the Institutional Animal Use and Care Committee of Xiamen University, China.

We selected vidofludimus, vidofludimus sodium salt and vidofludimus calcium salt to test the hepatoprotective effects of our compounds. Compounds were solved with DMSO and then prepared to work concentration with 40% HBC (2-hydroxypropyl- β -cyclodextrin) in which the final work concentrations of compounds are 10 mg/kg body weight in 200 μ l injection volume and the concentration of DMSO is 10%. Compounds were intraperitoneal (i.p.) injected once daily for five days. Six hours after the fifth injection, 500 mg/kg body weight of APAP solved in PBS was i.p. injected to the mice. 24 hours later, mice were sacrificed. Part of each liver was fixed in 4% paraformaldehyde, and the liver histology characterization was analyzed by haematoxylin and eosin (H&E) staining with paraffin-embedded sections by standard procedures. Other liver tissues were collected for detecting the reduced GSH levels using Reduced Glutathione Kit (Nanjing Jiancheng Bioengineering Institute, China), and the mRNA expression of genes involved in liver repairing, such as *GCLM* and *UGT1a1*, by RT-PCR. *GCLM* is the gene encoding the modifier subunit of the glutamate cysteine ligase GCLM, which is the rate-limiting enzyme in GSH biosynthesis. *UGT1a1* is a uridine diphosphate glucuronosyltransferase (UDP-glucuronosyltransferase, UDPGT), an enzyme of the glucuronidation pathway that transforms small lipophilic molecules, such as steroids, bilirubin, hormones, and drugs, into water-soluble, excretable metabolites. Mutations in this gene cause serious problems for bilirubin metabolism. The serums were collected to measure enzymes activities including AST, ALT, ALP and LDH using kits from Nanjing Jiancheng Bioengineering Institute, China.

For Real-time quantitative PCR (RT-qPCR), Total RNA was isolated using Tissue RNA kit (Omega Bio-Tek, GA). The first strand cDNA was obtained by TAKARA reverse transcription kit. RT-qPCR was performed on a CFX96™ Real-Time PCR Detection System (Bio-Rad) using SYBR Premix Ex Taq™ (TAKARA). Relative mRNA expression levels were normalized to GAPDH levels.

Results: As shown in Figure 2, the pathological sections in control group displayed obvious cell infiltration, vacuolization and necrosis in hepatic lobule. There were a large number of inflammatory cell infiltration, cell turbidity, dissolved karyopycnosis or broken in lobules and portal area. And liver cell cords were also blurred. Compared to the severe liver injury in the control group, pre-treated with vidofludimus, vidofludimus sodium salt and vidofludimus calcium salt obviously protected liver from injury. After the treatment with the compounds, the activities of serum AST, ALT, ALP and LDH were dramatically lower (Table 3), the reduced GSH levels in liver tissues were increased (Table 3), and the expression levels of *GCLM* and *UGT1A1* were significantly upregulated than those in the vehicle control group (Table 3). These results indicated the protective and repairing functions of the compounds in the pre-treatment of APAP-induced liver injury, demonstrating that vidofludimus and its pharmaceutically acceptable salts have therapeutic effects on liver injury.

Soluble oligomeric amyloid beta ($A\beta$) species are now considered to be of major pathological importance in Alzheimer's disease (AD). $A\beta$ -induced oxidative stress can be

due to either an increase in ROS or a decrease in endogenous antioxidants like GSH and the activities of antioxidant enzymes. Up-regulation of reduced GSH may be protective against the oxidative and neurotoxic effects of oligomeric A β . Thus, our results showed that vidofludimus treatment efficaciously increased the level of GSH by upregulating the expression of GCLM, indicating the potential therapeutic roles of our compounds in treating AD.

Table 3. The effects of vidofludimus and its salt forms on the biomarkers for liver health.

		vehicle	Vidofludimus	vidofludimus sodium salt	vidofludimus calcium salt
Serum	AST (U/L)	409 $\pm$ 92	105 $\pm$ 23 ^a	113 $\pm$ 48	122 $\pm$ 35
	ALT (U/L)	679 $\pm$ 151	133 $\pm$ 44 ^a	124 $\pm$ 29	139 $\pm$ 46
	ALP (U/L)	29.1 $\pm$ 1.73	20.8 $\pm$ 3.68 ^a	19.5 $\pm$ 3.3 ^a	18.0 $\pm$ 2.56 ^a
	LDH (U/L)	5458 $\pm$ 191	2466 $\pm$ 850 ^a	NA	NA
Liver	GSH (μ M/g liver)	1.95 $\pm$ 0.35	3.3. $\pm$ 0.5	NA	NA
	mRNA level of <i>GCLM</i>	1.00 $\pm$ 0.08	1.49 $\pm$ 0.22 ^a	NA	NA
	mRNA level of <i>UGT1A1</i>	1.00 $\pm$ 0.05	1.61 $\pm$ 0.24 ^a	NA	NA
	mRNA level of <i>OSTα</i>	1.02 $\pm$ 0.26	2.81 $\pm$ 0.57 ^a	NA	NA

a, p<0.05 (Student's t-test), n=2 per group. NA, not available.

Example 4. Effects of compounds on metabolic diseases mouse model.

Methods: In this example, we used ob/ob mouse (B6/JNju-Lep^{em1Cd25}/Nju) as an obese mouse model, and db/db mouse (BKS.Cg-Dock7m +/+ Leprdb/JNju) as an animal model of type II diabetes. These model mice were used to detect the functions of our compounds on metabolic diseases.

We first selected vidofludimus, its sodium salt and calcium salt to test their metabolic regulatory functions using ob/ob and db/db mice. 10-11 week-old male mice were maintained under environmentally controlled conditions with free access to water. Animal experiments were conducted in the barrier facility of the Laboratory Animal Center, Xiamen University, approved by the Institutional Animal Use and Care Committee of Xiamen University, China. In the example, mice were fed with high-fat diet (HFD, Research Diets, D12492) and treated with either vehicle (40% HBC) or compounds (10 mg/kg body weight) dissolved in vehicle by intra-peritoneally (i.p.) injection once a day for 10 days. After the last compound-treatment, mice were fasted for 6 hours with free access to water, and then sacrificed. Part of each liver was fixed in 4% paraformaldehyde for haematoxylin and eosin (H&E) staining; part of each liver was frozen for oil red O staining, and other liver tissues were stored in liquid nitrogen for triglyceride and cholesterol measurement using Triglyceride Assay Kit and Cholesterol Assay Kit (Applygen, Beijing, China; Nanjing Jiancheng Bioengineering Institute, China). Liver histology characterization was analyzed through H&E staining with paraffin-embedded sections by standard procedures. For oil red O staining, fresh liver tissues were embedded in optimum cutting temperature compound (OCT) and cryosectioned. The sections were fixed in 4% paraformaldehyde in PBS, and were stained with 0.3% oil red O according to standard procedures. The serums were collected to measure serum glucose, cholesterol and triglyceride levels using glucose oxidase method (Applygen, Beijing, China), Cholesterol Assay Kit and Triglyceride Assay

Kit (Nanjing Jiancheng Bioengineering Institute, China), respectively. The serum levels of uric acid (UA) and ALP were measured with kits from Nanjing Jiancheng Bioengineering Institute; the mouse high sensitivity c-reactive protein, hs-crp ELISA KIT (BioLab, Beijing, China) was used to measure the serum CRP level, and the Ultra-Sensitive Mouse Insulin ELISA Kit (Crystal Chem., USA) was used to measure the serum insulin level.

