CHOLANE DERIVATIVES FOR THE TREATMENT AND THE PREVENTION OF FXR AND TGR5GP-BAR1 RECEPTORS MEDIATED DISEASES

Cross-Reference to Related Applications

This Patent Application claims priority from Italian Patent Application No. 102024000006631 filed on March 25, 2024, the entire disclosure of which is incorporated herein by reference.

Technical Field of the Invention

The present invention relates to compounds having the basic structure of cholanes, in particular derivatives of ursodesoxycholic acid, BAR501 and BAR502, which can be used in the treatment and/or prevention of FXR and TGR5/GP-BAR1 receptor-mediated diseases.

State of the Art

Bile acids are signal molecules that interact with two types of dedicated cell receptors: intracellular nuclear receptors and cell surface receptors.

Nuclear receptors comprise the farnesoid X receptor (FXR), identified as the sensor of endogenous bile acids (Makishima et al Science 1999, 284, 1362, Parchi et al Science 1999, 284, 1365).

Highly expressed in entero-hepatic tissues (liver and intestine), FXR regulates bile acid homeostasis and certain metabolic pathways, including lipid and glucose metabolism (Zhang et al. Proc. Natl. Acad. Sci. USA 2006, 103, 1006). FXR agonists also have anti-inflammatory, anti-fibrotic activity and exert anti-tumour effects (Renga et al. PHASEB J. 2012, 26, 3021-3031).

The membrane receptor that binds bile acids (GP-BAR1-1, M-BAR1, GP-BAR1, TGR5) belongs to the G-protein-coupled receptor superfamily (Takeda et al FEBS Lett 2002, 520, 97; Kawamata et al. J. Biol. Chem. 2003, 278, 9435).

The interaction of TGR5/GP-BAR1 with specific ligands results in an increase in intracellular levels of cyclic AMP (cAMP) with subsequent activation of an intracellular signalling cascade. GP-BAR1 is highly expressed in the liver and intestine but also in other tissues and organs such as muscle, brain, adipose tissue, macrophages and endothelial cells. In muscle and adipose tissue, TGR5/GP-BAR1 increases energy expenditure and oxygen consumption (Watanabe et al. Nature 2006, 439, 484). In L-type entero-endocrine cells, activation of TGR5/GP-BAR1 stimulates the secretion of glucagon-like peptide (GLP-1), an incretin that promotes insulin release by the pancreas, thereby regulating blood glucose levels, gastrointestinal motility and appetite (Thomas et al. Cell. Metab. 2009, 10, 167).

Chemically, bile acids are derivatives of cholesterol. The molecular repertoire of bile acids is first generated in the liver with the production of primary bile acids such as cholic acid (CA) and chenodeoxycholic acid (CDCA). The microbiological transformation of bile acids in the intestine generates secondary bile acids such as deoxycholic acid (DCA) and lithocholic acid (LCA). In the human body, bile acids are conjugated with glycine and taurine. Among bile acids, CDCA is the most potent endogenous activator of the nuclear FXR receptor, while the bile acids LCA and Tauro-LCA (TLCA) are the natural agonists that most potently activate the TGR5/GP-BAR1 receptor.

Ursodeoxycholic acid (UDCA), which accounts for about 3% of total bile acids in humans, is nearly inactive at the FXR receptor. In fact, UDCA has been shown to be effective in the treatment of liver and biliary tract diseases and is the drug of first choice in the treatment of primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), intrahepatic cholestasis gravidarum (ICP), and less common in the treatment of cholestatic diseases in both adults and children. UDCA is also considered a safe molecule with a clinical history of minimal side effects, even when used in large doses, whereas activation of FXR by bile acids or semisynthetic bile acids is associated with hepatocyte necrosis and apoptosis resulting in inflammation and liver fibrosis (Fiorucci et al. Mini Rev. Med. Chem. 2011, 11, 753). Furthermore, the use of FXR agonists in the treatment of PBC generates cholestatic pruritus as a serious side effect, and a recent study showed that the TGR5/GP-BAR1 receptor could be the molecular target involved in the development of this side effect (Alemi et al. J. Clin. Invest. 2.013, 123, 1513-1530).

The molecules **BAR501** and **BAR502** were first described in WO 2015/181275 as modulators of FXR and TGR5/GP-BAR1 receptor activity.

The aim of the present invention is to identify new compounds active as FXR and TGR5/GP-BAR1 receptor modulators with improved bioavailability.

Subject and Summary of the Invention

This aim is achieved by compounds according to claim 1, a pharmaceutical composition according to claim 9 and their use according to claims 10 and 11.

Brief Description of the Drawings

The present invention will now be described in detail with reference to the figures of the attached drawings, wherein:

Figure 1 illustrates (A-C) the results of quantitative real-time PCR analysis of the expression of pro-inflammatory genes (A) IL-1β; (B) IL-6; (C) CXCL2 following administration of the compounds of the invention; (D-F) Effects on differentiation-related gene expression following administration of the compounds of the invention.

Preferred Embodiments of the Invention

The following paragraphs provide the chemical characteristics of the compounds according to the invention and are intended to apply uniformly to the entire description and all claims unless a broader definition is expressly provided.

The term “alkyl”, as used here, refers to saturated aliphatic hydrocarbons. This term includes linear or branched chains.

Non-limiting examples of alkyl groups according to the invention are, for example, methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl, tert-butyl and the like.

Unless otherwise indicated, the term “substituted”, as used here, means that one or more hydrogen atoms of the above-mentioned groups are replaced with another non-hydrogen atom, or functional group, provided that normal valences are maintained and the substitution results in a stable compound.

Those skilled in the art of organic chemistry will appreciate that many organic compounds can form complexes

with solvents in which they are reacted or from which they are precipitated or crystallised. These complexes are known as “solvates”. For example, a complex with water is known as a “hydrate”. Solvates of the compounds of the invention fall within the scope of protection of the invention. The compounds of formula (I) can be easily isolated in association with solvent molecules by crystallisation or evaporation of an appropriate solvent to provide the corresponding solvates.

The compounds of formula (I) can be in crystalline form. In some embodiments, the crystalline forms of the compounds of formula (I) are polymorphic.

The present invention also includes isotopically labelled compounds, which are identical to those listed in formula (I), but differ in that one or more atoms are replaced by an atom having an atomic mass or mass number that differs from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into the compounds of the invention include isotopes of hydrogen, carbon, nitrogen, and oxygen such as ^{2}H, ^{3}H, ^{11}C, ^{13}C, ^{14}C, ^{15}N, ^{17}O.

The compounds of the present invention that contain the above-mentioned isotopes and/or other isotopes of other atoms fall within the scope of protection of the present invention. The isotopically labelled compounds of the present invention, for example those in which radioactive isotopes such as ^{3}H and ^{14}C are incorporated, are useful in assays of tissue distribution of drug and/or substrate. Tritiated isotopes, i.e. ^{3}H and carbon-14, i.e. ^{14}C, are particularly preferred for their ease of preparation and

detectability. The isotopes ^{11}C are particularly useful in PET (positron emission tomography). In addition, substitution with heavier isotopes such as deuterium, i.e. ^{2}H, may provide certain therapeutic advantages resulting from increased metabolic stability, e.g. increased *invivo half-life* or reduced dosage requirements, and may therefore be referred to in certain circumstances. The isotopically labelled compounds of formula (I) of the present invention can generally be prepared by performing the procedures described in the schemes and/or examples below, substituting a non-isotopically labelled reagent for a readily available isotopically labelled reagent.

Certain groups/substituents included in the present invention may be present as isomers. Consequently, in some embodiments, the compounds of formula (I) can have axial asymmetries and, correspondingly, can exist in the form of optical isomers such as a form (*R*), a form (*S*) and the like. The present invention includes within the scope of protection all such isomers, including racemates, enantiomers and mixtures thereof.

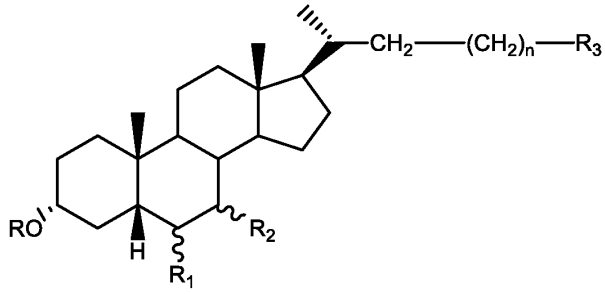
In particular, the scope of protection of the present invention includes all stereoisomeric forms, including enantiomers, diastereoisomers and mixtures thereof, including racemates, and the general reference to compounds of formula (I) includes all stereoisomeric forms, unless otherwise indicated.

In general, from the compounds of the invention one should considered as excluded those compounds (if any) that are chemically very unstable, either *on their own* or in water, so as to be clearly unsuitable for pharmaceutical use

through all routes of administration, whether oral, parenteral or otherwise. Such compounds are known to the skilled chemist.

Finally, the compounds of formula (I) can form salts.

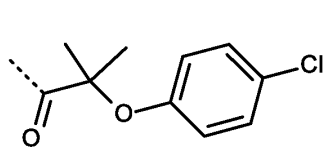
According to a **first aspect** of the invention a compound of formula (I) is provided:



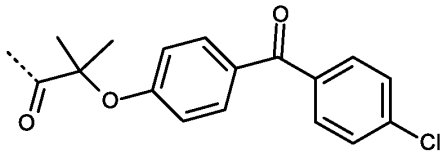
(I)

wherein:

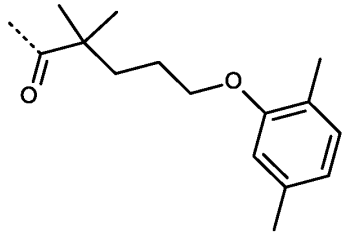
R is selected from the group consisting of H,



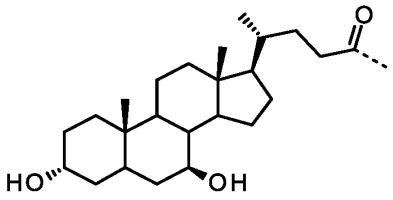
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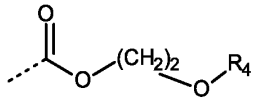
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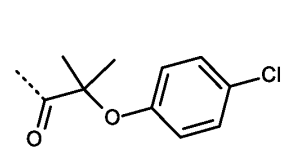
R_1 is selected from the group consisting of H and ethyl;

R_2 is OH

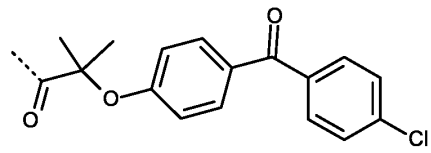
R_3 is selected from the group consisting of O-R_4,



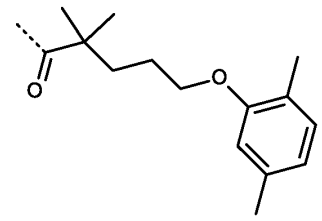
R_4 is selected from the group consisting of H,



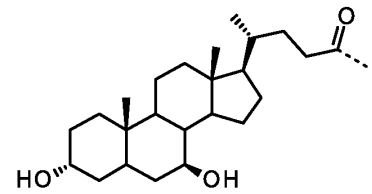
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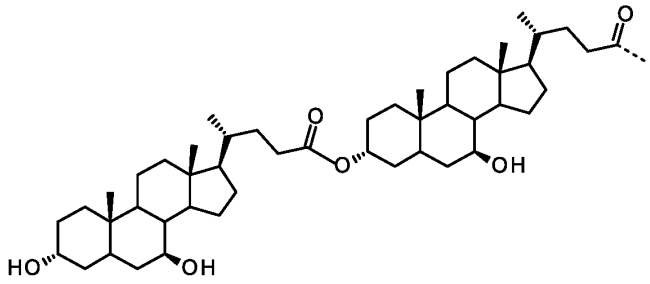
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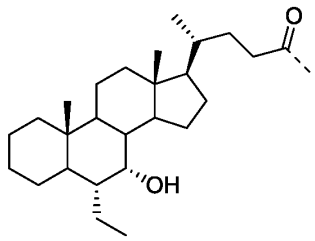


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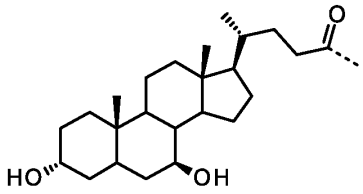


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n is selected from the group consisting of 1 and 2

or its pharmaceutically acceptable salts or solvates provided that

- R and R_4 are not H at the same time; or
- when R and R_1 are H, R_2 is βOH and n is 1, R_4 is not



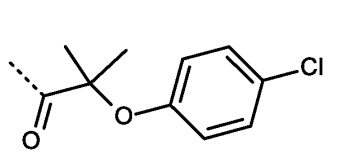
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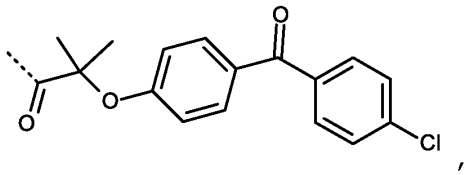
In a first embodiment R_1 is α-ethyl, R_2 is α-OH and n is 1.

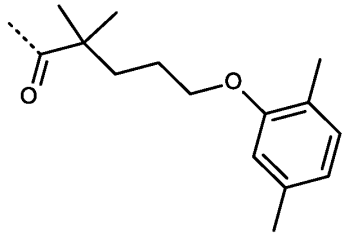
In an alternative embodiment R_1 is β-ethyl, R_2 is β-OH and n is 2.

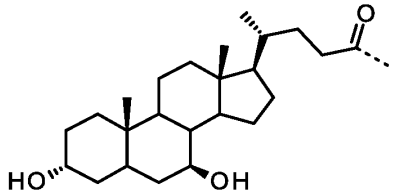
In a further embodiment R_1 is H, R_2 is β-OH and n is 1.

In one embodiment R_1 is α-ethyl, R_2 is α-OH, n is 1, R is selected from the group consisting of H,



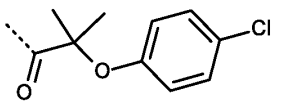
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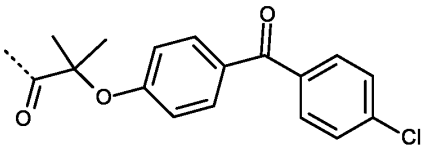
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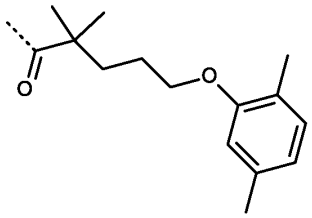
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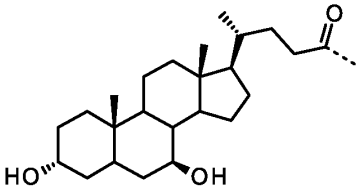
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R_3 is O-R_4 and R_4 is selected from the group consisting of H,

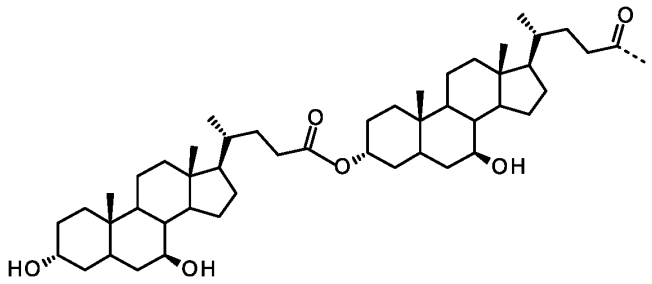
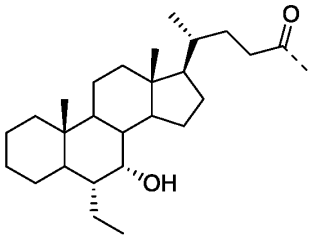


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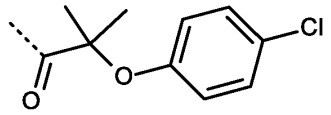
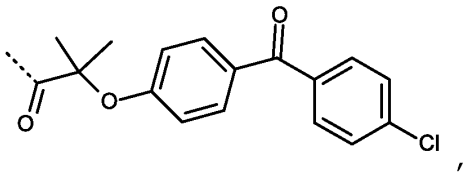
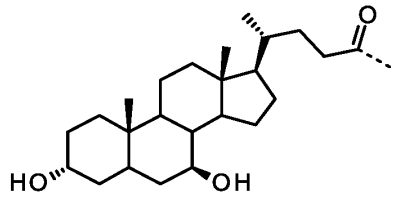
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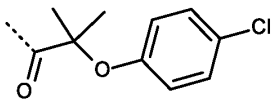
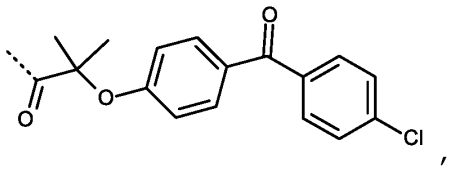
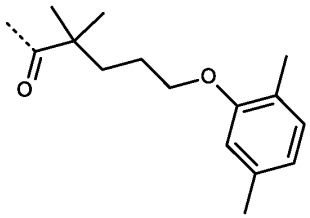
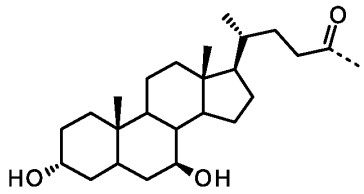
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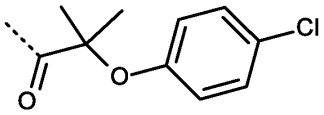
In an alternative embodiment R_1 is β-ethyl, R_2 is β-OH, n is 2, R is selected from the group consisting of H,

R_3 is O-R_4 and R_4 is selected from the group consisting of

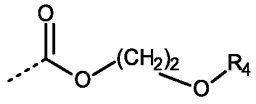





In a further embodiment R_1 is H, R_2 is β-OH, n is 1, R is selected from the group consisting of H and

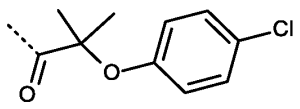


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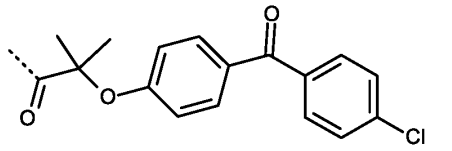
R_3 is



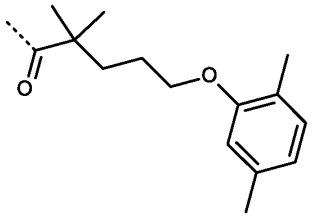
and R_4 is selected from the group consisting of



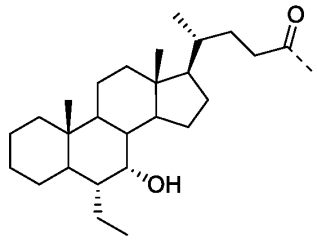
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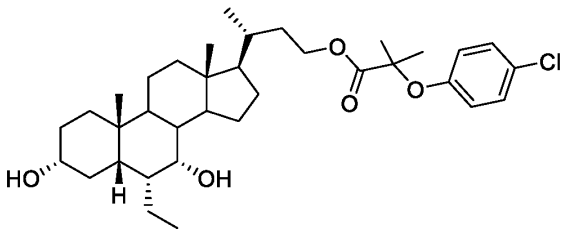


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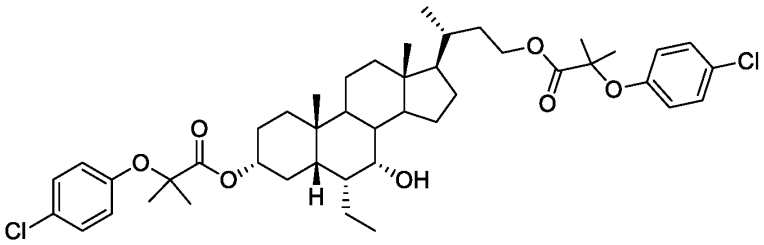


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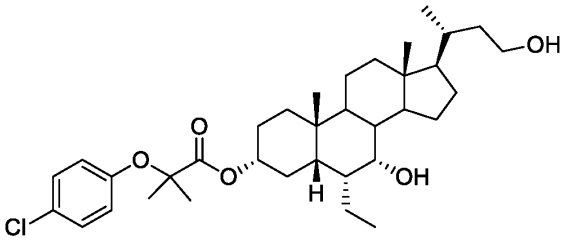
In one embodiment, the compounds of formula (I) are selected from the group consisting of:



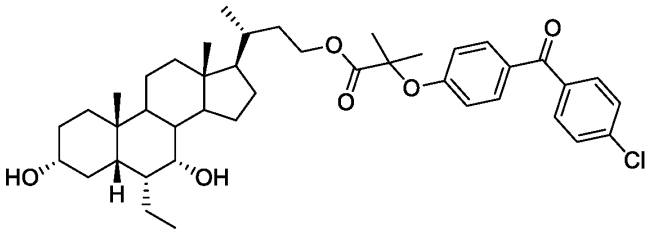
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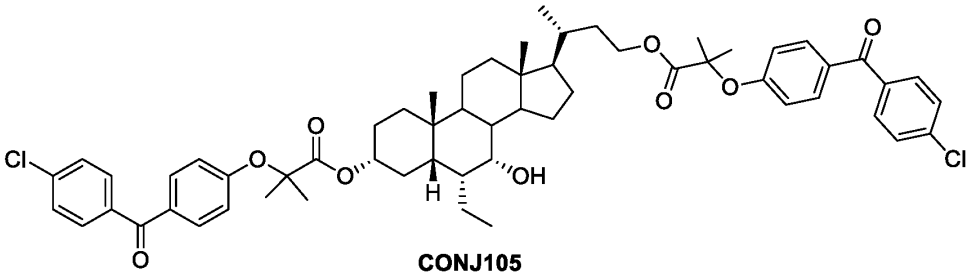
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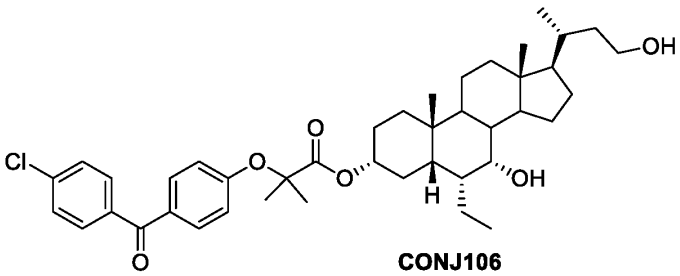
CONJ103



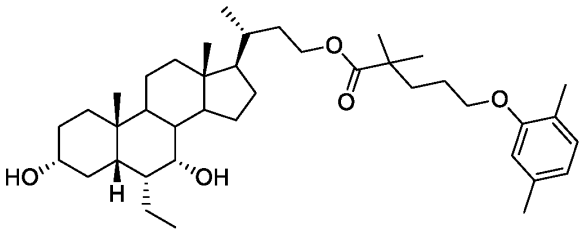
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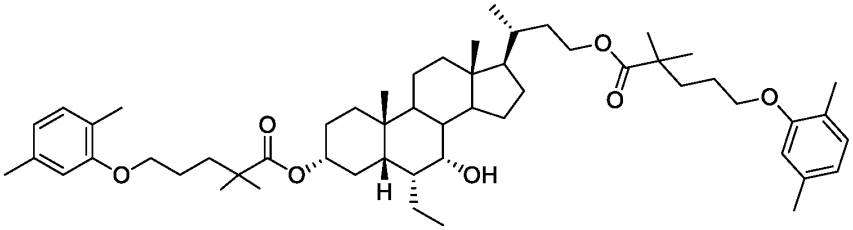
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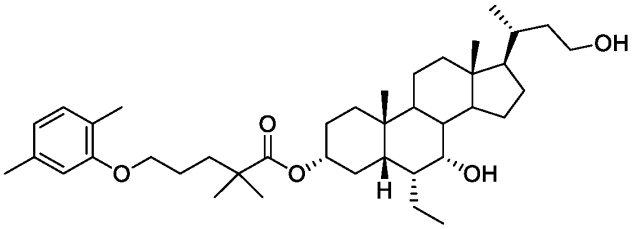
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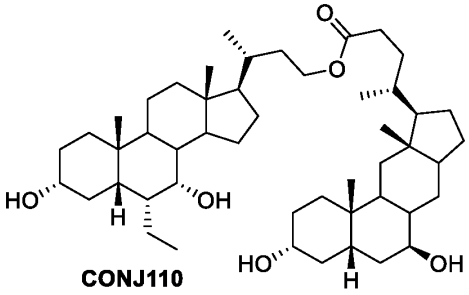
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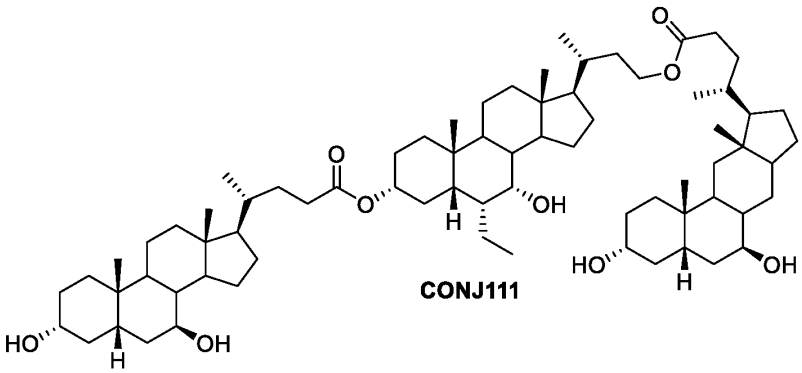
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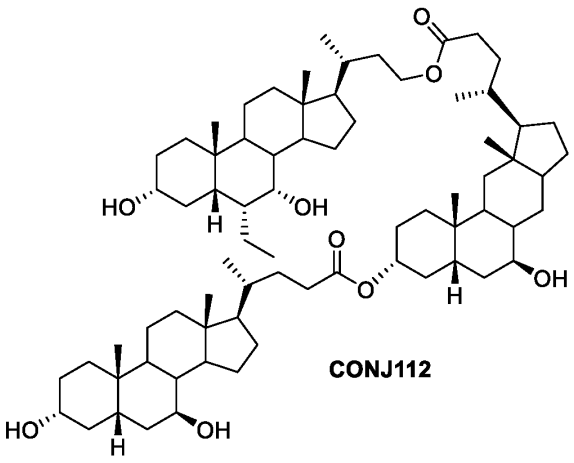
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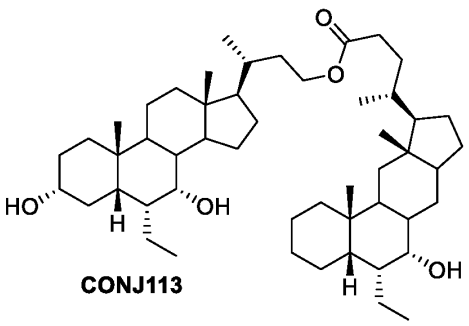
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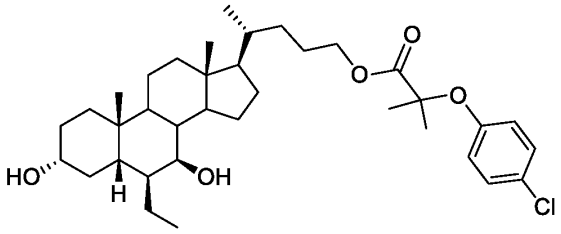
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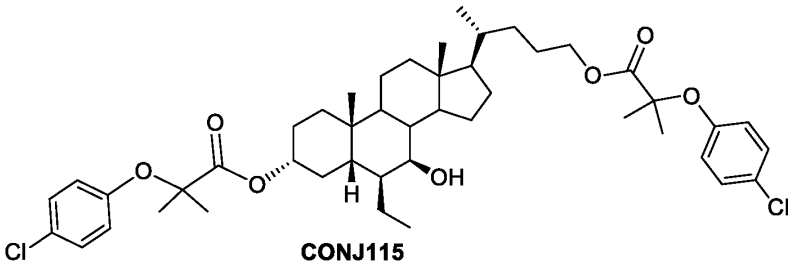
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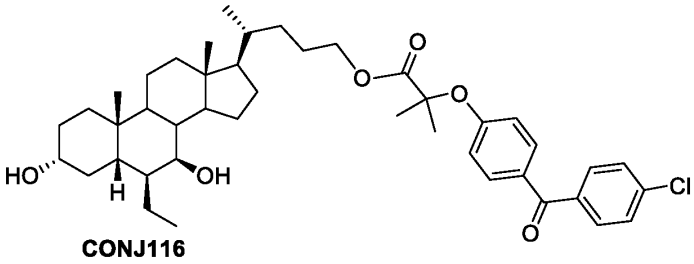
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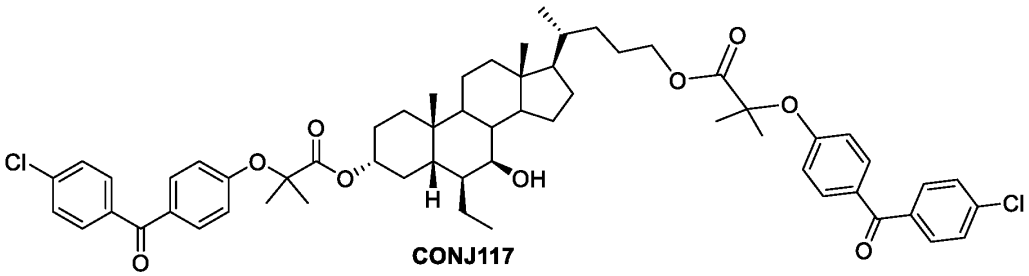
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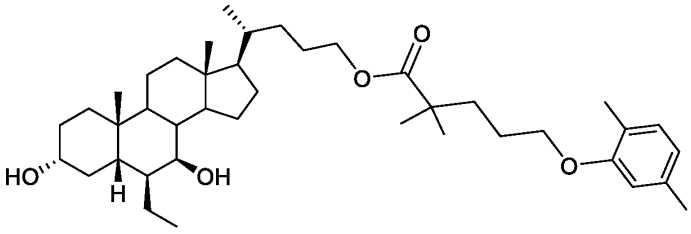
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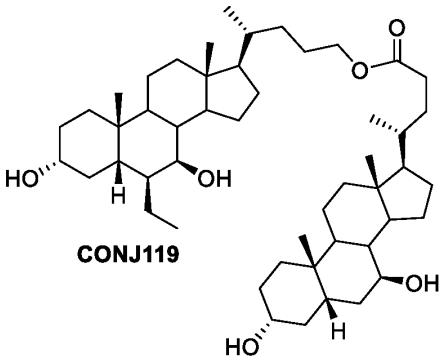
CONJ116



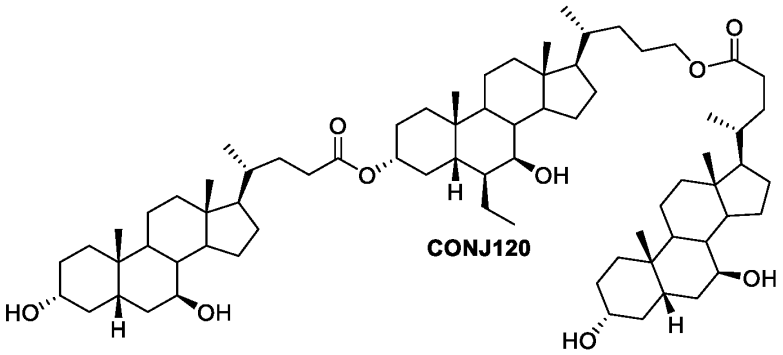
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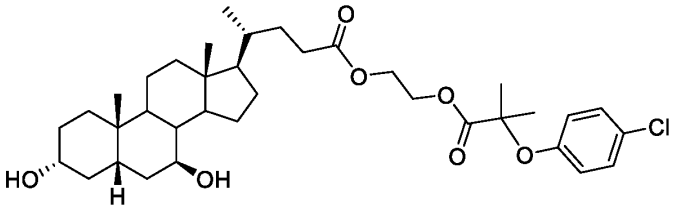
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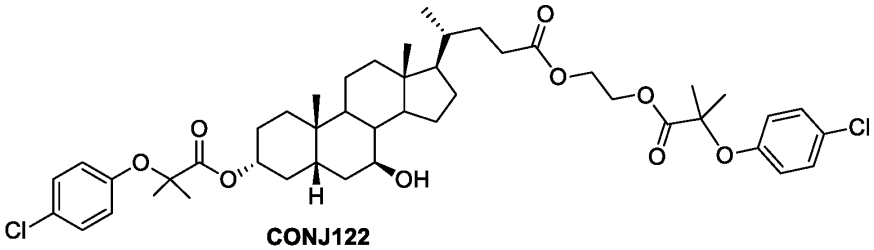
CONJ119



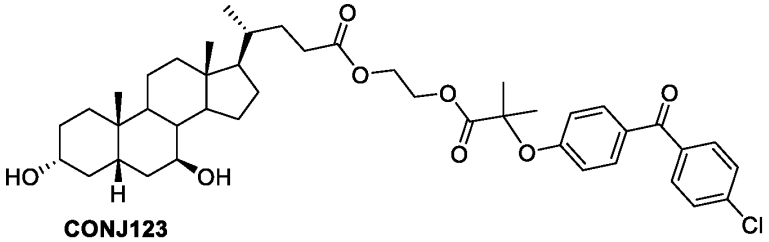
CONJ120



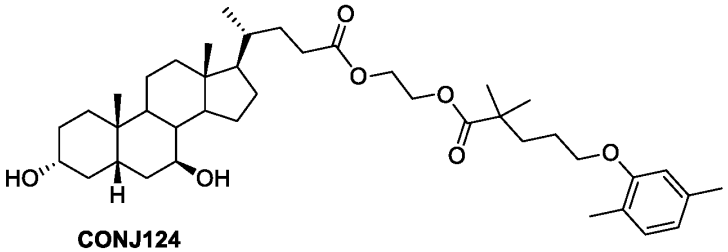
CONJ121



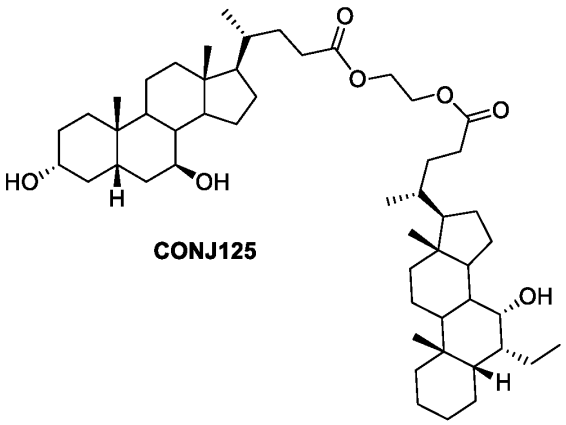
CONJ122



CONJ123



CONJ124



CONJ125

A **second aspect** of the present invention relates to a pharmaceutical composition comprising a compound of Formula (I) as illustrated above and at least one pharmaceutically acceptable excipient.