We then selected synthetic compounds to test their regulatory functions on metabolism using ob/ob mouse model. 10-11 week-old male mice were treated with compounds (10 mg/kg body weight) for 14 d as mentioned above in this example. Serum metabolic parameters were measured. Liver and intestine tissues were stored in liquid nitrogen for gene expression analysis by RT-qPCR.

All data are expressed as means $\pm$ s.e.m. Statistical significance was analyzed using Student's t-test. P values less than 0.05 were considered significant.

Results: After compound treatment for 10 days, the liver from mice treated with compounds displayed obvious difference in pathology. As shown in Table 4 and Table 5, the liver/body weight ratios were significantly decreased in compounds treated ob/ob and db/db mice. Importantly, the hepatic triglyceride and cholesterol levels were all significantly decreased in compounds treated mice. Moreover, as shown in Figure 3A and Figure 4A, the histological examination of liver sections obtained from vehicle treated mice showed the extensive existence of vesicular hepatocyte vacuolation, while compounds treatment efficaciously reversed the liver from hepatic steatosis in db/db mice with disappeared hepatic lipid accumulation and showed tight compact structure of the liver cells. The images of liver sections from oil red O staining further confirmed the therapeutic effects of compounds on hepatic lipid accumulation. As shown in Figure 3B and Figure 4B, liver sections from vehicle treated mice showed abundant lipid accumulation, especially containing many large lipid droplets, while liver sections from mice treated with compounds dramatically reduced the lipid accumulation. Patients suffering from non-alcoholic fatty liver disease (NAFLD) display a variety of hepatic dysfunctions, ranging from abnormal triglyceride accumulation in hepatocytes (steatosis) to steatohepatitis (non-alcoholic steatohepatitis, NASH) with fibrosis. Our results demonstrate that our compounds are efficacious drug candidates for treating fatty liver diseases, such as NAFLD and NASH. These data also indicate the therapeutic effects of our compounds on obesity.

Table 4. The effects of vidofludimus and its salt forms on the metabolic parameters of HFD-fed ob/ob mice.

	Vehicle	Vidofludimus	vidofludimus sodium salt	vidofludimus calcium salt
Liver/body weight (%)	5.96 $\pm$ 0.48	4.97 $\pm$ 0.31 ^a	4.78 $\pm$ 0.5 ^a	4.85 $\pm$ 0.28 ^a
Liver triglyceride (mg/g liver)	163.1 $\pm$ 19.6	128.2 $\pm$ 13.7 ^a	124.0 $\pm$ 17.1 ^a	115.1 $\pm$ 21.3 ^a
Liver cholesterol (mg/g liver)	10.35 $\pm$ 2.11	5.59 $\pm$ 0.45 ^b	5.41 $\pm$ 0.79 ^a	5.27 $\pm$ 1.02 ^a
Serum triglyceride (mg/dL)	156.0 $\pm$ 25.1	106.7 $\pm$ 16.7 ^a	110.4 $\pm$ 15.1 ^a	94.6 $\pm$ 22.5 ^a
Serum cholesterol (mg/dL)	152.9 $\pm$ 30.1	94.3 $\pm$ 12.9 ^a	105.3 $\pm$ 18.2 ^a	84.2 $\pm$ 22.7 ^a
Serum UA (μ M)	175.3 $\pm$ 33.7	89.8 $\pm$ 31.4 ^a	103.4 $\pm$ 26 ^a	96.6 $\pm$ 33.5 ^a
Serum BUN (mM)	11.18 $\pm$ 0.83	7.66 $\pm$ 1.15 ^a	8.21 $\pm$ 0.43 ^a	7.24 $\pm$ 1.24 ^a

a, $p < 0.05$; b, $p < 0.01$ (Student's t-test), $n = 3-4$ per group

Table 5. The effects of vidofludimus and its salt forms on the metabolic parameters of

615 HFD-fed db/db mice.

	Vehicle	Vidofludimus	vidofludimu s sodium salt	vidofludimus calcium salt
Liver/body weight (%)	6.41±0.92	4.56±0.35 ^a	4.81±0.29 ^a	4.37±0.56 ^a
Liver triglyceride (mg/g liver)	77.3±18.8	31.7±11.0 ^b	35.2±10.3 ^a	29.4±14.4 ^a
Liver cholesterol (mg/g liver)	6.98±0.75	5.33±1.10 ^a	5.7±0.5 ^a	5.2±0.9 ^a
Serum glucose (mg/dL)	514.4±32.1	436.2±17.7 ^b	442.1±15.4 ^b	415.3±22.2 ^b
Serum cholesterol (mg/dL)	200.0±11.7	89.5±28.6 ^c	110.1±25.4 ^c	82.4±31.6 ^c
Serum BUN (mM)	6.11±0.85	2.25±0.38 ^c	2.77±0.42 ^c	2.41±0.45 ^c

a, p<0.05; b, p<0.01; c, p<0.001 (Student's t-test), n=3-4 per group

Table 6. The treatment of compounds down-regulated the serum metabolic parameters in HFD-fed ob/ob mice.

Compound	Triglyceride (mg/dL)	Glucose (mg/dL)	Cholesterol (mg/dL)	Compound	Triglyceride (mg/dL)	Glucose (mg/dL)	Cholesterol (mg/dL)
Vehicle	146.1±13.2	12.9±0.2	166.8±11.9	FD10	107.5±31.3	10.0±0.7 ^b	126.6±5.1
FD1	74±1.7 ^b	9.9±2.5 ^a	114.5±13.9 ^a	FD11	88.5±18.6 ^a	7.5±1.0 ^c	105.8±2.1
FD2	69.6±6.8 ^b	7.1±0.1 ^c	129.7	FD12	78.8±5.1 ^b	7.6±0.2 ^c	136.5±6.1
FD5	86±4.0 ^b	9.4±0.7 ^b	120.9±15.4 ^a				

620 a, p<0.05; b, p<0.01; c, p<0.001 (Student's t-test), n=1-3 per group.

Table 7. The compounds regulated mRNA levels of genes related to metabolism in liver of HFD-fed ob/ob mice.

Compound	PPARα	SREBP1C	G6PC	Compound	PPARα	SREBP1C	G6PC
Vehicle	1.00±0.22	1.00±0.21	1.00±0.15	FD9	1.28±0.07	0.23±0.13 ^a	0.26±0.19 ^a
FD1	2.28±0.11 ^a	0.45±0.17 ^a	0.39±0.13 ^a	FD10	2.34±0.08 ^b	0.34±0.14 ^a	0.23±0.14 ^a
FD2	1.52±0.05 ^a	0.28±0.14 ^a	0.26±0.16 ^a	FD11	1.97±0.03 ^a	0.40±0.17 ^a	0.32±0.16 ^a
FD3	2.51±0.24 ^a	0.53±0.23 ^a	0.58±0.25	FD12	1.74±0.10 ^a	0.36±0.08 ^a	0.40±0.11 ^a
FD5	1.69±0.27	0.47±0.19 ^a	0.31±0.17 ^a	FD13	2.19±0.10 ^a	0.51±0.22 ^a	0.44±0.21 ^a

a, p<0.05; b, p<0.01 (Student's t-test), n=2-3 per group

625

The serum cholesterol levels were significantly lowered in compounds treated ob/ob and db/db mice (Table 4, Table 5 & Table 6, indicating the therapeutic effects of the compounds on hypercholesterolemia. The serum triglyceride levels were significantly lowered in compounds treated ob/ob mice (Table 4 and Table 6), indicating the therapeutic effects of the compounds on hypertriglyceridemia. The serum glucose levels were significantly lowered in compounds treated mice (Table 5 and Table 6), indicating the therapeutic effects of our compounds on hyperglycemia and diabetes.

630

High levels of blood triglycerides and glucose are the alert indicators of cardiovascular disease. These indicators reflect the high risk for development of cardiovascular disease. In this example, the compounds treatment significantly decreased the blood levels of glucose and triglyceride, indicating their therapeutic effects on cardiovascular diseases.

635

Studies demonstrated that high total cholesterol level is positively correlated with the degree of carotid atherosclerotic plaque lesions. In this example, compounds treatment

efficaciously decreased the serum cholesterol levels, indicating their therapeutic effects on atherosclerosis.

PPAR α controls lipid flux in the liver by modulating FA transport and β -oxidation, and improves plasma lipid profiles by decreasing triglyceride (TG) levels and increasing high-density lipoprotein cholesterol levels. In addition, PPAR α activation inhibits inflammatory genes induced by NF- κ B, and decreases the expression of acute-phase response genes. Accordingly, PPAR α deficiency increases susceptibility to NAFLD, NASH, hepatic inflammation and acute phase responses (see e.g., Ip E., et al., (2003) Hepatology; 38: 123–132). It has been reported that expression of PPAR α in human liver is reduced in patients with non-alcoholic steatohepatitis or infected with the hepatitis C virus. PPAR α effectively induces the expression of numerous genes involved in lipid metabolic pathways, including fatty acid elongation and desaturation, synthesis and breakdown of triglycerides and lipid droplets, gluconeogenesis, bile acid metabolism (see e.g., Kersten S. and Stienstra R., (2017) Biochimie. 136:75-84). SREBP-1c is responsible for regulating the genes required for de novo lipogenesis. G6PC is a key enzyme in glucose homeostasis. As shown in Table 7, our compounds increased the expression of PPAR α and decreased the expression of SREBP1c and G6PC in liver.

Table 8. The treatment of compounds regulated mRNA levels of collagens in liver.

Compound	α 1(I)collagen	α 2(I)collagen	Compound	α 1(I)collagen	α 2(I)collagen
Vehicle	1.00 $\pm$ 0.18	1.00 $\pm$ 0.14	FD11	0.52 $\pm$ 0.17 ^a	0.73 $\pm$ 0.19
FD2	0.53 $\pm$ 0.14 ^a	0.52 $\pm$ 0.16 ^a	FD12	0.35 $\pm$ 0.14 ^a	0.37 $\pm$ 0.16 ^a
FD3	0.37 $\pm$ 0.17 ^a	0.45 $\pm$ 0.17 ^a	FD13	0.36 $\pm$ 0.17 ^a	0.48 $\pm$ 0.14 ^a

a, p<0.05 (Student's t-test), n=2-3 per group

NAFLD with excessive fat accumulation in liver will affect the blood and oxygen supplies to liver and the metabolism of liver organ, resulting in amounts of cell swelling, inflammatory infiltration and necrosis in liver. Once fibrosis and false lobules appear, cirrhosis will happen and the risk of liver cancer will be greatly increased. The levels of various collagen contents are higher in patients with liver cirrhosis. As shown in Table 8, the compounds decreased the mRNA levels of α 1(I)collagen and α 2(I)collagen, suggesting their effects in preventing NAFLD and cirrhosis.

Table 9. The treatment of compounds regulated FXR target genes and inflammatory genes.

Compound	Relative mRNA levels in liver			Relative mRNA levels in intestine		
	α -SMA	IL-6	IL-1 β	FGF15	IL-1 β	IL6
Vehicle	1.00 $\pm$ 0.19	1.00 $\pm$ 0.20	1.00 $\pm$ 0.27	1.03 $\pm$ 0.34	1.00 $\pm$ 0.27	1.00 $\pm$ 0.22
FD2	0.70 $\pm$ 0.14	0.74 $\pm$ 0.21	0.73 $\pm$ 0.35	5.38 $\pm$ 0.74 ^a	0.81 $\pm$ 0.07	0.04 $\pm$ 0.01 ^a
FD3	0.41 $\pm$ 0.23 ^a	0.85 $\pm$ 0.22	0.35 $\pm$ 0.27 ^a	3.66 $\pm$ 0.78	0.66 $\pm$ 0.15	0.07 $\pm$ 0.02 ^a
FD5	0.58 $\pm$ 0.19	0.815 $\pm$ 0.24	0.59 $\pm$ 0.44	2.64 $\pm$ 0.44 ^a	0.59 $\pm$ 0.14	0.08 $\pm$ 0.03 ^a
FD9	0.82 $\pm$ 0.15	0.68 $\pm$ 0.15	0.38 $\pm$ 0.10 ^a	2.79 $\pm$ 0.40 ^a	0.68 $\pm$ 0.14	0.11 $\pm$ 0.02 ^a

FD10	0.47±0.16 ^a	0.64±0.14	0.48±0.17 ^a	4.21±1.11	0.88±0.10	0.05±0.01 ^a
FD12	0.42±0.11 ^a	0.77±0.13	0.31±0.12 ^a	5.73±1.44	0.31±0.11 ^a	0.04±0.01 ^a
FD13	0.45±0.15 ^a	0.46±0.12 ^a	0.41±0.19 ^a	2.71±0.52	0.72±0.17	0.06±0.02 ^a

a, p<0.05 (Student's t-test), n=2-3 per group

FGF15 plays an important role in feedback inhibition of hepatic bile acid synthesis. FXR directly binds to the response element of the promoter of FGF15 and regulates its expression. As shown in Table 9, the synthetic compounds increased the mRNA levels of FGF15, and efficaciously lowered the mRNA levels of inflammatory genes in both liver and intestine tissues, indicating their therapeutic effects on hepatitis and enteritis.

Table 10. The treatment of compounds decreased the levels of UA and CRP.

Compound	UA (μM)	CRP (μg/mL)	Compound	UA (μM)	CRP (μg/mL)
Vehicle	76.1±3.1	0.148±0.006	FD5	50±9.2	0.089±0.024 ^a
FD2	63.0±3.1 ^a	0.080±0.005 ^c	FD12	47.8±12.3	0.074±0.016 ^b
FD3	39.1	0.082	FD13	50±3.1 ^a	0.080±0.016 ^b

a, p<0.05; b, p<0.01; c, p<0.001 (Student's t-test), n=1-3

per group

High blood concentrations of uric acid can lead to gout and are associated with other medical conditions including diabetes and the formation of ammonium acid urate kidney stones. C-reactive protein (CRP) is synthesized by the liver in response to factors released by macrophages and fat cells (adipocytes) (see e.g. Lau DC et al., (2005) Am J Physiol Heart Circ Physiol. 288: H2031–41.). Elevated levels of CRP are commonly found in gouty arthritis. As shown in Table 4 & 10, our compounds decreased the serum level of UA and CRP, indicating the potential therapeutic effects of these compounds in treating gout and gouty arthritis.