A person skilled in the art is familiar with a whole variety of such excipient compounds suitable for formulating a pharmaceutical composition.

The compounds of the invention, together with a conventionally employed excipient may be placed in the form of pharmaceutical compositions and relative unit dosages, and in such form they may be used as solids, such as tablets or filled capsules, or liquids such as solutions, suspensions, emulsions, elixirs, or capsules filled with the same, all for oral use, or in the form of sterile injectable solutions for parenteral administration (including subcutaneous and intravenous use).

Such pharmaceutical compositions and relative unit dosage forms may comprise ingredients in conventional proportions, with or without additional active ingredients or compounds, and such unit dosage forms may contain any effective amount of the active ingredient commensurate with

the expected daily dosage range to be used.

The pharmaceutical compositions containing a compound of this invention can be prepared in a manner known in the pharmaceutical technology and comprise at least one active compound. Generally, the compounds of this invention are administered in a pharmaceutically effective quantity. The amount of the compound actually administered will typically be determined by a physician, in light of the relevant circumstances, including the disease to be treated, the route of administration chosen, the actual compound administered, the age, the weight, and the response of the individual patient, the severity of the patient's symptoms, and the like.

The pharmaceutical compositions of the present invention can be administered through a variety of routes, including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, intranasal and pulmonary routes. The compositions for oral administration may take the form of bulk liquid solutions or suspensions, or bulk powders. Most commonly, however, the compositions are presented in unit dosage forms to facilitate accurate dosing. The term “unit dosage forms” refers to physically discrete units suitable as unit dosages for humans and other mammals, each unit containing a predetermined amount of active material calculated to produce the desired therapeutic effect, in combination with a suitable pharmaceutical excipient. Typical unit dosage forms include ampoules or syringes of the pre-filled, pre-measured liquid compositions or pills, tablets, capsules or similar in the case of solid compositions.

Liquid forms suitable for oral administration may include a suitable aqueous or non-aqueous vehicle with buffers, suspending and dispersing agents, dyes, flavours and the like. Solid forms may include, for example, any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, tragacanth gum or gelatin; an excipient such as starch or lactose; a disintegrating agent such as alginic acid, Primogel or corn starch; a lubricant such as magnesium stearate; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavouring agent such as peppermint, methyl salicylate, or orange flavour.

The injectable compositions are typically based on a saline solution or sterile injectable phosphate buffered saline solution or other injectable carriers known in the art.

The pharmaceutical compositions may be in the form of tablets, pills, capsules, solutions, suspensions, emulsions, powders, suppositories and as sustained release formulations.

If desired, the tablets may be coated using standard aqueous or non-aqueous techniques. In certain embodiments, such compositions and preparations may contain at least 0.1 percent of active compound. The percentage of active compound in these compositions can certainly be varied, of course, and can be conveniently between about 1 percent and about 60 percent of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that the therapeutically active dosage is obtained. The active compounds may also be administered intranasally as,

for example, liquid droplets or sprays.

The tablets, the pills, the capsules, and the like may also contain a binder such as gum tragacanth, acacia, corn starch, or gelatin; excipients such as calcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose, or saccharin. When a unit dosage form is a capsule, it may contain, in addition to the materials of the above type, a liquid carrier such as a fatty oil. Various other materials may be present as coatings or to modify the physical shape of the dosage unit. For example, the tablets may be coated with shellac, sugar, or both. A syrup or elixir may contain, in addition to the active ingredient, sucrose as a sweetening agent, methyl and propyl parabens as preservatives, a dye and a flavouring agent such as cherry or orange flavour. To prevent decomposition during the transit through the upper portion of the gastrointestinal tract, the composition may be a coated enteric formulation.

Compositions for pulmonary administration include, but are not limited to, dry powder compositions consisting of the powder of a compound of Formula (I) or a relative salt, and of the powder of a suitable carrier and/or lubricant. Compositions for pulmonary administration may be inhaled from any suitable dry powder inhaler device known to the person skilled in the art.

The administration of the compositions is performed under a protocol and at a dosage that is sufficient to reduce inflammation and pain in the subject. In some embodiments, in the pharmaceutical compositions of the present invention

the active ingredient or the active ingredient(s) are generally formulated in dosage units. The dosage unit may contain 0.1 to 1000 mg of a compound of Formula (I) per dosage unit per daily administration.

In some embodiments, the effective amounts for a specific formulation will depend on the severity of the disease, disorder or condition, on the previous therapy, on the health status of the individual and on the response to the drug. In some embodiments the dose is in the range from 0.001% by weight to about 60% by weight of the formulation.

When used in combination with one or more of the other active ingredients, the compound of the present invention and the other active ingredient may be used in lower doses than when each is used individually.

Regarding formulations with respect to any variety of routes of administration, methods and formulations for drug administration are illustrated in Remington's Pharmaceutical Sciences, 17th edition, Gennaro et al. Eds., Mack Publishing Co., 1985, and Remington’s Pharmaceutical Sciences, Gennaro AR ed. 20th edition, 2000, Williams & Wilkins PA, USA, and Remington: The Science and Practice of Pharmacy, 21th edition, Lippincott Williams & Wilkins Eds., 2005; and in Loyd V. Allen and Howard C. Ansel, Ansel’s Pharmaceutical Dosage Forms and Drug Delivery Systems, 10th edition, Lippincott Williams & Wilkins Eds., 2014.

The components described above for orally administered or injectable compositions are purely representative.

The compounds of this invention can also be administered in sustained-release forms or by sustained-release drug delivery systems.

A **third aspect** of the present invention refers to a compound of Formula (I) as illustrated above for use as a medicament.

A compound of Formula (I) as illustrated above may be used in the prevention and/or in the treatment of a disorder selected from the group consisting of gastrointestinal disorders, liver disorders, cardiovascular disorders, vascular disorders, pulmonary disorders, methabolic disorders, infectious diseases, cancer, renal disorders, inflammatory disorders including immunomediated disorders and neurological disorders.

In one embodiment, immune-mediated inflammatory disorders include autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, Syogren's disease, scleroderma also known as systemic sclerosis, spondyloarthritis, vasculitis, sarcoidosis, Mediterranean fever, and other inherited auto-inflammatory diseases, polymyositis dermatomyositis and Behcet's syndrome.

In one embodiment, the infectious diseases are selected from the group of acquired immunodeficiency syndrome (AIDS) and associated disorders, B virus and C virus infections.

In one embodiment, neurological disorders include Alzheimer's disease and other forms of dementia, Parkinson's disease and other movement disorders, amyotrophic lateral sclerosis and other motor neuron disorders, multiple sclerosis and other demyelinating diseases, ischaemic stroke, myasthenia and muscular dystrophies.

In one embodiment, liver disorders include primary biliary cirrhosis (PBC), cerebrotendinous xanthomatosis (CTX), primary sclerosing cholangitis (PSC), drug-induced

cholestasis, intrahepatic cholestasis gravidarum, cholestasis associated with parenteral nutrition, cholestasis associated with bacterial proliferation or sepsis, autoimmune hepatitis, chronic viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), liver transplantation, congenital liver fibrosis, granulomatous liver disease, intra- or extra-hepatic malignant tumour, Wilson's disease, haemochromatosis, and alpha 1-antitrypsin deficiency.

In one embodiment, gastrointestinal disorders include inflammatory bowel disease (IBD) (including Crohn's disease, ulcerative rectocolitis and indeterminate colitis), irritable bowel syndrome (IBS), bacterial proliferation, acute and chronic pancreatitis, malabsorption, post-radiation colitis, and microscopic colitis.

In one embodiment, renal disorders include diabetic nephropathy, hypertensive nephropathy, chronic glomerulonephritis, including chronic transplant glomerulonephritis, chronic tubulo-interstitial diseases and vascular disorders of the kidney.

In one embodiment, cardiovascular disorders include atherosclerosis, arteriosclerosis, dyslipidaemia, hypercholesterolaemia, hypertriglyceridaemia, hypertension also known as arterial hypertension, cardiac inflammatory disorders including myocarditis and endocarditis, cardiac ischaemia, stable angina and unstable angina myocardial infarction, cerebrovascular disorders including ischaemic stroke.

In one embodiment, vascular disorders include

cardiopulmonary diseases such as pulmonary hypertension, peripheral artery disease (PAD), also known as peripheral vascular disease (PVD), peripheral arterial occlusive disease and peripheral arterial obliterative disease.

In one embodiment, pulmonary disorders include asthma, cystic fibrosis, obstructive respiratory disease, interstitial lung disease including, but not limited to, primary or secondary pulmonary fibrosis.

In one embodiment, the metabolic disease is selected from the group consisting of insulin resistance, metabolic syndrome, type I and type II diabetes, hypoglycaemia, adrenal cortex disorders including adrenal cortex insufficiency. The metabolic pathology further includes obesity and conditions associated with bariatric surgery.

In one embodiment, cancer is selected from the group comprising liver cancer, bile duct cancer, oesophageal cancer, pancreatic cancer, gastric cancer, colorectal cancer, breast cancer, ovarian cancer and the condition associated with resistance to chemotherapy.

Further characteristics of the present invention will become apparent from the following description of some merely illustrative and non-limiting examples.

EXAMPLE 1. Preparation of bile acid-fibre conjugates

Starting with **BAR502** and **BAR501**, a coupling reaction was carried out between the alcohol function of the two derivatives and the acid function of the fibrates (clofibric acid (**A**), fenofibric acid (**B**) and gemfibrozil (**C**)) to obtain the different conjugates. The **UDCA** was initially esterified with ethylene glycol (glyc) and subsequently conjugated with acids via the same coupling reaction.

General procedures:

Reaction a). The carboxylic acids [clofibric acid (**A**), fenofibric acid (**B**) and gemfibrozil (**C**)] were dissolved in anhydrous DMF (10 mL), and then DIPEA (8 eq) and the coupling reagents EDC·HCl (4 eq) and HOBt (4 eq) were added. After 30 min, the corresponding compound (**BAR502**, **BAR501**, **23-acetyl-BAR502** or **UDCA-glyc**) was added to the mixture. The reaction was allowed under stirring at room temperature overnight. At the end of the reaction, the solvent was removed under vacuum and the reaction crude was extracted with H_2O/EtOAc (50 mL) three times. The organic phases were anhydrified with Na_2SO_4, filtered and concentrated under vacuum.

Reaction b). **BAR502** (100 mg, 0.25 mmol) was dissolved in anhydrous pyridine (3 mL) and acetic anhydride (25 µL, 0.25 mmol). After 1h, the pyridine was removed under vacuum and the residue was placed in water (10 mL) and extracted with ethyl acetate (3×10 mL) three times. The organic phases were anhydrified with Na_2SO_4, filtered and evaporated to dryness under vacuum to give the product **23-acetyl-BAR502** alone.
Selected values of ^{1}H NMR (400 MHz, CD_3OD): δH 4.11 (2H, m, H_2-23), 3.65 (1H, s, H-7β), 3.53 (1H, s, H-3β), 2.00 (3H, s, $COCH_3$), 0.96 (3H, d, J = 6.5 Hz, H_3-21), 0.91 (6H, ovl, H_3-19 and H_3-25), 0.67 (3H, s, H_3-18).

Reaction c). The deacetylation reaction was performed using a catalytic amount of *p*-toluensulphuric acid in $CHCl_3$/MeOH (5:3). At the end of the reaction, it is neutralised with a saturated solution of $NaHCO_3$, the solvent is evaporated and the residue is extracted DCM (3 x 20 mL) and H_2O. The organic phases were anhydrified with Na_2SO_4, filtered and evaporated to dryness under vacuum to give the product that was

subsequently purified by HPLC.

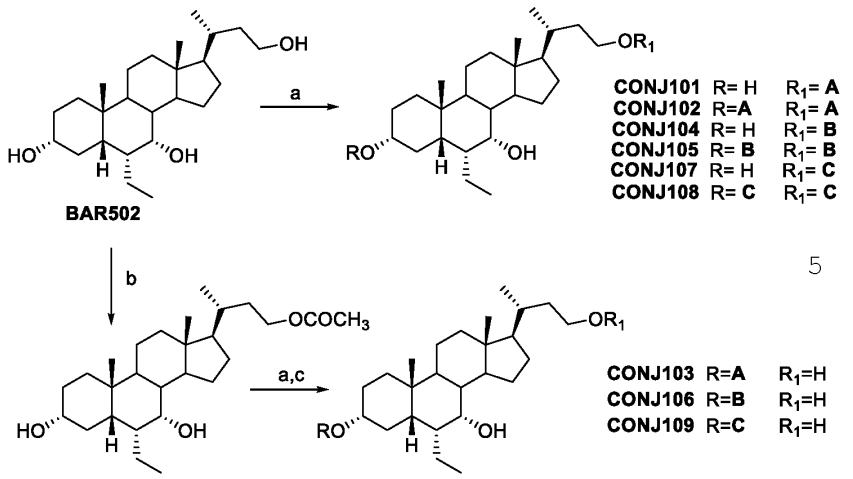
Reaction d). The ursodeoxycholic acid (**UDCA**) (500 mg, 1.27 mmol) was dissolved in anhydrous THF (10 mL) and anhydrous ethylene glycol (10 mL). A stoichiometric amount of *p*-toluenesulphonic acid was added to the mixture and the reaction was kept at room temperature for 3 h. Once the reaction was complete, water (5 mL) was added, the mixture was brought to neutrality with a 1N NaOH solution and then extracted with H_2O/DCM (50 mL) three times. The organic phases were anhydrified with Na_2SO_4, filtered and evaporated to dryness to give the product **UDCA-glyc.**

Selected values of ^1H NMR (400 MHz, CD_3OD): δ_H 4.12 (2H, t, J = 5.0 Hz, H_2-Glyc), 3.73 (2H, t, J = 5.0 Hz, H_2-Glyc), 3.48 (2H, ovl, H-3β and H-7α), 2.41 (1H, s, H-23a), 2.28 (1H, m, H-23b), 0.96 (3H, s, H_3-19), 0.89 (3H, d, J= 7.0 Hz, H_3-21), 0.71 (3H, s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_c 173.1, 72.1, 71.9, 66.5, 62.0, 56.3, 55.4, 44.4 (2C), 42.9, 40.7, 39.8, 37.9, 37.4, 35.8, 35.5, 34.6, 31.7, 31.5, 30.9, 29.0, 27.5, 23.9, 21.8, 19.0, 12.7.

EXAMPLE 1A. Synthesis of BAR502 conjugates with fibrates (CONJ101-109).

The reaction of **BAR502** with the different carboxylic acids [clofibric acid (**A**), fenofibric acid (**B**) and gemfibrozil (**C**) (3 eq)], activated in situ using coupling reagents, yielded the compounds **CONJ101-109.**



a) Different acids (**A**-**C**), EDC•HCl, HOBt, DIPEA, after 30' **BAR502** in anhydrous DMF; b) acetic anhydride in anhydrous pyridine; c) *p*-toluenesulphonic acid in $CHCl_3$:MeOH (5:3).

EXAMPLE 1A-1. Preparation of CONJ101 and CONJ102.

Purification in HPLC, on a reversed-phase column (Luna C18, 10 µm, 10 mm i.d. x 250 mm) and MeOH/H_2O 95:5 as eluent (flow 3 mL/min), of the reaction between **BAR502** (200 mg, 0.51 mmoles) and clofibric acid (**A**) (3 eq), yielded **CONJ101** (70.7 mg, t_R = 9.7 min) and **CONJ102** (24.3 mg, t_R = 18.8 min).

CONJ101: $C_{35}H_{53}ClO_5$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.20 (2H, d, *J* = 8.7 Hz, H-3″ and H-5″), 6.77 (2H, d, *J* = 8.7 Hz, H-2″ and H-6″), 4.22 (1H, m, H-23a), 4.19 (1H, m, H-23b), 3.71 (1H, s, H-7β), 3.44 (1H, m, H-3β), 1.59 (6H, s, H_3-3′ and H_3-4′), 0.92 (6H, ovl, H_3-21 and H_3-25), 0.90 (3H, s, H_3-19), 0.60 (3H, s, H_3-18).

s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_c 174.3, 153.9, 129.2 (2C), 126.8, 119.8 (2C), 79.4, 72.3, 70.9, 63.7, 55.9, 50.3, 45.2, 42.6, 41.2, 39.8, 39.6, 35.4, 34.4, 33.9, 33.1, 33.0, 30.6, 28.2,

25.4, 25.1 (2C), 23.6, 23.0, 22.2, 20.7, 18.5, 11.6, 11.5.

CONJ102: $C_{45}H_{62}Cl_2O_7$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.20 (4H, d, *J* = 8.7 Hz, H-3″ and H-5″), 6.80-6.77 (4H, d, *J* = 8.7 Hz, H-2″ and H-6″), 4.62 (1H, m, H-3β), 4.21 (1H, m, H-23a), 4.19 (1H, m, H-23b), 3.71 (1H, s, H-7β), 1.59-1.57 (12H, s, H_3-3′ and H_3-4′), 0.92 (6H, ovl, H_3-21 and H_3-25), 0.91 (3H, ovl, H_3-19), 0.60 (3H, s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_c 174.1, 173.3, 154.1 (2C), 129.1 (2C), 128.9 (2C), 127.0, 126.9, 120.4 (2C), 120.1 (2C), 79.5, 79.3, 76.0, 70.5, 63.7, 55.8, 50.3, 45.0, 42.7, 41.0, 39.9, 39.4, 35.5, 35.0, 34.3, 33.0, 32.9, 29.2, 28.3, 26.4, 25.6, 25.3, 25.2, 25.1, 23.6, 23.1, 22.1, 20.7, 18.6, 11.6 (2C).

EXAMPLE 1A-2. Preparation of CONJ104 and CONJ105.

HPLC purification, on a reversed-phase column (Luna C18, 10 µm, 10 mm i.d. x 250 mm) and MeOH/H_2O 95:5 as eluent (flow 3 mL/min), of the reaction between **BAR502** (50 mg, 0.127 mmoles) and fenofibric acid (**B**) (3 eq), yielded **CONJ104** (32 mg, t_R = 11.1 min) and **CONJ105 (**4.6 mg, t_R = 31.2 min).

CONJ104: $C_{42}H_{57}ClO_6$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.74 (2H, d, *J* = 8.6 Hz, H-3″ and H-5″), 7.70 (2H, d, *J* = 8.4 Hz, H-2‴ and H-6‴), 7.45 (2H, d, *J* = 8.4 Hz, H-3‴ and H-5‴), 6.85 (2H, d, *J* = 8.6 Hz, H-2″ and H-6″), 4.22 (1H, m, H-23a), 4.20 (1H, m, H-23b), 3.68 (1H, s, H-7β), 3.40 (1H, m, H-3β), 1.68 (6H, s, H_3-3′ and H-4′), 0.89 (6H, ovl, H_3-21 and H_3-25), 0.87 (3H, ovl, H_3-19), 0.58 (3H, s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 194.0, 174.0, 159.5, 138.5, 136.5, 132.0 (2C), 131.0 (2C), 130.0, 128.5 (2C), 117.2 (2C), 79.0, 72.3, 70.8, 63.8, 55.8, 50.3, 44.9, 42.3, 41.2, 39.6, 39.3,

35.4, 34.2, 33.9, 32.9, 32.7, 30.4, 28.2, 25.4, 25.1 (2C), 23.3, 23.0, 21.8, 20.5, 18.5, 11.7, 11.4.

CONJ105: $C_{59}H_{70}Cl_2O_9$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.73 (8H, ovl, H-3″ and H-5, H-2‴ and H-6‴), 7.45 (4H, ovl, H-3‴ and H-5‴), 6.87 (4H, ovl, H-2″ and H-6″), 4.64 (1H, m, H-3β), 4.22 (1H, m, H-23a), 4.18 (1H, m, H-23b), 3.67 (1H, s, H-7β), 1.69 (12H, s, H_3-3′ and H-4′), 0.82 (9H, ovl, H_3-21 and H_3-25, H_3-19), 0.56 (3H, s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 194.6, 194.3, 174.0, 173.3, 160.0, 159.8, 138.5 (2C), 136.6 (2C), 132.2 (2C), 132.1 (2C), 131.4 (2C), 131.3 (2C), 130.4, 130.3, 128.7 (4C), 117.5, 117,4, 79.7, 79.5, 70.6, 64.1, 56.2, 50.6, 45.3, 42.9, 41.3, 40.1, 39.6, 35.7, 35.2, 34.6, 33.3, 33.2, 29.4, 28.4, 26.6, 25.7 (4C), 25.6, 25.5, 23.8, 23.3, 22.4, 20.8, 18.7, 11.8 (2C).

EXAMPLE 1A-3. Preparation of CONJ107 and CONJ108.

The same coupling reaction between **BAR502** (50 mg, 0.127 mmol) and gemfibrozil (C) (3 eq), followed by purification in HPLC, using a reversed-phase column (Luna C18, 10 µm, 10 mm i.d. x 250 mm) and MeOH/H_2O 95:5 as eluent (flow 3 mL/min), yielded **CONJ107** (38 mg, t_R = 11.6 min) and **CONJ108** (1.6 mg, t_R= 37.6 min).

CONJ107: $C_{40}H_{64}O_5$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.01 (1H, d, *J* = 7.4 Hz, H-3″), 6.66 (1H, d, *J* = 7.4 Hz, H-4″), 6.61 (1H, s, H-6″), 4.09 (2H, m, H_2-23), 3.91 (2H, t, *J* = 5.5 Hz, H-5′), 3.68 (1H, s, H-7β), 3.42 (1H, m, H-3β), 2.31 (3H, s, H_3-8″), 2.18 (3H, s, H_3-7″), 1.22 (6H, s, H_3-6′ and H_3-7′), 0.97 (3H, d, *J* = 6.5 Hz, H_3-21), 0.91 (3H, t, *J* = 7.4 Hz, H_3-25), 0.90 (3H, s, H_3-19), 0.63 (3H, s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 178.0, 156.3, 136.3, 130.4, 123.7, 120.4, 111.6, 72.5, 70.8, 68.0, 62.5, 56.0, 50.4, 45.2, 42.8, 41.9, 41.1, 40.0, 39.5, 37.1, 35.5 (2C), 34.6, 33.9, 33.1 (2C), 30.6, 28.3, 25.2 (3C), 23.6, 23.0, 22.3, 21.4, 20.7, 18.8, 15.7, 11.5 (2C).

CONJ108: $C_{55}H_{84}O_7$

Selected values of ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.00 (2H, d, *J* = 7.4 Hz, H-3″), 6.66 (2H, d, *J* = 7.4 Hz, H-4″), 6.61 (2H, s, H-6″), 4.53 (1H, m, H-3β), 4.11 (2H, m, H_2-23), 3.91 (4H, m, H-5′), 3.69 (1H, s, H-7β), 2.30 (6H, s, H_3-8″), 2.18 (6H, s, H_3-7″), 1.20 (12H, ovl, H_3-6′ and H_3-7′), 0.97 (3H, d, *J* = 6.5 Hz, H_3-21), 0.91 (6H, ovl, H_3-19 and H_3-25), 0.65 (3H, s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 177.9, 177.8, 156.5 (2C), 136.1 (2C), 129.9 (2C), 123.4 (2C), 120.9 (2C), 111.2 (2C), 74.3, 70.4, 67.7 (2C), 62.3, 55.8, 50.2, 44.9, 42.6, 41.6 (2C), 40.9, 39.5, 39.1, 36.9 (2C), 35.4, 34.8, 34.4, 32.8 (2C), 29.2 (4C), 27.3, 26.5, 24.9 (4C), 24.7, 23.3, 22.8, 21.8, 21.1, 20.5, 18.3, 15.4, 11.4 (2C).

EXAMPLE 1A-4. Preparation of CONJ103.

The **23-acetyl-BAR502** was subjected to the coupling reaction with clofibric acid (**A**) under the same conditions as described above, followed by deacetylation using a catalytic amount of *p*-toluenesulphonic acid in $CHCl_3$:MeOH (5:3). At the end of the reaction, the mixture was neutralised with a saturated solution of $NaHCO_3$. After removing the solvent under vacuum, the reaction residue was diluted with water and extracted with DCM (3 x 20 mL). The organic phases were anhydrified with Na_2SO_4, filtered and evaporated to dryness to obtain 200 mg of raw extract. The reaction mixture was

purified in HPLC, using a reversed-phase column (Luna C18, 10 µm, 10 mm i.d. x 250 mm) and MeOH/H_2O 95:5 as eluent (flow 3 mL/min), yielding **CONJ103** (35 mg, t_R = 10.0 min)

CONJ103: $C_{35}H_{53}ClO_5$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.18 (2H, d, *J* = 8.9 Hz, H-3″ and H-5″), 6.80 (2H, d, *J* = 8.7 Hz, H-2″ and H-6″), 4.63 (1H, m, H-3β), 3.72 (1H, s, H-7β), 3.68 (2H, ovl, H_2-23), 1.56 (6H, s, H_3-3′ and H_3-4′), 0.96 (3H, d, *J* = 6.5 Hz, H_3-21), 0.91 (6H, ovl, H_3-19 and H_3-25), 0.67 (3H, s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 173.2, 154.0, 129.3 (2C), 126.8, 120.5 (2C), 79.6, 76.0, 70.6, 60.8, 56.4, 53.4, 50.5, 45.1, 42.8, 41.1, 39.9, 39.5, 38.9, 35.6, 35.0, 33.1, 32.8, 29.6, 28.3, 26.5, 25.5, 25.1, 23.1, 22.1, 20.7, 18.8, 11.7, 11.6.

EXAMPLE 1A-5. Preparation of CONJ106.

CONJ106 was obtained by the same coupling reaction between **23-acetyl-BAR502** (30 mg, 0.127 mmol) and phenofibric acid (B) (3 eq) followed by deacetylation. The reaction mixture purified by HPLC, using a reversed-phase column (Luna C18, 10 µm, 10 mm i.d. x 250 mm) and MeOH/H_2O 90:10 as eluent (flow 3 mL/min), yielded **CONJ106** (8 mg, t_R = 21 min).

CONJ106: $C_{42}H_{57}ClO_6$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.72 (4H, ovl, H-3′′, H-5′′, H-2′′′ and H-6′′′), 7.45 (2H, d, *J* = 8.5 Hz, H-3′″ and H-5′″), 6.88 (2H, d, *J* = 8.8 Hz, H-2″ and H-6″), 4.64 (1H, m, H-3β), 3.67 (3H, ovl, H-7β and H_2-23), 1.67 (3H, s, CH_3-3′), 1.66 (3H, s, CH_3-4′), 0.95 (3H, d, *J* = 6.5 Hz, CH_3-21), 0.90 (6H, ovl, CH_3-19 and CH_3-25), 0.67 (3H, s, CH_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 194.5, 173.0, 160.0, 138.3,

136.5, 131.9 (2C), 131.3 (2C), 130.1, 128.4 (2C), 117.3 (2C), 79.6, 76.4, 70.5, 60.9, 56.3, 50.5, 45.2, 42.7, 41.1, 40.0, 39.6, 38.9, 35.6, 35.0, 33.1, 32.9, 29.2, 28.4, 26.4, 25.6, 25.5, 23.7, 23.0, 22.2, 20.7, 18.8, 11.7 (2C).

EXAMPLE 1A-6. Preparation of CONJ109.

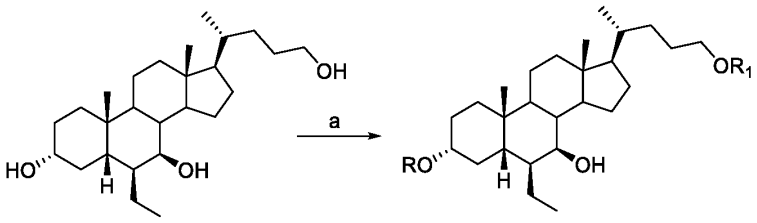
The same coupling reaction between **23-acetyl-BAR502** (30 mg, 0.127 mmol) and gemfibrozil (C) (3 eq) followed by deacetylation and purification by HPLC, using a reversed-phase column (Luna C18, 10 µm, 10 mm i.d. x 250 mm) and MeOH/H_2O 95:5 as eluent (flow 3 mL/min), yielded **CONJ109** (5 mg, tR = 24 min).

CONJ109: $C_{40}H_{64}O_5$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.01 (1H, d, *J* = 7.4 Hz, H-3″), 6.66 (1H, d, *J* = 7.4 Hz, H-4″), 6.61 (1H, s, H-6″), 4.54 (1H, m, H-3β), 3.92 (2H, t, J = 5.5 Hz, H-5′), 3.68 (3H, ovl, H-7β and H_2-23), 2.31 (3H, s, CH_3-8″), 2.18 (3H, s, CH_3-7″), 1.20 (6H, s, CH_3-6′ and CH_3-7′), 0.97 (3H, d, *J* = 6.5 Hz, CH_3-21), 0.92 (6H, ovl, CH_3-19 and CH_3-25), 0.68 (3H, s, CH_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 177.4, 157.0, 136.4, 130.2, 123.5, 120.7, 112.0, 74.5, 70.8, 68.1, 60.8, 56.4, 50.5, 45.1, 42.8, 42.0, 41.2, 40.0, 39.5, 39.0, 37.1, 35.6, 35.1, 33.3, 32.9, 29.5, 28.4, 26.6, 25.3, 25.2, 25.0, 23.7, 23.1, 22.2, 21.4, 20.8, 18.8, 15.8, 11.8, 11.7.

EXAMPLE 1B. Preparation of conjugates between BAR501 and fibrates (CONJ115-118).



BAR501

a) Different acids (**A**-**C**), EDC•HCl, HOBt, DIPEA, after 30' **BAR501** in anhydrous DMF.

EXAMPLE 1B-1. Preparation of CONJ114 and CONJ115.

Treatment of **BAR501** (50 mg, 0.123 mmol) with clofibric acid (**A**) (3 eq), resulted in the mixture of **CONJ114** and **CONJ115**, efficiently separated by HPLC, on a reversed-phase column (Luna C18; 10 μM; 10 mm i.d. x 250 mm) and MeOH/H_2O 95:5 as eluent (flow 3 mL/min). This resulted in 11.3 mg of **CONJ114** (t_R = 12.0 min) and 1.8 mg of **CONJ115** (t_R = 37.3 min).

CONJ114: $C_{36}H_{55}ClO_5$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.18 (2H, d, J= 8.8 Hz, H-3″ and H-5″), 6.77 (2H, d, *J* = 8.8 Hz, H-2″ and H-6″), 4.12 (2H, m, H_2-24), 3.80 (1H, dd, *J* = 9.9, 6.0 Hz, H-7α), 3.59 (1H, m, H-3β), 1.58 (6H, s, H-3′ and H-4′), 0.89 (3H, d, *J* = 6.5 Hz, H_3-21), 0.97 (3H, s, H_3-19), 0.96 (3H, t, *J* = 7.4 Hz, H_3-26), 0.65 (3H, s, H_3-26).