Table 11. The treatment of compounds decreased serum ALP level in HFD-fed ob/ob mice. (n=2-3 per group)

Compound	ALP (U/L)	Compound	ALP (U/L)
Vehicle	38.5±6.8	FD9	28.8±4.9
FD1	30.1±0.1	FD10	30.3±2.9
FD2	31.3±1.4	FD11	27.7±6.5

Except in liver disease or hepatitis, elevated ALP is also commonly found in diseases and conditions including biliary obstruction, bone conditions, osteoblastic bone tumors, osteomalacia, osteoporosis, etc. As shown in Table 11, there is a trend for some of the synthetic compounds to decrease the serum levels of ALP. These data suggested that our compounds might be curative in treating bone conditions including osteoporosis.

Table 12. The treatment of compounds decreased serum BUN levels in HFD-fed ob/ob mice. (n=2-3 per group)

Compound	BUN (mM)	Compound	BUN (mM)
Vehicle	5.94±0.55	FD5	4.60±1.04
FD1	4.77±0.03	FD10	5.45±0.44
FD3	4.89±0.27	FD12	4.73±0.33

The blood urea nitrogen (BUN) concentration will increase rapidly when the filtration ratio in glomerular decrease lower than 50%. Various renal parenchymal diseases, including glomerulonephritis, interstitial nephritis, acute and chronic renal failure, renal lesions and renal destructive lesions, can increase the BUN levels. Therefore, BUN is a main indicator for kidney function, as well as the uremia. The BUN levels were measured with the Urea Assay kit (Nanjing Jiancheng Bioengineering Institute, China) in this example, and the results showed that the compounds decreased the BUN levels in HFD-fed ob/ob mice (Table 4, 5 & 12), suggesting the therapeutic effects of our compounds on various kidney diseases including glomerulonephritis, interstitial nephritis, acute and chronic renal failure, renal lesions, renal destructive lesions and uremia that have increased BUN.

Example 5. Effects of compounds on ob/ob mice administrated by oral gavage.

Methods: Compounds were prepared to work concentration with 0.5% (w/v) carboxymethylcellulose (CMC-Na) in PBS. 7-8 week-old male ob/ob mice were treated with vidofludimus (5 mg/kg body weight), other compounds (20 mg/kg body weight), or 0.5% CMC-Na as vehicle controls by daily oral gavage for 4 weeks. Liver tissues and serum samples were collected for metabolic parameters measurement as mentioned in example 4. The serum level of free fatty acids (FFA) was measured with kits from Nanjing Jiancheng Bioengineering Institute.

Results: As shown in Table 13, compounds treatment by orally gavage decreased the activities of AST and ALT, indicating the safety of these compounds to liver functions, and also their hepatoprotective functions.

Table 13. The effects of compounds on the serum AST and ALT.

Compound	AST (U/L)	ALT (U/L)	Compound	AST (U/L)	ALT (U/L)
Vehicle	73.15±10.13	89.35±2.56	FD13	61.67±5.11	73.82±9.17 ^a
FD6	42.54	52.66	FD20	54.69	72.25
FD9	47.60±8.12	64.82±8.59 ^a	FD29	39.16±5.73 ^a	47.60±6.21 ^b
FD10	36.80±4.30 ^a	46.59±0.95 ^c	FD31	55.03±11.94	65.49±7.64 ^a
FD11	57.39	74.27	vidofludimus	50.98±20.53	69.88±28.17
FD12	53.00±0.48	67.18±7.16 ^a			

a, p<0.05; b, p<0.01; c, p<0.001 (Student's t-test). n=1-3 per group.

The levels of the serum FFA (Table 14), triglycerides (Table 15), UA (Table 16) and the hepatic level of total cholesterol (Table 17) of mice were reduced in the compounds treated mice, and the mRNA expression levels of inflammatory factors (Table 18) in liver tissues were down-regulated in the compounds treated mice. As discussed in example 4, these data suggest that our compounds are potential drug candidates for treating diseases such as fatty liver diseases, hypertriglyceridemia, hepatitis, gout and gouty arthritis. This example also indicates that orally administrated with our compounds are also efficacious in treating metabolic diseases.

Table 14. The effects of compounds on the serum level of FFA.

Compound	FFA (mM)	Compound	FFA (mM)
Vehicle	1.94±0.06	FD12	1.72±0.08 ^a
FD4	1.22	FD13	1.63±0.06 ^b
FD6	1.17	FD20	1.35±0.26 ^a

FD9	1.74±0.06 ^a	FD29	0.83
FD10	1.39±0.39	FD31	1.25±0.39 ^a
FD11	1.57±0.61	vidofludimus	1.32±0.06 ^b

a, p<0.05; b, p<0.01 (Student's t-test). n=1-3 per group.

Table 15. The effects of compounds on the serum level of triglycerides. (n=2-3 per group)

Compound	Triglycerides (mg/dL)	Compound	Triglycerides (mg/dL)
Vehicle	129.56±11.09	FD29	108.86±3.62
FD9	108.86±1.03	FD20	111.78±0.52
FD10	109.95±5.17	vidofludimus	116.34±6.97

Table 16. The effects of compounds on the serum level of UA. (n=2-3 per group)

Compound	UA (mM)	Compound	UA (mM)
Vehicle	220.88±25.98	FD29	175.76±38.01
FD20	123.23±25.71	FD31	135.35±82.85

Table 17. The effects of compounds on the level of hepatic cholesterol.

Compound	Total Cholesterol (mg/g)	Compound	Total Cholesterol (mg/g)
Vehicle	4.18±1.11	FD13	1.71±0.26 ^a
FD6	2.89	FD20	2.63±0.19
FD9	2.26±0.05	FD29	3.19±1.41
FD10	2.53±0.38	FD31	1.58±0.07 ^a
FD12	1.92±0.64	vidofludimus	2.35±0.26

a, p<0.05 (Student's t-test). n=1-3 per group.

Table 18. The effects of compounds on the mRNA levels of inflammatory factors in liver tissues.

Compound	α -SMA	IL-1 β	Compound	α -SMA	IL-1 β
Vehicle	1.06±0.21	1.00±0.25	FD12	0.35±0.16 ^a	0.48±0.27
FD4	0.37	0.77	FD13	0.38±0.21 ^a	0.57±0.23
FD6	0.42	0.49	FD20	0.60±0.25	0.46±0.19 ^a
FD9	0.54±0.34	0.38±0.19 ^a	FD29	0.32±0.19 ^a	0.55±0.23 ^a
FD10	0.27	0.46±0.21	FD31	0.34±0.19 ^a	0.66±0.17
FD11	0.42±0.18 ^a	0.38±0.16 ^a	Vidofludimus	0.32±0.22 ^a	0.53±0.24

a, p<0.05 (Student's t-test). n=1-3 per group.

Example 6. Therapeutic effects on DSS-induced enteritis.

Methods: Dextran sodium sulfate (DSS) challenge in mice causes intestinal inflammation and injury that resembles human colitis (see e.g. Wirtz et al., (2007) Nature protocols 2, 541-546). In this example, mouse model with DSS-induced intestinal inflammation was used to test the therapeutic effects of our compounds in treating enteritis.