^{13}C NMR (400 MHz, $CDCl_3$): δ_C 174.0, 154.1, 129.1 (2C), 127.1, 120.4 (2C), 79.5, 74.6, 71.2, 66.1, 55.5, 54.8, 49.3, 44.3, 43.9, 40.4, 39.9, 39.8, 39.3, 35.9, 35.2, 34.7, 31.9, 29.9, 28.6, 27.4, 25.5, 25.4, 25.3, 25.2, 22.2, 20.8, 18.6, 14.4, 12.2.

CONJ115: $C_{46}H_{64}Cl_2O_7$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.20 (4H, d, *J* = 8.8 Hz, H-3″ and H-5″), 6.79 (4H, d, *J* = 8.8 Hz, H-2″ and H-6″), 4.82 (1H, m, H-3β), 4.14 (2H, m, H_2-24), 3.73 (1H, dd, *J* = 9.9, 6.0 Hz, H-7α), 1.59 (6H, s, H-3′ and H-4′),

1.58 (6H, s, H-3′ and H-4′), 0.90 (3H, d, *J* = 6.5 Hz, H_3-21), 0.98 (3H, s, H_3-19), 0.97 (3H, t, *J* = 7.5 Hz, H_3-26), 0.66 (3H, s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 173.7 (2C), 154.2 (2C), 129.1 (4C), 127.0 (2C), 120.4 (4C), 79.4 (2C), 74.6, 74.3, 66.1, 55.6, 54.9, 48.2, 43.8, 43.4, 40.8, 39.9, 39.8, 35.3, 34.7 (2C), 33.8, 31.9, 29.9, 28.7, 27.3, 26.1, 25.4 (4C), 25.2, 22.2, 20.8, 18.7, 14.2, 12.1.

EXAMPLE 1B-2. Preparation of CONJ116 and CONJ117.

Purification in HPLC, on a reversed-phase column (Luna C18, 10 µm, 10 mm i.d. x 250 mm) and MeOH/H_2O 95:5 as eluent (flow 3 mL/min), of the reaction between **BAR501** (50 mg, 0.123 mmoles) and fenofibric acid (**B**) (1 eq), yielded the compounds **CONJ116** (6.6 mg, t_R = 13.0 min) and **CONJ117** (0.6 mg, t_R = 40.6 min).

CONJ116: $C_{43}H_{59}ClO_6$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.75 (2H, d, *J* = 8.7 Hz, H-3′ and H-5′), 7.72 (2H, d, *J* = 8.4 Hz, H-2″ and H-6″), 7.46 (2H, d, *J* = 8.4 Hz, H-3″ and H-5″), 6.87 (2H, d, *J* = 8.7 Hz, H-2′ and H-6′), 4.15 (2H, m, H_2-24), 3.78 (1H, m, H-7α), 3.61 (1H, m, H-3β), 1.68 (6H, s, H_3-7′e H_3-8′), 0.98 (6H, ovl, H_3-19 and H_3-26), 0.88 (3H, d, *J* = 6.5 Hz, H_3-21), 0.64 (3H, s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 194.1, 173.7, 159.6, 138.3, 136.2, 131.9 (2C), 131.1 (2C), 130.1, 128.5 (2C), 117.2 (2C), 79.3, 74.4, 71.1, 66.2, 55.4, 54.7, 49.4, 44.3, 43.7, 40.3, 39.9, 39.5, 39.3 35.8, 35.1, 34.6, 31.8, 29.9, 28.5, 27.3, 25.5, 25.4 (2C), 25.0, 22.1, 20.8, 18.6, 14.4, 12.1.

CONJ117: $C_{60}H_{72}Cl_2O_9$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.75 (4H, d, *J*

= 8.6 Hz, H-3′ and H-5′), 7.72 (4H, d, *J* = 8.5 Hz, H-2″ and H-6″), 7.46 (4H, d, *J* = 8.5 Hz, H-3″ and H-5″), 6.87 (4H, d, *J* = 8.6 Hz, H-2′e H-6′), 4.81 (1H, m , H-3β), 4.15 (2H, m, H_2-24), 3.64 (1H, m, H-7α), 1.68 (12H, ovl, H_3-7′ and H-8′), 0.96 (6H, ovl, H_3-19 and H_3-26), 0.87 (3H, d, *J* = 6.6 Hz, H_3-21), 0.62 (3H, s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 194.4 (2C), 173.6 (2C), 159.7 (2C), 138.5 (2C), 136.7 (2C), 132.3 (4C), 131.6 (4C), 130.4 (2C), 128.9 (4C), 117.6 (4C), 79.6 (2C), 75.1, 74.7, 66.5, 55.8, 55.6, 55.1, 48.4, 43.6, 41.0, 40.0, 39.9 35.4, 34.9, 32.1, 29.9, 28.7, 27.3, 26.1, 25.7 (4C), 25.3, 25.2, 22.7, 22.2, 21.2, 18.9, 14.4, 12.3.

EXAMPLE 1B-3. Preparation of CONJ118.

The mixture of the coupling reaction between **BAR501** (50 mg, 0.123 mmol) and gemfibrozil (**C**) (1 eq), was purified by HPLC, using a reversed-phase column (Luna C18, 10 µm, 10 mm i.d. x 250 mm) and MeOH/H_2O 95:5 as eluent (flow 3 mL/min), yielding **CONJ118** (10.2 mg, t_R = 20.2 min).

CONJ118: $C_{41}H_{66}O_5$

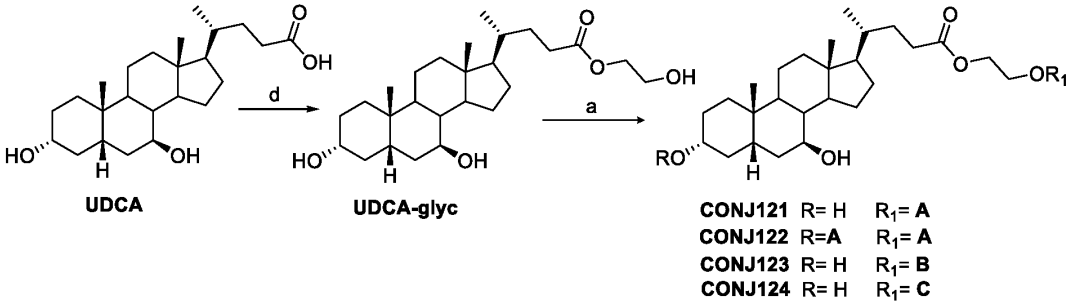
Selected values of ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.01 (1H, d, *J* = 7.4 Hz, H-3″), 6.67 (2H, d, *J* = 7.4 Hz, H-4″), 6.61 (1H, s, H-6″) 4.04 (2H, m, H_2-24), 3.92 (2H, t, *J* = 5.3 Hz, H_2-1′) 3.79 (1H, m, H-7α), 3.61 (1H, m, H-3β), 2.32 (3H, s, H_3-8″), 2.18 (3H, s, H_3-7″), 1.22 (6H, s, H_3-5′ and H_3-6′), 0.98 (6H, ovl, H_3-26 and H_3-19), 0.94 (3H, d, *J* = 6.6 Hz, H_3-21), 0.67 (3H, s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 177.9, 156.9, 136.4, 130.2, 123.5, 120.6, 111.9, 74.6, 71.2, 67.9, 64.9, 55.4, 54.9, 49.3, 44.3, 43.8, 42.1, 40.3, 39.9, 39.8, 39.3, 37.1, 35.8, 35.2, 34.7, 32.1, 29.9, 28.7, 27.4, 25.5, 25.3, 25.2 (2C),

25.1, 22.2, 21.4, 20.8, 18.7, 15.8, 14.5, 12.2.

EXAMPLE 1C. Preparation of conjugates between UDCA and fibrates (CONJ121-124)

Treatment of the **UDCA** (500 mg, 1.27 mmol) with anhydrous ethylene glycol (0.177 mmoles) led to the formation of the esterified intermediate (**UDCA-glyc**) (200 mg), which was divided into three different aliquots and subjected to coupling reaction with the three different activated acids *in situ* [clofibric acid (**A**), fenofibric acid (**B**) and gemfibrozil (**C**)].



(d) *p*-TsOH, ethylene glycol in anhydrous THF; (a) Different acids (**chlofibric acid (A)**, **fenofibric acid (B)**, **gemfibrozil (C)**), EDC•HCl, HOBt, DIPEA, after 30' **UDCA-Glyc** in anhydrous DMF.

EXAMPLE 1C-1. Preparation of CONJ121 and CONJ122.

Treatment of **UDCA-glyc** (50 mg, 0.123 mmol) with clofibric acid (**A**) (3 eq), yielded a mixture of **CONJ121** and **CONJ122**, efficiently separated by HPLC using a reversed-phase column (Luna C18, 10 µm, 10 mm i.d. x 250 mm) and MeOH/H_2O 92:8 as eluent (flow 3 mL/min). This resulted in **CONJ121** (43.7 mg, t_R = 7.1 min) and **CONJ122** (3.8 mg, t_R = 15.7 min).

CONJ121: $C_{36}H_{53}ClO_7$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.20 (2H, d, *J* = 8.9 Hz, H-3″ and H-5″), 6.80 (2H, d, *J* = 8.9 Hz, H-2″ and H-6″), 4.38 (2H, dd, J = 5.8, 3.4 Hz, glyc), 4.28 (2H, dd,

J = 5.8,3.4 Hz glyc), 3.59 (2H, m, H-3β and H-7α), 2.29 (1H, m, H-23a), 2.15 (1H, m, H-23b), 1.59 (6H, s, H_3-3′ and H-4′), 0.95 (3H, s, H_3-19), 0.91 (3H, d, *J* = 6.4 Hz, H_3-21), 0.67 (3H, s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 173.8 (2C), 153.9, 129.2 (2C), 127.4, 120.7 (2C), 79.4, 71.4, 71.3, 63.1, 61.6, 55.6, 54.8, 43.8, 43.7, 42.4, 40.1, 39.1, 37.3, 36.8, 35.2, 34.9, 34.0, 30.9, 30.8, 30.3, 28.6, 26.8, 25.3 (2C), 23.3, 21.1, 18.3, 12.1.

CONJ122: $C_{46}H_{62}Cl_2O_9$

Selected values of ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.20 (4H, d, *J* = 8.9 Hz, H-3″ and H-5″), 6.81-6.79 (4H, d, *J* = 8.9 Hz, H-2″ and H-6″), 4.77 (1H, m, H-3β), 4.38 (2H, dd, *J* = 5.9, 3.4 Hz, glyc), 4.29 (2H, dd, *J* = 5.9, 3.4 Hz glyc), 3.54 (1H, m, H-7α), 2.29 (1H, m, H-23a), 2.16 (1H, m, H-23b), 1.60-1.58 (12H, s, H_3-3′ and H-4′), 0.96 (3H, s, H_3-19), 0.91 (3H, d, *J* = 6.5 Hz, H_3-21), 0.67 (3H, s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 175.1, 174.8, 174.5, 155.1 (2C), 129.6, (4C), 127.2 (2C), 120.8 (4C), 80.5, 75.4, 71.6, 63.5, 62.0, 56.2, 55.3, 44.6, 44.2, 43.2, 42.6, 40.4, 39.4, 36.9, 35.6, 34.9, 33.2, 31.3, 31.1, 29.9, 28.9, 27.1, 26.5, 25.6 (4C), 23.5, 21.5, 18.6, 12.3.

EXAMPLE 1C-2. Preparation of CONJ123.

The reaction mixture between **UDCA-glyc** (50 mg, 0.123 mmol) and fenofibric acid (**B**) (1 eq), was purified by HPLC on a reversed-phase column (Luna C18; 10 μM; 10 mm i.d. x 250 mm) and MeOH/H_2O 95:5 as an eluent (flow 3 mL/min) resulting in the compound **CONJ123** (26.4 mg, t_R = 8.2 min).

CONJ123: $C_{43}H_{57}ClO_8$

Selected values of ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.75 (2H, d, *J*

= 8.6 Hz, H-3″ and H-5″), 7.72 (2H, d, *J* = 8.4 Hz, H-2‴ and H-6′″), 7.46 (2H, d, *J* = 8.4 Hz, H-3‴ and H-5′″), 6.89 (2H, d, *J* = 8.6 Hz, H-2″ and H-6″), 4.40 (2H, m, glyc), 4.27 (2H, m, glyc), 3.59 (2H, m, H-3β and H-7α), 2.27 (1H, m, H-23a), 2.15 (1H, m, H-23b), 1.69 (6H, s, H_3-3′ and H-4′), 0.95 (3H, s, H_3-19), 0.88 (3H, d, *J* = 6.3 Hz, H_3-21), 0.66 (3H, s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 194.1, 173.8, 173.5, 159.5, 138.4, 136.3, 131.9 (2C), 131.2 (2C), 130.5, 128.6 (2C), 117.4 (2C), 79.3, 71.4, 71.3, 63.2, 61.6, 55.7, 54.8, 43.8, 43.7, 42.4, 40.1, 39.1, 37.3, 36.8, 35.1, 34.9, 34.0, 30.9, 30.8, 30.3, 28.6, 26.8, 25.4 (2C), 23.3, 21.1, 18.3, 12.1.

EXAMPLE 1C-3. Preparation of CONJ124.

The reaction mixture obtained by treatment of **UDCA-glyc** (50 mg, 0.123 mmoles) with gemfibrozil (C) (1 eq), was purified by HPLC on a reversed-phase column (Luna C18, 10 µm, 10 mm i.d. x 250 mm) and using MeOH/H_2O 95:5 as eluent (flow 3 mL/min) obtaining **CONJ124** (17 mg, t_R = 12.0 min).

CONJ124: $C_{41}H_{64}O_7$

Selected values of ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.01 (1H, d, *J* = 7.5 Hz, H-3″), 6.67 (1H, d, *J* = 7.5 Hz, H-4″), 6.61 (1H, s, H-6″), 4.28 (4H, s, glyc), 3.92 (2H, t, *J* = 5.5 Hz, H-1′), 3.58 (2H, br s, H-3β and H-7α), 2.35 (2H, m, H-23a), 2.32 (3H, s, H_3-8″), 2.22 (2H, m, H-23b), 2.19 (3H, s, H_3-7″), 1.20 (6H, s, H_3-5′ and H_3-6′), 0.95 (3H, s, H_3-19), 0.90 (3H, d, *J* = 6.5 Hz, H_3-21), 0.66 (3H, s, H_3-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 177.8, 173.9, 156.7, 136.5, 130.5, 123.5, 120.6, 111.7, 71.5, 71.3, 67.7, 62.1, 61.9, 55.5, 54.8, 43.7 (2C), 42.4, 42.1, 40.0, 39.1, 37.2, 37.0, 36.8, 35.2, 34.8, 34.0, 31.1, 30,9, 30.2, 28.6, 26.8, 25.1 (3C),

23.4, 21.4, 21.1, 18.3, 15.7, 12.1.

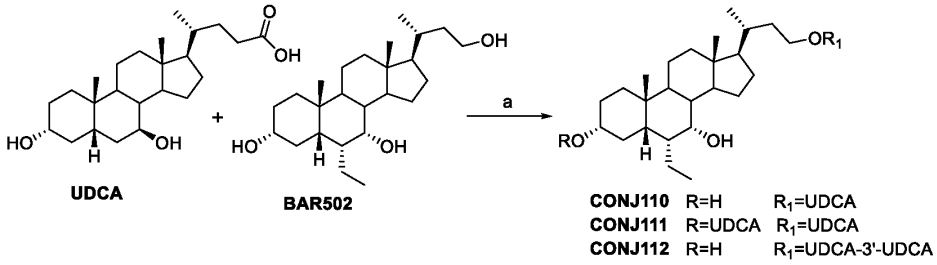
EXAMPLE 2. PREPARATION OF DIMERIC OR TRIMERIC BA CONJUGATES.

The same coupling reactions discussed above were used to obtain the different dimeric or trimeric BA conjugates.

The carboxylic function of **UDCA** and of **BAR704** was activated *in situ* with coupling reagents (EDC HCl (4 eq) and HOBt (4 eq) and diisopropylethylamine (8 eq) in anhydrous DMF. After 30 min, 1 eq of the alcohols **BAR502, BAR501** and **UDCA-Glyc** were added to the reactions, and kept at room temperature overnight. When the reaction is complete, the solvent is removed under vacuum and the crude is extracted with H_2O/EtOAc three times. The organic phases are anhydrified with Na_2SO_4, filtered and concentrated under vacuum to obtain dimeric or trimeric derivatives.

EXAMPLE 2A-1. Preparation of CONJ110-CONJ112.