Compounds were prepared to work concentration with 0.5% (w/v)

carboxymethylcellulose (CMC-Na) in PBS. 8-9 week-old male wild-type mice were treated with compounds (20 mg/kg body weight) or 0.5% CMC-Na as vehicle controls by daily oral gavage for 13 days. Mice received 2.5% (w/v) DSS in drinking water on days 4-13. Ilea were collected for measurement of the inflammatory genes expression, and colons were fixed in 10% formalin and embedded in paraffin for H&E staining.

Results: As shown in Figure 5, DSS challenge to mice resulted in goblet cell loss, colonic epithelial damage and ulceration. Compared with the vehicle control, compounds treatment obviously improved the colonic epithelial damage and ulceration, with much less goblet cell loss, which is consistent with the larger ratio of final/initial body weight of mice with compounds treatment (Table 19).

Inflammatory cytokines are produced during inflammation, and thus are markers of the inflammatory reaction. In this example, we detected the levels of various inflammatory cytokines including COX2, IL-1 β , IL-17 and MIP-1 α in ilea from the treated mice. As shown in Table 20, compounds treatment decreased the inflammatory cytokines levels in DSS-induced colitis models, demonstrating the therapeutic effects of compounds in treating enteritis.

Table 19. Compounds improved final/initial body weight ratio.

Compound	Ratio of final/initial body weight (%)	Compound	Ratio of final/initial body weight (%)
Vehicle	70.0 $\pm$ 2.2	FD6	86.3 $\pm$ 2.5 ^a
FD1	81.5	FD9	75.0 $\pm$ 9.1
FD2	83.4 $\pm$ 6.1	FD10	77.9 $\pm$ 2.0 ^a
FD3	84.9 $\pm$ 7.3	FD11	74.8 $\pm$ 7.21
FD4	81.7 $\pm$ 12.7	FD12	118.4 $\pm$ 10.9 ^a
FD5	83.4 $\pm$ 2.2 ^c	FD13	77.5 $\pm$ 4.5

a, p<0.05; c, p<0.001 (Student's t-test), n=2-3 per

group

Table 20. Compounds regulated mRNA expression of FXR target gene and inflammatory factors.

Compound	FGF15	COX2	MCPI	IL-17	IL-1 β
Vehicle	1.66 $\pm$ 0.31	17.62 $\pm$ 2.52	10.10 $\pm$ 1.60	20.39 $\pm$ 4.19	5.16 $\pm$ 0.55
FD1	2.41 $\pm$ 0.40	13.65 $\pm$ 1.58	4.97 $\pm$ 1.05 ^a	11.75 $\pm$ 2.14 ^a	3.08 $\pm$ 0.79 ^a
FD3	193.69 $\pm$ 8.32 ^a	9.14 $\pm$ 1.85 ^a	2.27 $\pm$ 0.42 ^b	9.80 $\pm$ 1.04 ^a	4.00 $\pm$ 0.26
FD12	67.19 $\pm$ 9.25 ^a	3.25 $\pm$ 0.76 ^c	4.45 $\pm$ 0.99 ^c	2.20 $\pm$ 1.04 ^c	3.50 $\pm$ 0.56 ^b
FD13	19.83 $\pm$ 3.78 ^a	4.37 $\pm$ 1.01 ^a	1.48 $\pm$ 0.39 ^b	1.89 $\pm$ 0.90 ^b	1.31 $\pm$ 0.16 ^b

a, p<0.05; b, p<0.01; c, p<0.001 (Student's t-test), n=2-3 per group

Example 7. Therapeutic effects on arthritis.

Methods: As mentioned above in the BACKGROUND, TNF α secreted from the macrophages has becoming an indicator of activity in arthritis and a target for treating arthritis. In this example, 8-week-old male C57BL/6J mice were injected with sterile thioglycolic acid broth into the peritoneum. 3 days after injection, mice were sacrificed and injected with 10 mL of RPMI-1640 medium containing 5% FBS into the peritoneum to harvest peritoneal macrophages. Collected cells were centrifuged at 160 $\times$ g for 5 min, and the cell pellet was washed with PBS and centrifuged again. The cell pellet was then suspended in RPMI-1640 medium supplemented with 100 U/mL of penicillin, 100 μ g/mL

of streptomycin and 10% FBS, and plated into 12-well plates. After incubation at 37 °C for 3 h, the cells were washed with PBS three times to remove unattached cells such as neutrophils, and then cultured in the same RPMI-1640 medium. 24 h after the cells were plated into the wells, 10 uM of compounds were added into the culture medium. Additional 2 h later, 0.5 ug/mL of LPS were added into the culture medium, and cells were harvested in 22 h. The media were collected and the secreted TNF α in the medium were detected using the Mouse TNF α ELISA kit (NeoBioscience).

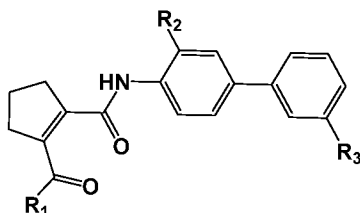
Results: As shown in Table 21, following the stimulation with LPS alone, peritoneal macrophages produced high level of TNF α . In combined with our compounds treatment, the production of TNF α were obviously suppressed, suggesting the roles of compounds in treating arthritis, such as RA, OA, JIA and so on.

Table 21. Compounds suppressed the production of TNF α in LPS-induced macrophages.

Compound treatment		Relative level of TNF α (%)	Compound treatment	Relative level of TNF α (%)
Vehicle		8.1	Vehicle	8.1
LPS	Vehicle	100	Vidofludimus sodium salt	78.2
	GW4064	64.6	Vidofludimus calcium salt	74.7
	OCA	68.7	FD9	83.4
	FD-1	83.1	FD10	72.2
	FD-2	84.4	FD11	70.4
	FD-3	74.8	FD12	84.4
	FD-4	88.6	FD13	83.3
	FD-5	72.8	FD20	59.5
	FD-6	68.9	FD29	58.3

What is claimed:

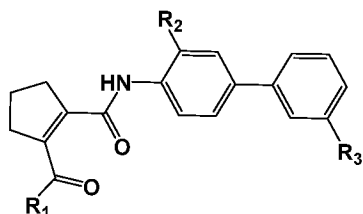
1. A method for treating an FXR-mediated process or disease in a mammal, comprising administering to the mammal a therapeutically effective amount of at least one compound having the formula:



or in a pharmaceutically acceptable carrier and/or diluent form thereof, wherein R1 is independently selected from hydrogen, hydroxy, methoxy, ethoxy, amino, methylamine, dimethylamine, trimethylamine, lower amines, or lower alkoxy group; R2 is independently selected from hydrogen, halogen, sulfur, or lower alkyl group; and R3 is methoxy, methyl, ethyl, propyl, 1-methylethyl, non-isomeric cyclopropyl, lower alkyl group or other hydrophobic groups.

2, The compound according to claim 1, wherein R1 is hydroxyl; R2 is independently selected from halogen; and R3 is a methoxy group.