The reaction resulting from the treatment of **BAR502** with **UDCA** yielded a mixture of compounds that were efficiently separated by HPLC using a reversed-phase column (Luna C18, 10 μM; 10 mm i.d. x 250 mm). Specifically, using a mixture of MeOH/H_2O 92:8 as an eluent (flow 3 mL/min) the compound **CONJ110** was obtained(10.1 mg, t_R = 37.6 min), while using a mixture of MeOH/H_2O 99:1 as eluent **CONJ111** (7.5 mg, t_R = 23.4 min) and the compound **CONJ112** (7.5 mg, t_R = 29.2 min) were obtained.



a) **UDCA**, EDC•HCl, HOBt, DIPEA, after 30' **BAR502** in anhydrous DMF.

CONJ110: $C_{49}H_{82}O_6$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 4.20 (1H, m, H-23a BAR502), 4.18 (1H, m, H-23b BAR502), 3.71 (1H, s, H-7β BAR502), 3.60 (2H, ovl, H-3β and H-7α UDCA) 3.44 (1H, m, H-3β BAR502), 2.24 (1H, m, H-23a UDCA), 2.21 (1H, m, H-23b UDCA), 0.98-0.90 (15H, ovl, H_3-19, H_3-21, H_3-25 BAR502, H_3-21 and H_3-19 UDCA), 0.68 (6H, s, H_3-18 BAR502 and H_3-18 UDCA).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 174.5, 72.5, 71.6, 71.4, 71.1, 62.6, 56.2, 55.8, 55.0, 50.7, 45.4, 44.0, 42.9, 42.5, 41.3, 40.3, 40.1, 39.7, 39.3, 37.5, 37.0, 35.6 (2C), 35.4, 35.0, 34.7, 34.2, 34.1, 33.3 (3C), 31.6, 31.2, 30.7, 30.5, 28.7, 28.5, 27.0, 23.8, 23.5, 23.3, 22.4, 21.3, 20.8, 18.9, 18.5, 12.3, 11.8 (2C).

CONJ111: $C_{73}H_{120}O_9$

Selected values of ^1H NMR (700 MHz, $CDCl_3$): δ_H 4.56 (1H, m, H-3β BAR502), 4.14 (1H, m, H-23a BAR502), 4.04 (1H, m, H-23b BAR502), 3.71 (1H, s, H-7β BAR502), 3.58 (4H, ovl, H-3β UDCA and H-7α UDCA) 2.33 (2H, m, H-23a UDCA and H-23a′ UDCA′), 2.20 (2H, m, H-23b UDCA and H-23b′ UDCA′), 0.97 (3H, d, J = 6.5 Hz, H_3-21 BAR502), 0.96 (6H, ovl, H_3-19 UDCA and H_3-19′ UDCA′), 0.93 (9H, m, H_3-21 BAR502, H_3-21 UDCA and H_3-21′ UDCA′), 0.90 (3H, t, J = 7.4 Hz, H_3-25 BAR502), 0.70 (6H, s, H_3-18, UDCA and H_3-18′ UDCA′), 0.69 (3H, s, H_3-18 BAR502).

^{13}C NMR (175 MHz, $CDCl_3$): δ_C 174.4 (2C), 74.5, 71.5 (4C), 70.9, 62.6, 56.0, 55.7 (2C), 55.0 (2C), 50.4, 45.0, 43.8 (2C), 43.7 (2C), 43.5, 42.3 (2C), 41.2, 40.2 (2C), 40.0, 39.6, 39.3 (2C), 37.3 (2C), 36.7 (2C), 35.2, 35.1 (2C), 35.0, 34.9 (2C), 34.7, 34.6, 34.0 (2C), 33.2, 33.1, 31.5 (2C), 31.0 (2C), 30.8, 30.2 (2C), 28.4 (3C), 26.8 (2C), 23.5, 23.3 (2C), 23.1, 22.0, 21.1 (2C), 20.8, 18.6, 18.4 (2C), 11.9 (3C),

11.6.

CONJ112: $C_{73}H_{120}O_9$

Selected values of ^1H NMR (700 MHz, $CDCl_3$): δ_H 4.70 (1H, m, H-3β UDCA), 4.14 (1H, m, H-23α, BAR502), 4.07 (1H, m, H-23b, BAR502), 3.70 (1H, s, H-7β, BAR502), 3.60 (3H, ovl, H-3β UDCA, H-3'β UDCA' and H-7α UDCA') 2.33 (2H, m, H-23a UDCA and H-23a' UDCA'), 2.19 (2H, m, H-23b UDCA and H-23b' UDCA'), 0.96 (3H, d, *J*=6.6 Hz, H_3-21 BAR502), 0.95 (6H, ovl, H_3-19 UDCA and H_3-19' UDCA'), 0.93 (6H, m, H_3-21 UDCA and H_3-21' UDCA'), 0.90 (3H, s, H_3-19 BAR502), 0.91 (3H, t, J=7.4 Hz, H_3-25 BAR502), 0.68 (6H, s, H_3-18, UDCA and H_3-18' UDCA'), 0.66 (3H, s, H_3-18 BAR502).

^{13}C NMR (175 MHz, $CDCl_3$): δ_H 174.2, 173.6, 73.3, 72.2, 71.4 (3C), 71.0, 62.3, 55.8 (2C), 55.7, 54.8 (2C), 50.6, 45.0, 43.7 (4C), 42.7, 42.2 (2C), 41.0, 40.0 (3C), 39.6, 39.1 (2C), 37.3, 37.0 (2C), 35.3 (2C), 35.0 (2C), 34.8 (2C), 34.7, 34.6, 34.0 (2C), 33.2, 33.1 (2C), 31.4 (2C), 31.0 (2C), 30.7, 30.3, 28.4 (3C), 26.7 (2C), 26.5, 23.3, 23.4 (2C), 22.8, 22.0, 20.8, 21.0 (2C), 18.6, 18.2 (2C), 11.9 (3C), 11.5.

EXAMPLE 2A-1. Preparation of CONJ113.

The reaction mixture between **BAR502** and **BAR704** with the previously described procedure was purified by HPLC using a reversed-phase column (Synergi fusion C18, 4 μM; 4.6 mm i.d. x 250 mm) and MeOH/H_2O 99.8:0.2 as eluent (flow 1 mL/min) providing **CONJ113** (1.3 mg, t_R = 14.4 min).

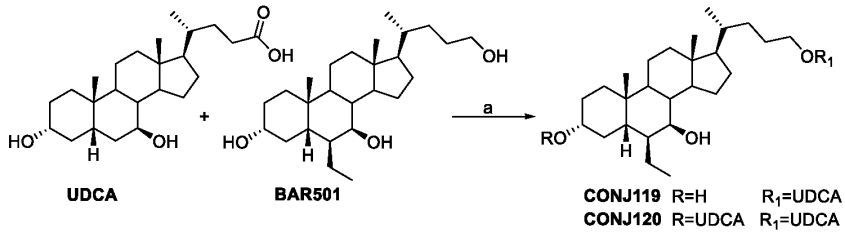
CONJ113: $C_{51}H_{86}O_5$

Values of ^1H NMR selected(700 MHz, $CDCl_3$): δ_H 4.15 (1H, m, H-23α), 4.06 (1H, m, H-23β, BAR502), 3.71 (2H, ovl, H-7β BAR502 and H-7β BAR704), 3.41 (1H, m, H-3β, BAR502), 2.34 (1H, m, H-23α, BAR704), 2.22 (1H, m, H-23β. BAR704), 0.99-0.97 (6H,

ovl, H_3-21 BAR502 and H_3-19 BAR704), 0.95-0.90 (12H, ovl, H_3-19, H_3-25 BAR502, H_3-26 BAR704 and H_3-21 BAR704), 0.68 (6H, s, H_3-18 BAR502 and H_3-18 BAR704).

EXAMPLE 2B-1. Preparation of BAR501-UDCA conjugates (CONJ119-120).

The treatment of **BAR501** (50 mg, 0.123 mmol) with **UDCA**, using the same procedure as previously described, yielded a mixture of compounds that was efficiently separated by HPLC using a reversed-phase column (Luna C18, 10 μM; 10 mm i.d. x 250 mm) and MeOH/H_2O 99:1 as eluent (flow 3 mL/min). The compounds **CONJ119** (13.9 mg, t_R = 15.0 min) and **CONJ120** (5.9 mg, t_R = 27.8 min) were obtained in this way.



a) **UDCA**, EDC•HCl, HOBt, DIPEA, after 30' **BAR501** in anhydrous DMF.

CONJ119: $C_{50}H_{84}O_6$

Selected values of ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 4.04 (2H, m, H_2-24 BAR501), 3.81 (1H, m, H-7α BAR501), 3.60 (3H, ovl, H-3β UDCA, H-7α UDCA and H-3β BAR501), 2.34 (1H, m, H-23a UDCA), 2.24 (1H, m, H-23b UDCA), 0.99 (3H, s, H_3-19 BAR501), 0.97 (3H, ovl, H_3-21 BAR501), 0.96 (6H, ovl, H_3-19 UDCA and H_3-26 BAR501), 0.94 (3H, ovl, H_3-21 UDCA), 0.69 (6H, s, H_3-18 UDCA and H_3-18 BAR501).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 174.3, 74.7, 71.5, 71.3, 71.2, 64.8, 55.7, 55.6, 54.9, 54.8, 49.4, 44.3, 43.9, 43.7 (2C), 42.5, 40.4, 40.1, 40.0, 39.8, 39.3, 39.2, 37.3, 36.8, 35.8, 35.2 (2C), 34.9, 34.7, 34.0, 32.0, 31.4, 31.2, 30.4, 29.9, 28.7, 28.6, 27.5, 26.9, 25.5, 25.2, 23.4, 22.1, 21.2, 20.8,

18.7, 18.4, 14.5, 12.2, 12.1.

CONJ120: $C_{74}H_{122}O_9$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 4.71 (1H, m, H-3β BAR501), 4.06 (1H, m, H-24a BAR501), 4.02 (1H, m, H-24b BAR501), 3.80 (1H, m, H-7α BAR501), 3.60 (4H, ovl, H-3β and H-7α UDCA, H-3'β and H-7'α UDCA'), 2.33 (2H, m, H-23a and H-23a' UDCA'), 2.24 (2H, m, H-23b and H-23b' UDCA'), 0.99-0.93 (21H, ovl, H_3-19, H_3-21 and H_3-26 BAR501, H_3-19 and H_3-21 UDCA, H_3-19' and H_3-21' UDCA'), 0.69 (9H, s, H_3-18 BAR501, H_3-18 UDCA and H_3-18' UDCA').

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 174.3, 173.8, 74.6, 73.2, 71.3 (4C), 64.8, 55.7 (3C), 54.8 (3C), 49.2, 43.9, 43.8 (3C), 43.7 (2C), 42.4 (2C), 40.6, 40.0 (3C), 39.7, 39.4, 39.2 (2C), 37.3 (2C), 36.8 (2C), 35.7, 35.2 (3C), 34.9 (2C), 34.7, 34.0 (2C), 31.9, 31.5 (2C), 31.1 (2C), 30.1 (2C), 29.9, 28.6 (3C), 27.4, 26.9 (2C), 25.4, 25.2, 23.4 (2C), 22.1, 21.0 (3C), 18.5 (3C), 14.4, 12.2 (3C).

EXAMPLE 2C-1. Preparation of the CONJ125 dimer.

The reaction mixture obtained by treatment of **UDCA-glyc** (50 mg, 0.123 mmol) with **BAR704 (D)** (1 eq), was purified on a reversed-phase column (Luna C18, 10 µm, 10 mm i.d. x 250 mm) and using MeOH/H_2O 95:5 as eluent (flow 3 mL/min) obtaining **CONJ125** (9.6 mg, t_R = 36.2 min)

CONJ125: $C_{52}H_{86}O_7$

Selected values of ^1H NMR (400 MHz, $CDCl_3$): δ_H 4.28 (4H, s, glyc), 3.69 (1H, s, H-7β BAR704), 3.59 (2H, m, H-3β and H-7α UDCA), 2.39 (2H, ovl, H-23a UDCA and BAR704), 2.25 (2H, ovl, H-23b UDCA and BAR704), 0.96 (3H, s, H_3-19 BAR704), 0.95 (3H, s, H_3-19 UDCA), 0.94 (3H, d, J = 6.5 Hz, H_3-21 BAR704), 0.90-

0.91 (6H, ovl, H_3-21 UDCA and H_3-26 BAR704), 0.69 (3H, s, H_3-18 BAR704), 0.67 (3H, s, H_3-18 UDCA).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 174.0, 173.9, 71.5, 71.4, 71.2, 62.1, 62.0, 55.8, 55.7, 54.9, 50.0, 46.9, 43.8, 43.7, 42.8, 42.4, 41.4, 40.1, 40.0, 39.7, 39.1, 38.0, 37.3, 36.9, 36.3, 35.4, 35.2, 34.9, 34.1, 33.4, 31.1 (2C), 30.9 (2C), 30.3, 28.6, 28.2, 27.6, 26.9, 24.2, 24.0, 23.7, 23.3, 22.2, 21.3, 21.2, 20.8, 18.4, 18.2, 12.1, 11.8, 11.7.

BIOLOGICAL DATA

The biological activity towards TGR5/GPBAR1 of the selected compounds (Table 1) was tested in vitro, using a cell model transfected with reporter genes for the TGR5/GPBAR1 receptor, in comparison to the control agonist, taurolithocholic acid (TLCA), a physiological ligand of the TGR5/GPBAR1 receptor.

HEK-293T cells were maintained in culture at 37°C in D-MEM (Dulbecco's Minimum Essential Medium) medium supplemented with 10% foetal bovine serum (FBS), 1% L-Glutamine, and 1% penicillin/streptomycin. Transfection experiments were performed using Fugene HD reagent (Promega) according to the manufacturer's instructions. The cells were plated in 24-well plates in numbers of $5x10^4$ cells/well. HEK-293T cells were transfected with: 200 ng of the pGL4.29 plasmid (Promega), a reporter vector containing the cAMP response element (CRE) cloned upstream of the luc2P luciferase reporter gene, 100 ng of the human pCMVSport6-GPBAR1 vector, and 100 ng of the pGL4.70 Renilla vector, a plasmid encoding for the human Renilla gene.

In the control experiments, the HEK-293T cells were transfected only with the pGL4.29 (Promega) and pGL4.70

Renilla vectors, to exclude any possibility that the compounds could activate CRE in a GPBAR1-independent manner.

24 hours after transfection, the cells were stimulated for 18h with 10 µM CDCA, TLCA, GW3965 or Rifaximin (10µM), used as control agents, or with the test compounds at the same concentration. After the treatments, the cells were lysed in 100 µL of lysis buffer (25 mM Tris-phosphate, pH 7.8; 2 mM DTT; 10% Glycerol; 1% Triton X-100); 10 µL of the cell lysate from each sample was tested for luciferase activity using the Dual Luciferase Reporter Assay Sistem (Promega Italia s.r.l., Milan, Italy) according to the manufacturer's instructions. Luminescence was measured using the Glomax 20/20 luminometer (Promega Italia s.r.l., Milan, Italy). Luciferase activity (Luciferase Reading Unit, RLU) was normalised using Renilla activity (Renilla Reading Unit, RRU).

To assess the activity of the selected compounds towards the nuclear receptor FXR, a cell-free AlphaScren assay was performed (Table 1). The ability of the compounds to mediate recruitment of the SRC1 co-activator was assessed using the AlphaScreen GST Detection Kit (PerkinElmer). In particular, the LBD of FXR fused with GST (10 nM) and the biotinylated peptide derived from SRC1 (CPSSHSSLTERHKILHRLLQEGSPS) at a concentration of 30 nM were used. The experiment was conducted in the presence of 20 µg/mL of the donor and acceptor beads and in a buffer containing Tris-HCl 50 mM (pH7.4), KCl 20 mM, DTT 1 mM and 0.1% BSA. The activity of the selected compounds was compared with that of chenodeoxycholic acid (CDCA), a primary bile acid that functions as an endogenous ligand of the FXR receptor.

The data on the activity of selected compounds within formula (I) towards FXR and TGR5/GPBAR1 receptors are described in Table 1. In Table 1, the efficacy of the selected compounds is reported in comparison to the reference compounds, CDCA for FXR, taurolithocholic acid (TLCA) for TGR5/GPBAR1 for which the activity was considered to be 100%. Each compound was tested at a concentration of 10 µM.

Table 1

Compounds	**FXR efficacy (% vs CDCA)**	**GPBAR1 efficacy (% vs TLCA)**
CONJ101	79 ± 3.8	145 ± 10
CONJ102	48 ± 5.5	101 ± 0.40
CONJ103	3.9 ± 0.1	46 ± 16
CONJ104	14 ± 1.8	41 ± 3.0
CONJ105	n.a.	30.2 ± 17
CONJ106	n.a.	40 ± 0.7
CONJ107	4.1± 0.6	14 ± 1.3
CONJ108	5.1 ± 1.9	28.5 ± 0.42
CONJ109	n.a.	29.4 ± 5.1

For **CONJ101** the anti-inflammatory activity was evaluated on a U937 cell line (a human monocytic/macrophage cell line)

The U937 cells were first stimulated with LPS (100 ng/mL), which induces a polarization towards a pro-inflammatory phenotype as shown by the increased expression of IL-1β, IL-6 and CXCL2 (Figure 1).