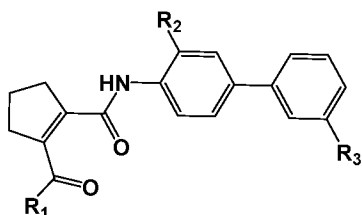
3, A pharmaceutical composition comprising a pharmaceutical acceptable vehicle and at least one compound having the formula:



or in a pharmaceutically acceptable carrier and/or diluent form thereof, wherein R1 is independently selected from amino, methylamine, dimethylamine, trimethylamine, or ethoxy group; R2 is independently selected from hydrogen, halogen, or lower alkyl group; and R3 is methoxyl, methyl, ethyl, propyl, 1-methylethyl, non-isomeric cyclopropyl, lower alkyl group or other hydrophobic groups.

4. The compound according to claim 3, wherein R1 is independently selected from amino, methylamine, or ethoxy group; R2 is independently selected from halogen; and R3 is methoxyl, methyl, ethyl, or propyl.

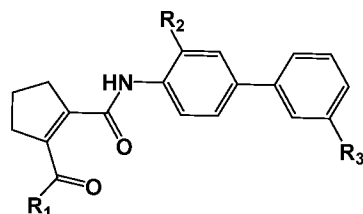
5, A pharmaceutical composition comprising a pharmaceutical acceptable vehicle and at least one compound having the formula:



or in a pharmaceutically acceptable carrier and/or diluent form thereof, wherein R1 is independently selected from hydroxyl or methoxyl group; R2 is independently selected from hydrogen, halogen, or lower alkyl group; R3 is methyl, ethyl, propyl, 1-methylethyl, non-isomeric cyclopropyl, lower alkyl group or other hydrophobic groups.

6, The compound according to claim 5, wherein R1 is hydroxyl; R2 is independently selected from halogen; and R3 is methyl, ethyl, or 1-methylethyl.

- 35 7, A pharmaceutical composition comprising a pharmaceutical acceptable vehicle and at least one compound having the formula:



or in a pharmaceutically acceptable carrier and/or diluent form thereof, wherein R1 is independently selected from hydrogen, hydroxy, methoxy, ethoxy, amino, methylamine, dimethylamine, trimethylamine, lower amines, or lower alkoxy group; R2 is independently selected from hydrogen, or lower alkyl group; and R3 is methoxyl, methyl, ethyl, propyl, 1-methylethyl, non-isomeric cyclopropyl, lower alkyl group or other hydrophobic groups.

8, The compound according to claim 7, wherein R1 is hydroxyl, methoxyl, amino, or methylamine; R2 is a methyl group; and R3 is methoxyl, methyl, ethyl, or 1-methylethyl.

- 45 9. The compound according to claim 3, 5 or 7 selected from the group consisting of the following:

N-(3-fluoro-3'-methoxy-[1,1'-biphenyl]-4-yl)cyclopent-1-ene-1,2-dicarboxamide
N¹-(3-fluoro-3'-methoxy-[1,1'-biphenyl]-4-yl)-N²-methylcyclopent-1-ene-1,2-dicarboxamide

- 50 ethyl

2-((3-fluoro-3'-methoxy-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylate
methyl

2-((3-fluoro-3'-methoxy-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylate

2-((3'-ethoxy-3-fluoro-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid

- 55 2-((3-fluoro-3'-methyl-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid;

2-((3'-ethyl-3-fluoro-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid;

2-((3-fluoro-3'-isopropyl-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid;

2-((3'-methoxy-3-methyl-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid;

- 60 2-((3-chloro-3'-methoxy-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid;

2-((3'-ethyl-3-methyl-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid;

2-((3,3'-dimethyl-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid;

2-((3'-isopropyl-3-methyl-[1,1'-biphenyl]-4-yl)carbamoyl)cyclopent-1-ene-1-carboxylic acid.

- 65 10. A method for treating an FXR-mediated process or disease in a mammal, comprising administering to the mammal a therapeutically effective amount of at least one compound of claims 2-9.

- 11, The method of claim 1 or 10, wherein the FXR-mediated process or disease is selected from: cholestasis; colitis; Alzheimer's disease; a chronic liver disease selected from primary biliary cirrhosis, primary sclerosing cholangitis, nonalcoholic fatty liver disease, and nonalcoholic steatohepatitis; a gastrointestinal disease selected from inflammatory bowel disease, irritable bowel syndrome, bacterial over-growth, and malabsorption; a cardiovascular disease selected from atherosclerosis, arteriosclerosis, dyslipidemia, hypercholesterolemia, and hypertriglyceridemia; a metabolic disease selected from insulin resistance, hyperglycemia, Type I and Type II diabetes, and obesity; a disorder related to bone formation such as osteoporosis, bone hyperplasia and osteoarthritis; an autoimmune

and inflammatory disease selected from rheumatoid arthritis, tendinitis or bursitis, fibromyalgia, muscular low back pain, chronic obstructive pulmonary disease (COPD), psoriasis, pelvic inflammatory disease (PID), asthma, pneumonia, polymyalgia rheumatica and gout; and a kidney disease selected from diabetic nephropathy, focal segmental glomerulosclerosis, chronic glomerulonephritis, interstitial nephritis, acute and chronic renal failure, renal lesions, renal destructive lesions and uremia.

12, A method for liver protection or treatment of hepatic injury comprising administering to the mammal a therapeutically effective amount of a compound of any one of claims 1-9.

13, A method for lowering alanine aminotransferase (ALT), aspartate aminotransferase (AST) or alkaline phosphatase (ALP) comprising administering to the mammal a therapeutically effective amount of a compound of any one of claims 1-9.

14, A method for lowering triglyceride comprising administering to the mammal a therapeutically effective amount of a compound of any one of claims 1-9.

15, A method for treating nonalcoholic fatty liver disease, nonalcoholic steatohepatitis or atherosclerosis comprising administering to the mammal a therapeutically effective amount of a compound of any one of claims 1-9.

16, A method for treating inflammatory bowel disease or rheumatoid arthritis comprising administering to the mammal a therapeutically effective amount of a compound of any one of claims 3-9.

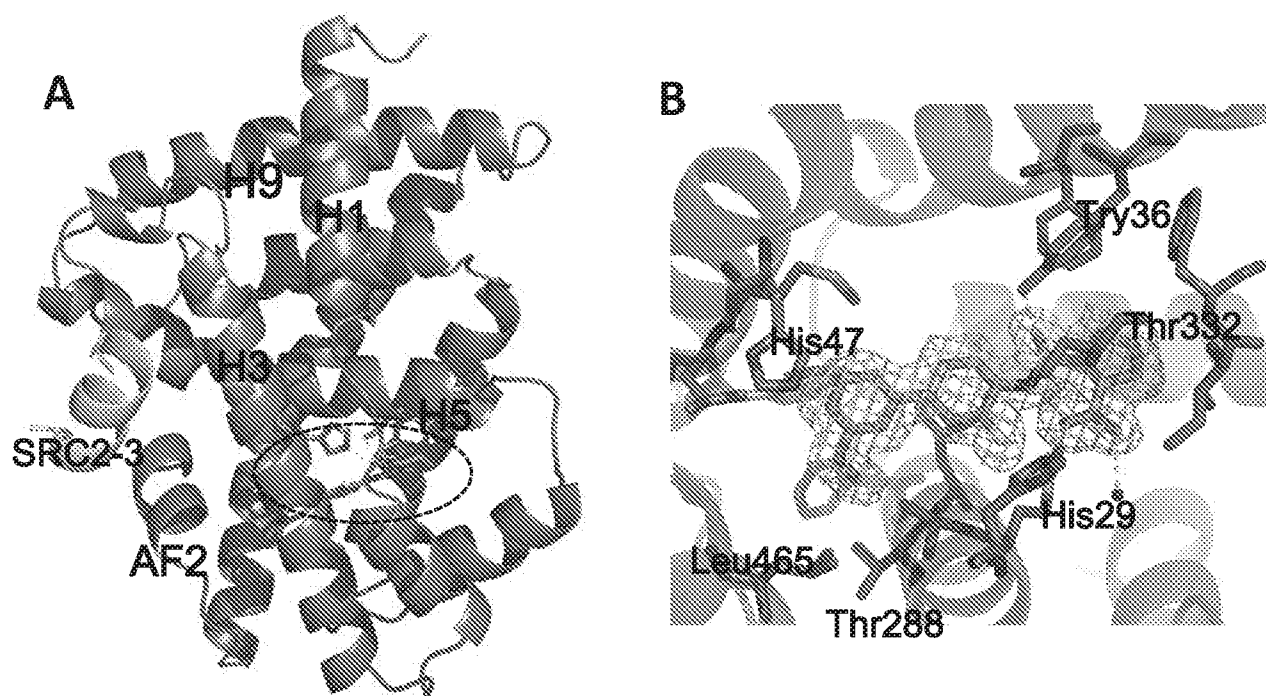


Fig. 1

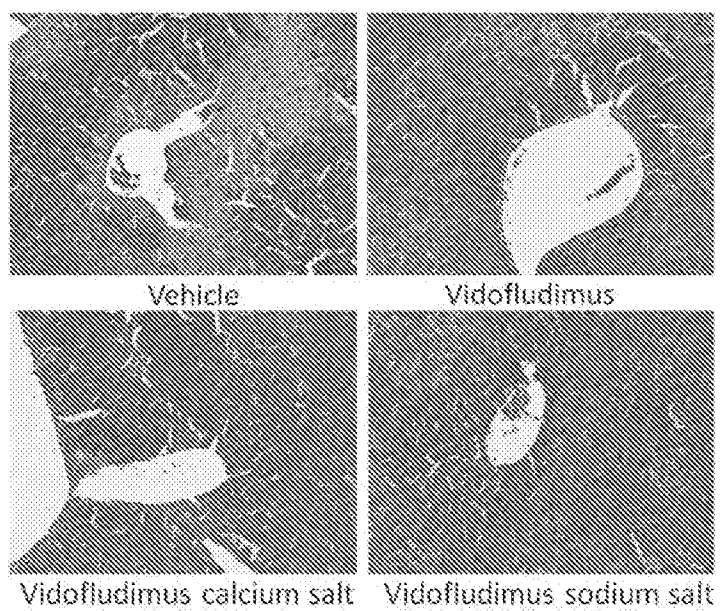


Fig. 2

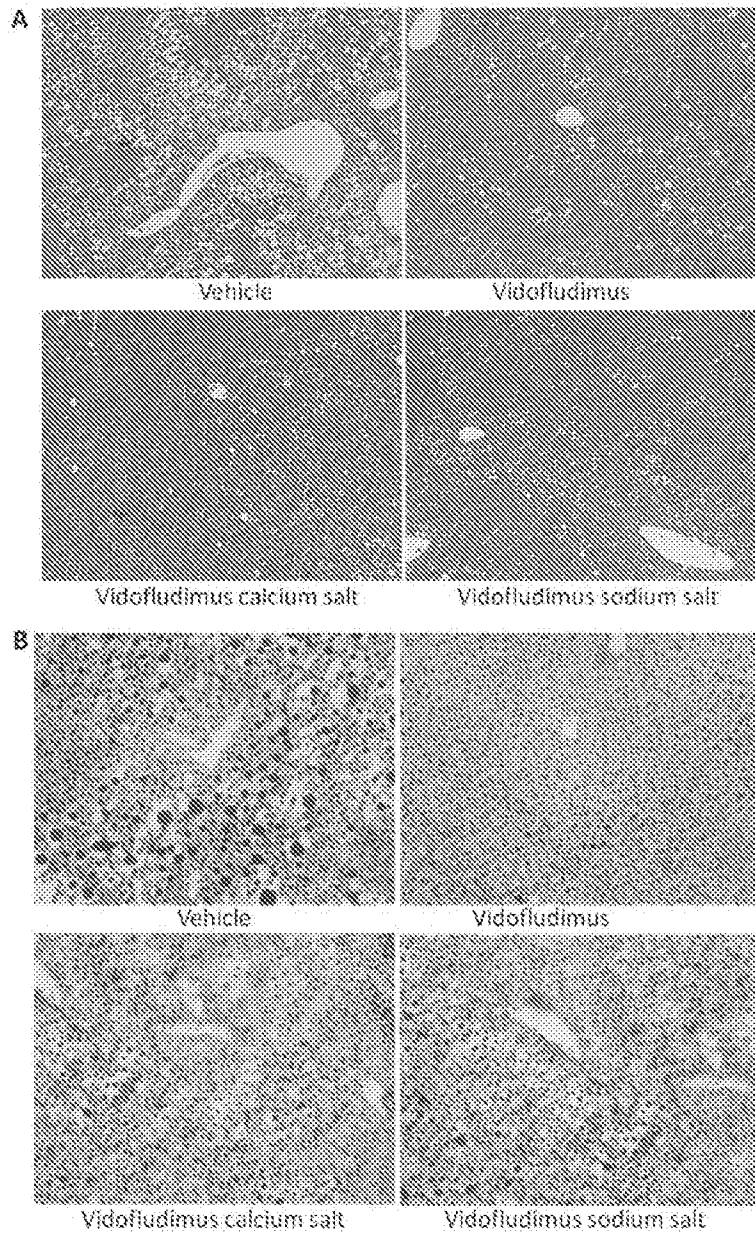


Fig.3

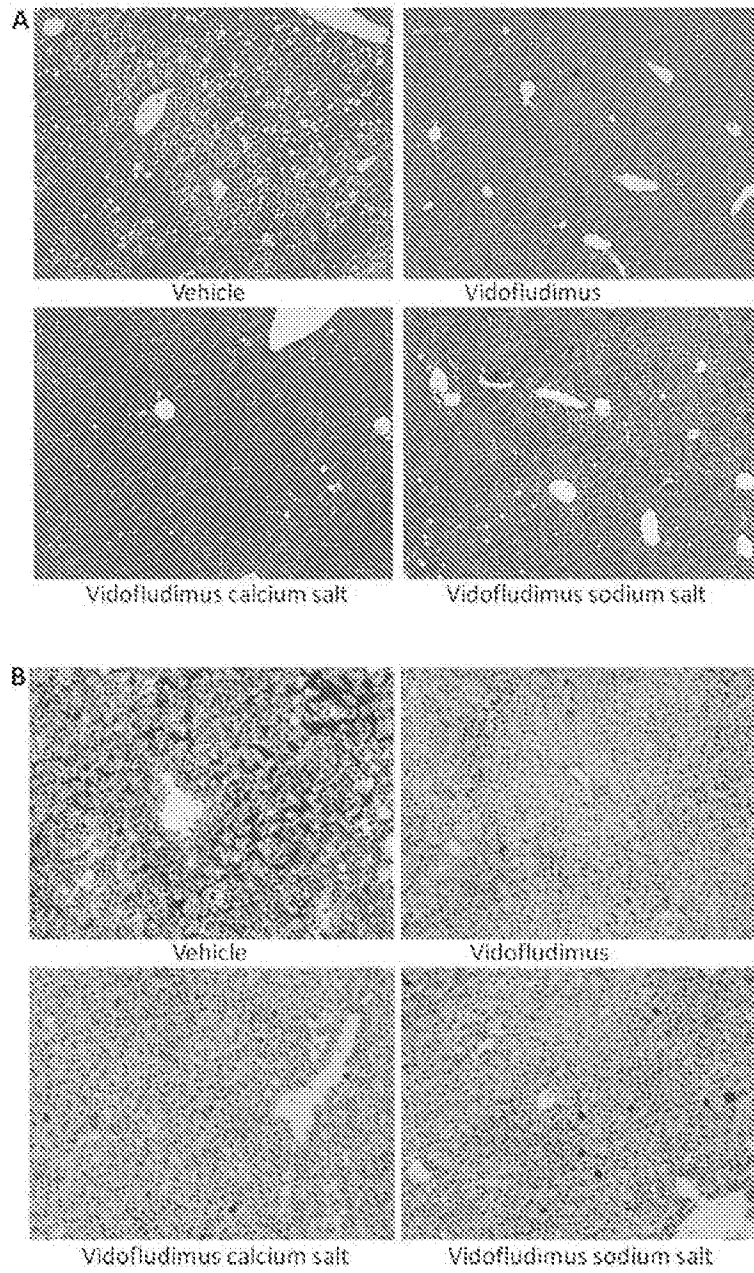


Fig. 4.

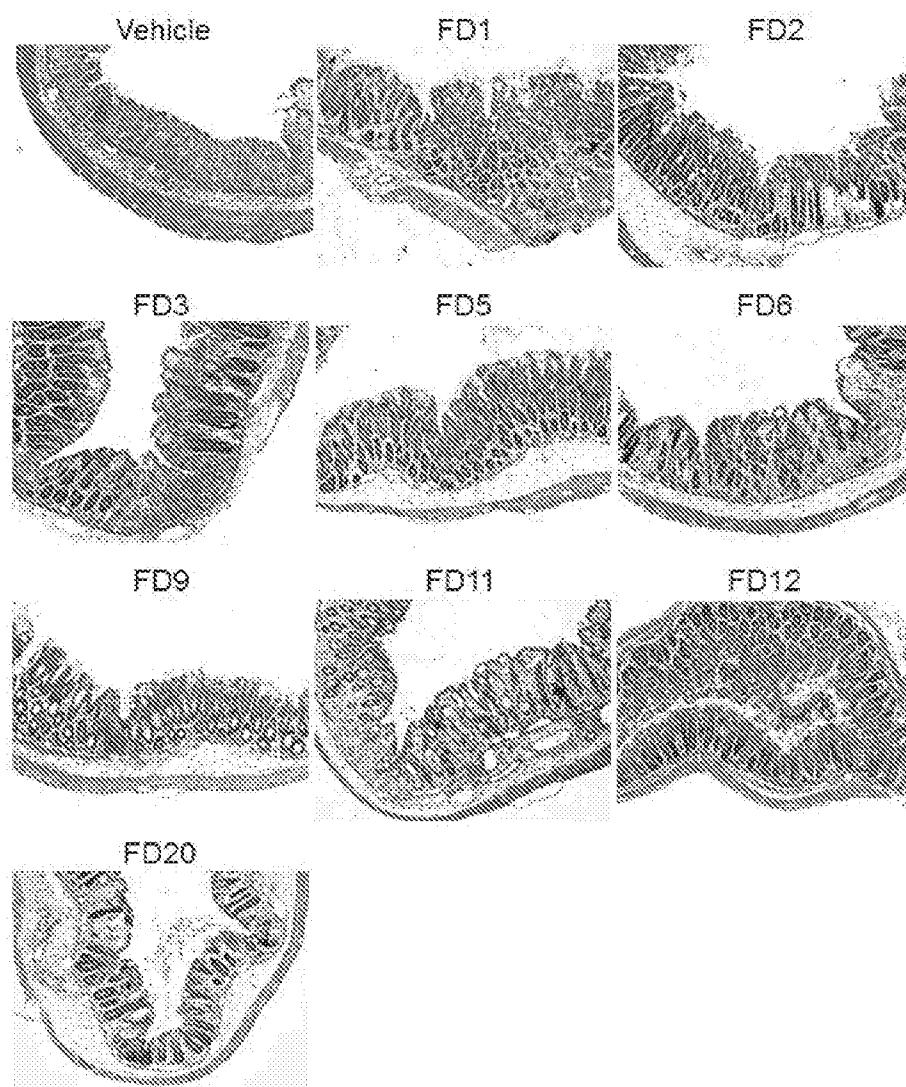


Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2018/079472

A. CLASSIFICATION OF SUBJECT MATTER		
C07C 233/59(2006.01)i; C07D 333/38(2006.01)i; A61K 31/196(2006.01)i; A61K 31/277(2006.01)i; A61K 31/341(2006.01)i; A61K 31/381(2006.01)i; A61K 31/42(2006.01)i; A61P 37/02(2006.01)i; A61P 1/16(2006.01)i		
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) A61P,A61K,C07C,C07D		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Data base: WPI, EPODOC, CNPAT, Chinese patent full text search system, EMBASE, CNKI, Search terms: vidofludimus, FXR, farnesoid x receptor, nuclear receptor, liver, nonalcoholic fatty liver, inflammatory, rheumatoid arthritis, inflammatory bowel disease, Xiamen university		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 103097338 A (4SC AG) 08 May 2013 (2013-05-08) claims 1-14, paragraph [0064]-[00130], table 1	1-8, 10, 11, 16
X	SUN, Xiuxiu et al. "Progress of drug development for ulcerative colitis" <i>World Clinical Drugs</i> , Vol. 34, No. 5, 31 May 2013 (2013-05-31), ISSN: 1672-9188, abstract, page 303, paragraph 2	1, 2, 10, 11, 16
A	SUN, Xiuxiu et al. "Progress of drug development for ulcerative colitis" <i>World Clinical Drugs</i> , Vol. 34, No. 5, 31 May 2013 (2013-05-31), ISSN: 1672-9188, abstract, page 303, paragraph 2	3-9, 12-15
☐ 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 29 May 2018		Date of mailing of the international search report 20 June 2018
Name and mailing address of the ISA/CN STATE INTELLECTUAL PROPERTY OFFICE OF THE P.R.CHINA 6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088 China		Authorized officer ZHANG, Ting
Facsimile No. (86-10)62019451		Telephone No. 86-(010)-62411123

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2018/079472

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: **1,10-16**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] The subject matter of claims 1, 10-16 relates to a method of treatment of the human or animal body by therapy and therefore does not warrant an international search according to the criteria set out in Pct Rule 39.1(iv). However, the search has been carried out and based on the use of the compositions in the manufacture of medicaments for treating corresponding diseases.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

International application No.
PCT/CN2018/079472

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	103097338	A	08 May 2013	JP	2013531667	A	08 August 2013
				WO	2012001151	A1	05 January 2012
				EP	2588447	A1	08 May 2013
.....							