The cells were treated with **BAR502**, clofibrate (5 and 10 µM), **CONJ101** (10 µM) and clofibric acid (10 µM).

All molecules showed anti-inflammatory activity by reducing the expression of pro-inflammatory cytokines. However, **CONJ101** showed the greatest efficacy in reducing the expression of both IL-1β and IL-6 and was the only compound able to reduce the expression of the chemokine CXCL2. Collectively, these data highlight the powerful anti-inflammatory effect of **CONJ101** (Figure 1A-C).

To assess the effect of the selected compound on the metabolic pathway, 3T3.L1 cells (a pre-adipocytic murine cell line) were used.

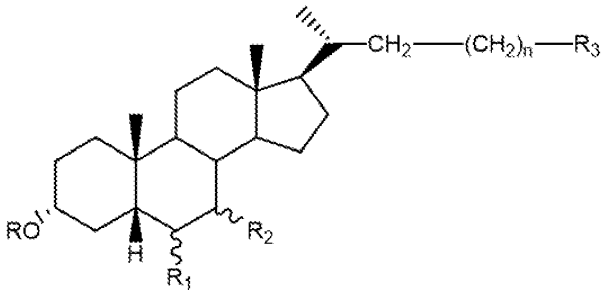
These cells were treated for 7 days with differentiation medium (DIM) alone or in combination with **BAR502**, clofibrate or **CONJ101** (Figure 1D-F).

DIM-induced adipocytic differentiation increases the expression of adiponectin, Pparγ and Cebp.

CONJ101 is able to reverse the DIM-induced effect as shown by the reduced expression of all markers of adipocyte differentiation (Figure 1D-F).

CLAIMS

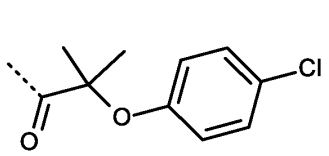
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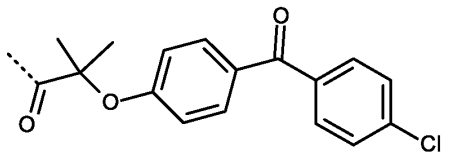
(I)

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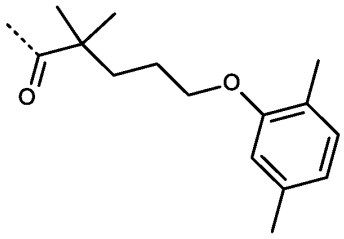
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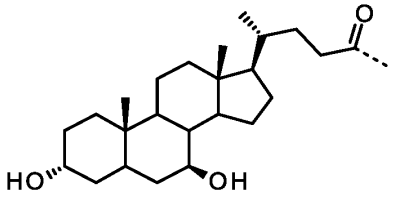
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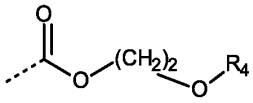


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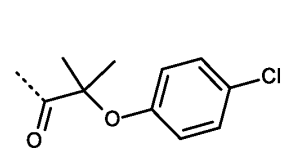
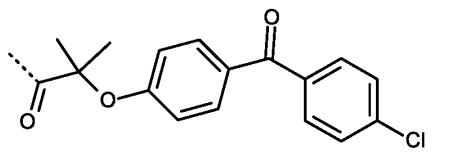
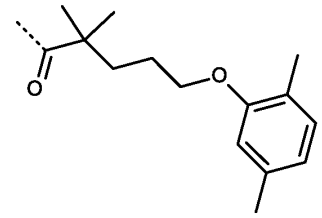
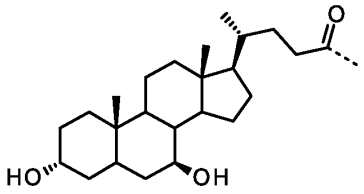
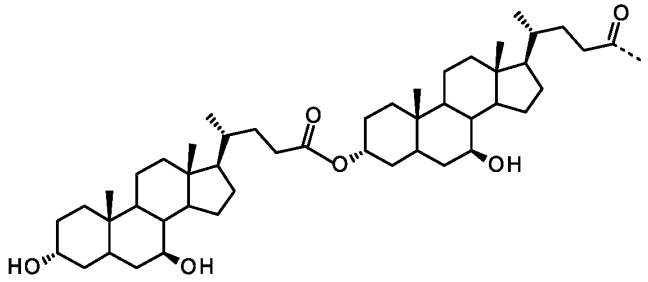
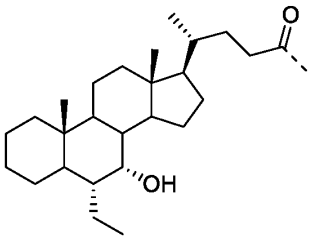
R_1 is selected from the group consisting of H and ethyl;

R_2 is OH

R_3 is selected from the group consisting of O-R_4,



R_4 is selected from the group consisting of H,

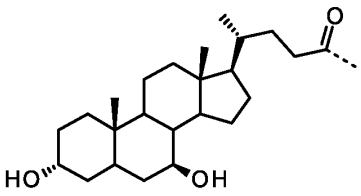







n is selected from the group consisting of 1 and 2

or its pharmaceutically acceptable salts or solvates

provided that

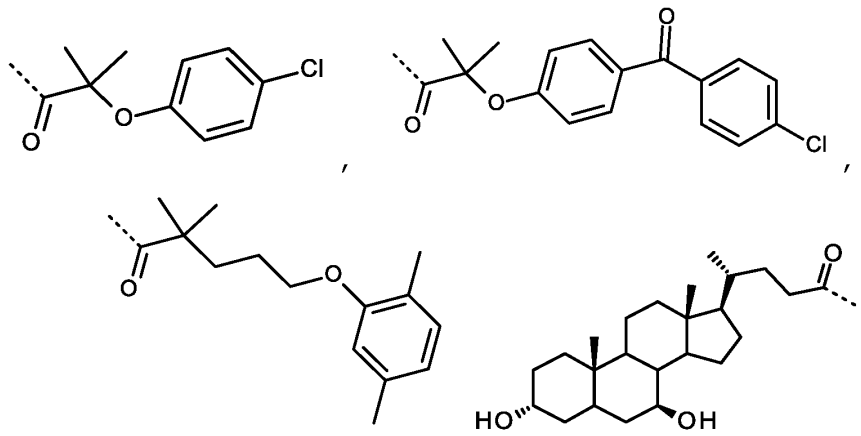
- R and R_4 are not H at the same time; or
- when R and R_1 are H, R_2 is βOH and n is 1, R_4 is not



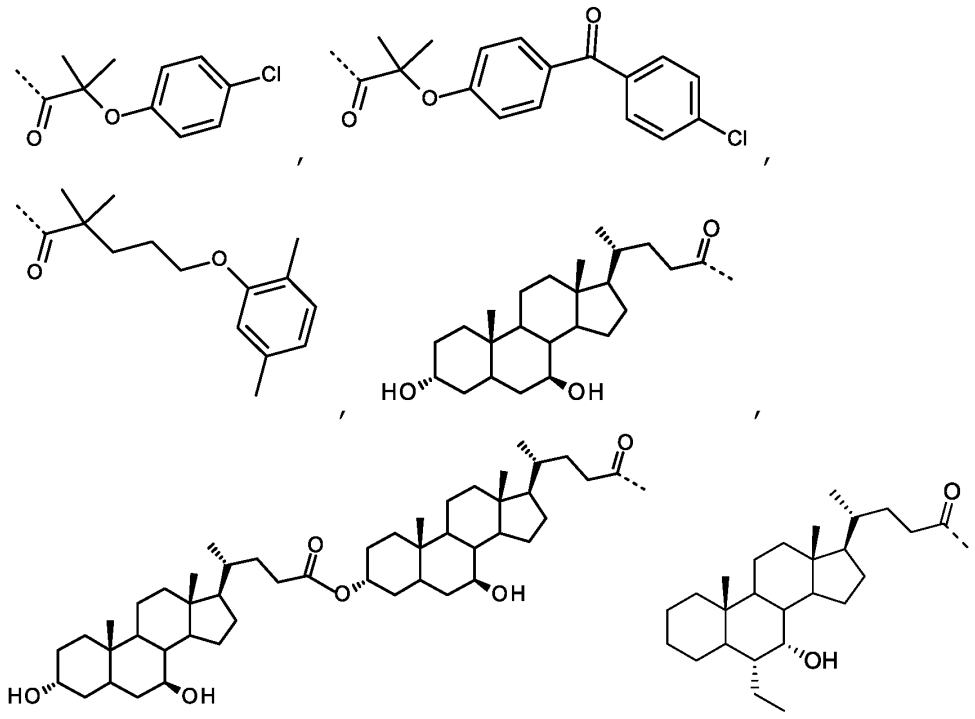
.

2.- Compound of formula (I) according to claim 1, characterized in that R_1 is α-ethyl, R_2 is α-OH and n is 1.

3.- Compound of formula (I) according to claim 2, characterized in that R is selected from the group consisting of H,

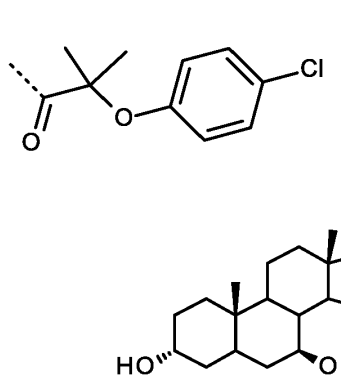


R_3 is O-R_4 and R_4 is selected from the group consisting of H,

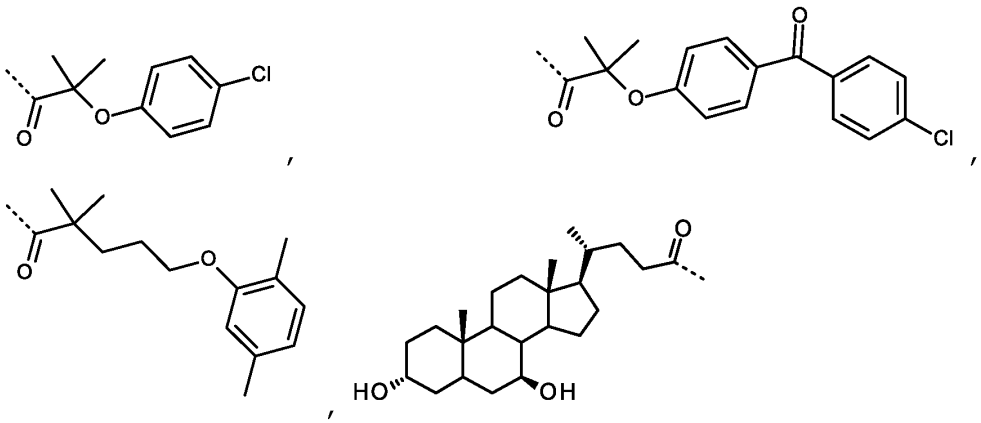


4.- Compound of formula (I) according to claim 1, characterized in that R_1 is β-ethyl, R_2 is β-OH and n is 2.

5.- Compound of formula (I) according to claim 4, characterized in that R is selected from the group consisting of H,

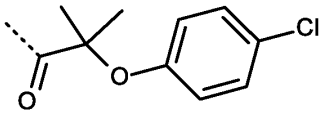


R_3 is O-R_4 and R_4 is selected from the group consisting of

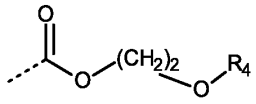


6.- Compound of formula (I) according to claim 1, characterized in that R_1 is H, R_2 is β-OH, n is 1.

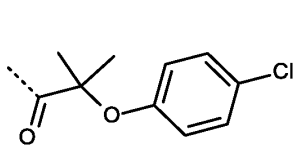
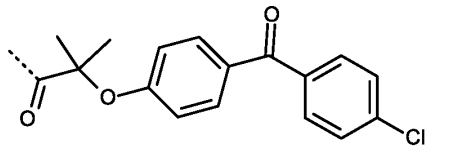
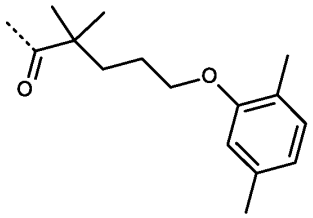
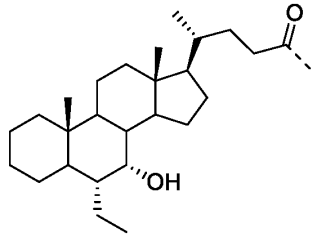
7.- Compound of formula (I) according to claim 6, characterized in that R is selected from the group consisting of H,



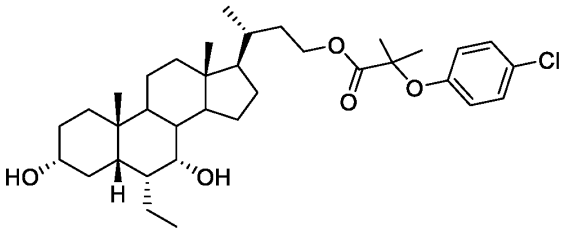
R_3 is



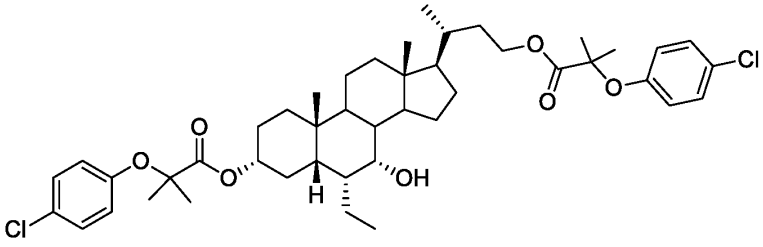
and R_4 is selected from the group consisting of


,

,

,

,

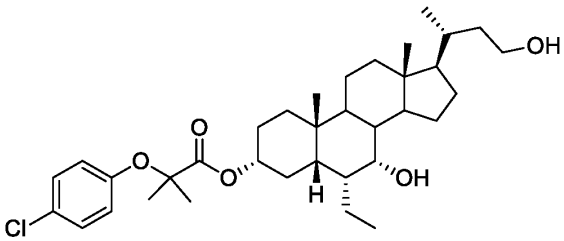
8.- Compound of formula (I) according to any of claims 1 to 7 characterized in being selected from the group consisting of:



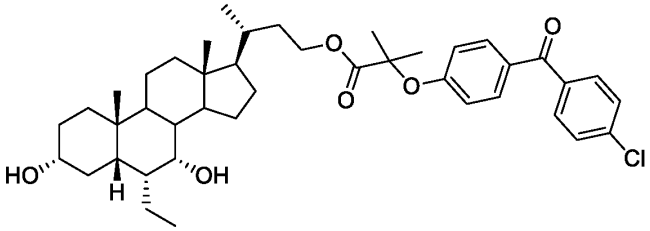
CONJ101



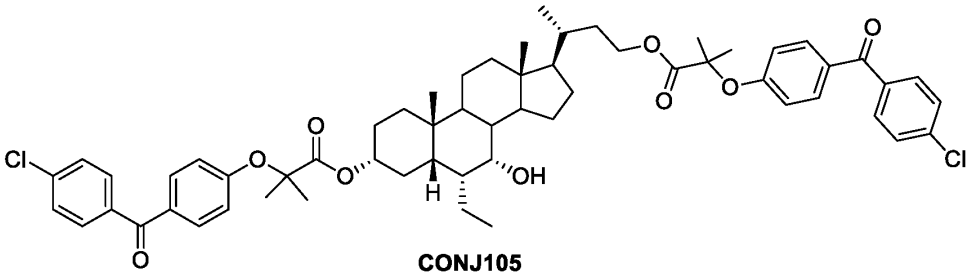
CONJ102



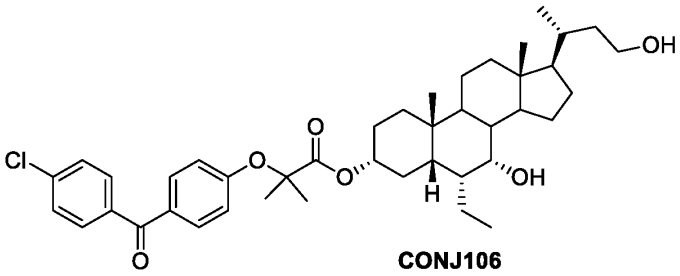
CONJ103



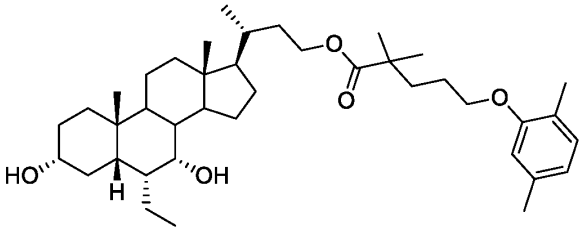
CONJ104



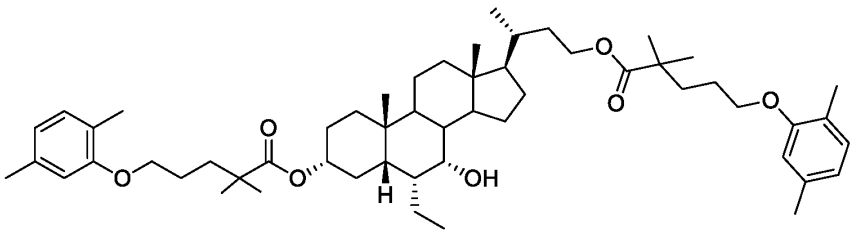
CONJ105



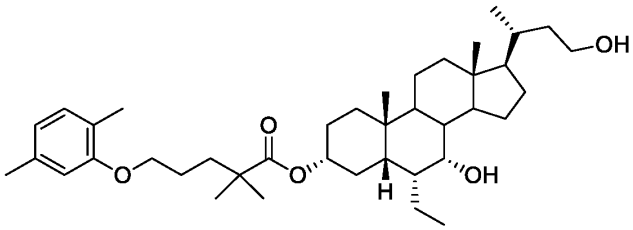
CONJ106



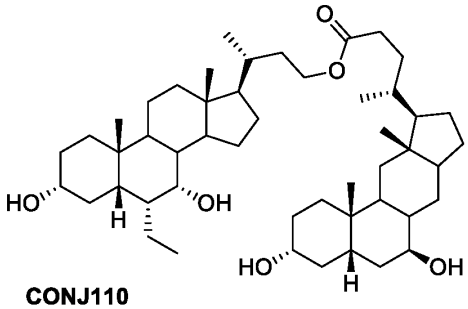
CONJ107



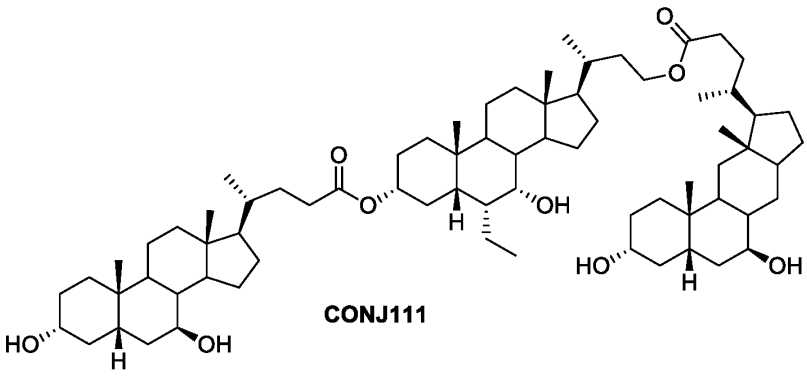
CONJ108



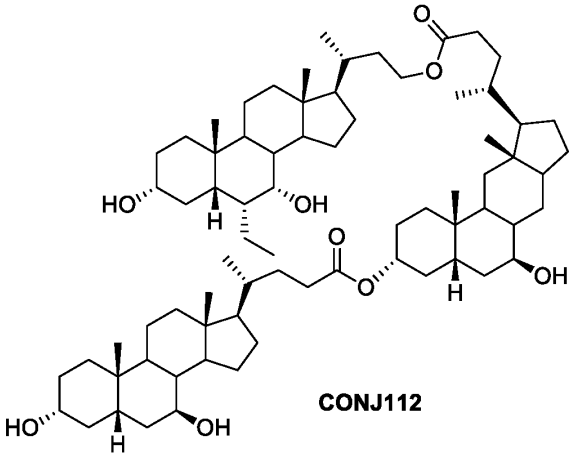
CONJ109



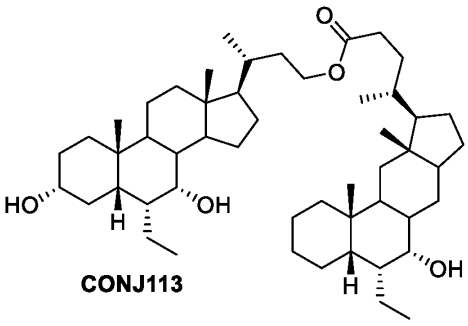
CONJ110



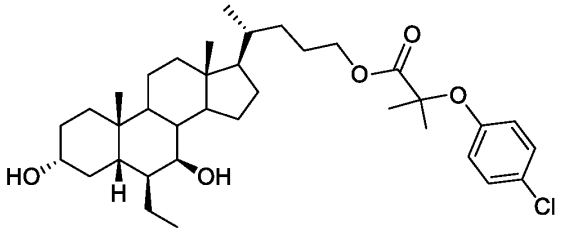
CONJ111



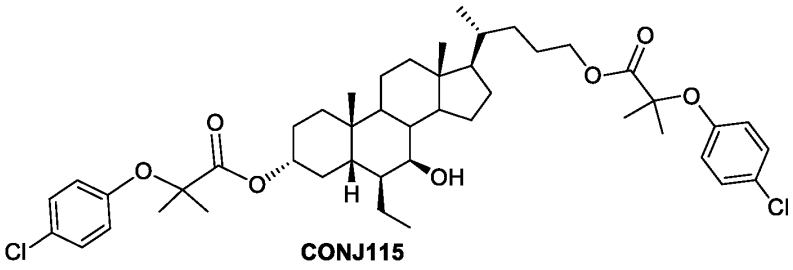
CONJ112



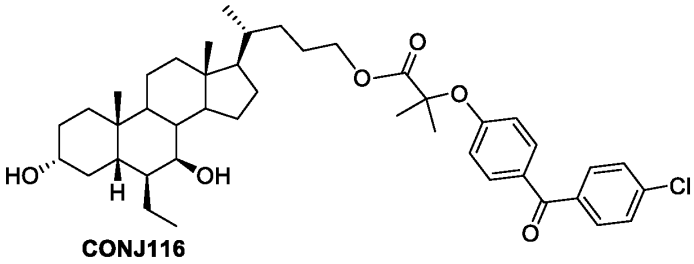
CONJ113



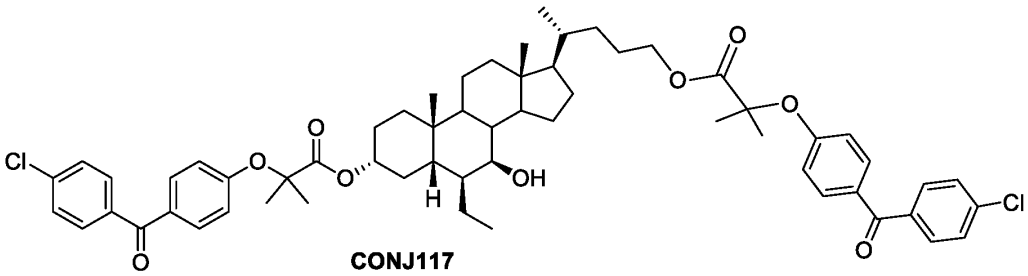
CONJ114



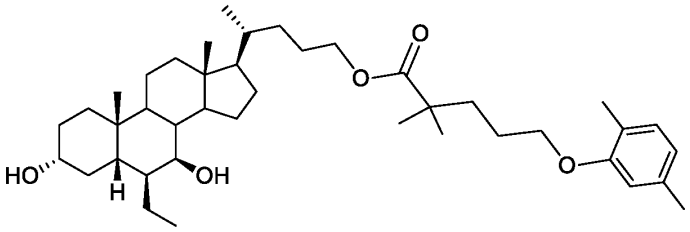
CONJ115



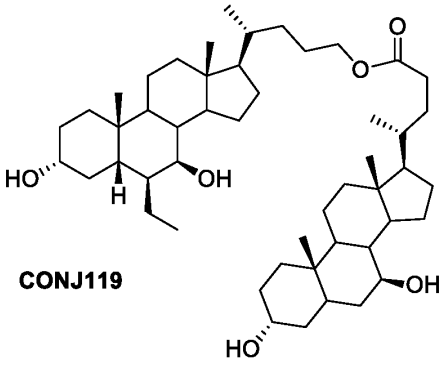
CONJ116



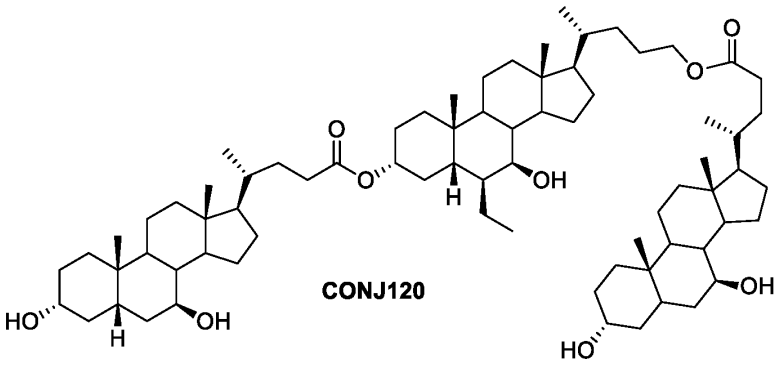
CONJ117



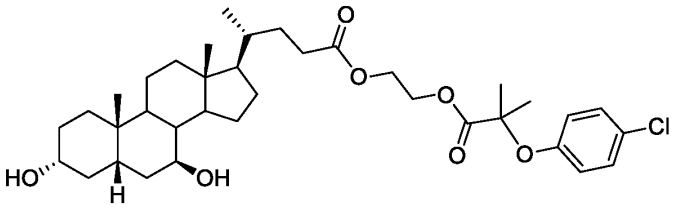
CONJ118



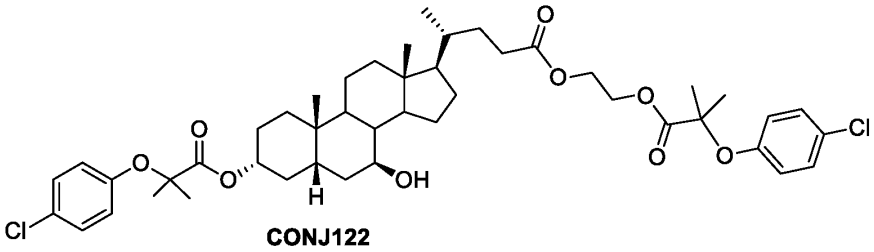
CONJ119



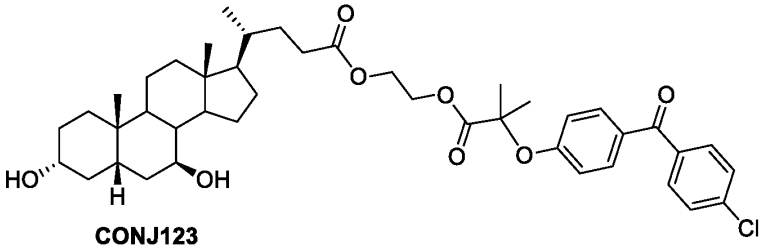
CONJ120



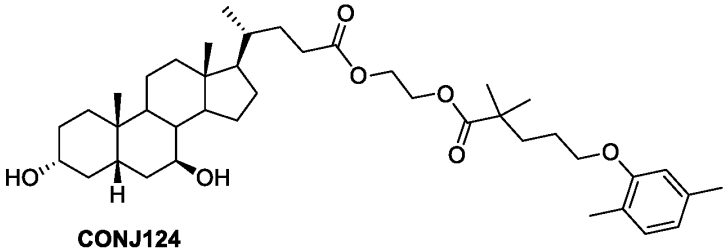
CONJ121



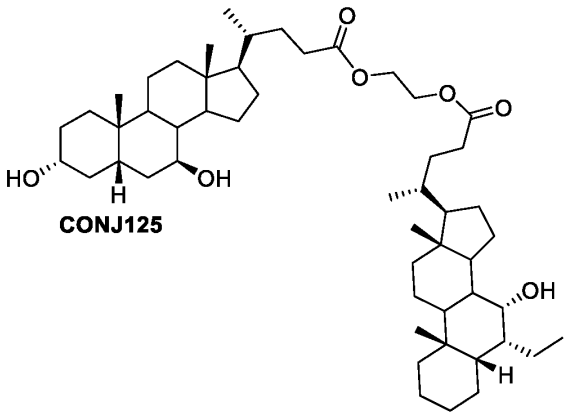
CONJ122



CONJ123



CONJ124



CONJ125

9.- Pharmaceutical composition comprising a compound of formula (I) according to any of claims 1 to 8 and at least one pharmaceutically acceptable excipient.

10.- Compound of formula (I) according to any of claims 1 to 8 for the use as a medicament.

11.- Compound of formula (I) according to any of claims 1 to 8 for the use in the prevention and/or in the treatment of a disorder selected from the group consisting of gastrointestinal disorders, liver disorders, cardiovascular disorders, vascular disorders, pulmonary disorders, methabolic disorders, infectious diseases, cancer, renal disorders, inflammatory disorders including immune-mediated disorders and neurological disorders.

12.- Compound of formula (I) for the use according to claim 11 characterized in that immune-mediated inflammatory disorders are selected from the group consisting of systemic lupus erythematosus, rheumatoid arthritis, Syogren's disease, scleroderma also known as systemic sclerosis, spondyloarthritis, vasculitis, sarcoidosis, Mediterranean fever, inherited auto-inflammatory diseases, polymyositis dermatomyositis and Behcet's syndrome; infectious diseases

are selected from the group consisting of acquired immunodeficiency syndrome (AIDS) and associated disorders, B virus and C virus infections; neurological disorders are selected from the group consisting of Alzheimer's disease and other dementias, Parkinson's disease and other movement disorders, amyotrophic lateral sclerosis and other motor neuron disorders, multiple sclerosis and other demyelinating diseases, ischaemic stroke, myasthenia and muscular dystrophies; liver disorders are selected from the group consisting of primary biliary cirrhosis (PBC), cerebrotendinous xanthomatosis (CTX), primary sclerosing cholangitis (PSC), drug-induced cholestasis, intrahepatic cholestasis gravidarum, cholestasis associated with parenteral nutrition, cholestasis associated with bacterial proliferation or sepsis, autoimmune hepatitis, chronic viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), liver transplantation, congenital liver fibrosis, granulomatous liver disease, intra- or extra-hepatic malignant tumour, Wilson's disease, haemochromatosis, and alpha 1-antitrypsin deficiency; gastrointestinal disorders are selected from the group consisting of inflammatory bowel disease (IBD) including Crohn's disease, ulcerative rectocolitis and indeterminate colitis, irritable bowel syndrome (IBS), bacterial proliferation, acute and chronic pancreatitis, malabsorption, post-radiation colitis, and microscopic colitis; renal disorders are selected in the group consisting of diabetic nephropathy, hypertensive nephropathy, chronic glomerulonephritis, including chronic transplant glomerulonephritis, chronic tubulo-interstitial

diseases and vascular disorders of the kidney; cardiovascular disorders are selected from the group consisting of atherosclerosis, arteriosclerosis, dyslipidaemia, hypercholesterolaemia, hypertension also known as arterial hypertension, cardiac inflammatory disorders including myocarditis and endocarditis, cardiac ischaemia, stable angina and unstable angina myocardial infarction, cerebrovascular disorders including ischaemic stroke; vascular disorders are selected from the group consisting of cardiopulmonary diseases such as pulmonary hypertension, peripheral artery disease (PAD), also known as peripheral vascular disease (PVD), peripheral arterial occlusive disease and peripheral arterial obliterative disease; pulmonary disorders are selected from the group consisting of asthma, cystic fibrosis, obstructive respiratory disease, interstitial lung disease including, but not limited to, primary or secondary pulmonary fibrosis; metabolic pathology is selected from the group consisting of insulin resistance, metabolic syndrome, type I and type II diabetes, hypoglycaemia, adrenal cortex disorders including adrenal cortex insufficiency, obesity and conditions associated with bariatric surgery cancer is selected in the group consisting of liver cancer, bile duct cancer, oesophageal cancer, pancreatic cancer, gastric cancer, colorectal cancer, breast cancer, ovarian cancer and the condition associated with resistance to chemotherapy.