Novel FXR (NR1H4) binding and activity modulating compounds

The present invention relates to compounds which bind to the NR1H4 receptor (FXR) and act as agonists or modulators of FXR. The invention further relates to the use of the compounds for the treatment and/or prophylaxis of diseases and/or conditions through binding of said nuclear receptor by said compounds.

Multicellular organisms are dependent on advanced mechanisms of information transfer between cells and body compartments. The information that is transmitted can be highly complex and can result in the alteration of genetic programs involved in cellular differentiation, proliferation, or reproduction. The signals, or hormones, are often low molecular weight molecules, such as peptides, fatty acid, or cholesterol derivatives.

Many of these signals produce their effects by ultimately changing the transcription of specific genes. One well-studied group of proteins that mediate a cell's response to a variety of signals is the family of transcription factors known as nuclear receptors, hereinafter referred to often as "NR". Members of this group include receptors for steroid hormones, vitamin D, ecdysone, cis and trans retinoic acid, thyroid hormone, bile acids, cholesterol-derivatives, fatty acids (and other peroxisomal proliferators), as well as so-called orphan receptors, proteins that are structurally similar to other members of this group, but for which no ligands are known. Orphan receptors may be indicative of unknown signalling pathways in the cell or may be nuclear receptors that function without ligand activation. The activation of transcription by some of these orphan receptors may occur in the absence of an exogenous ligand and/or through signal transduction pathways originating from the cell surface (D. J. Mangelsdorf et al., Cell 1995, 83, 835; R. M. Evans, Mol. Endocrinol. 2005, 19, 1429).

In general, three functional domains have been defined in NRs. An amino terminal domain is believed to have some regulatory function. It is followed by a DNA-binding domain hereinafter referred to as "DBD" which usually comprises two zinc finger elements and recognizes a specific Hormone Responsive Element hereinafter referred to as "HRE" within the promoters of responsive genes. Specific amino acid residues in the "DBD" have been shown to confer DNA sequence binding specificity (M. Schena and K. R. Yamamoto, Science 1988, 241, 965). A ligand-binding-domain hereinafter referred to as "LBD" is at the carboxy-terminal region of known NRs.

In the absence of hormone, the LBD appears to interfere with the interaction of the DBD with its HRE. Hormone binding seems to result in a conformational change in the NR and thus opens this interference (A. M. Brzozowski et al., Nature 1997, 389, 753). A NR without the LBD constitutively activates transcription but at a low level.

Coactivators or transcriptional activators are proposed to bridge between sequence specific transcription factors, the basal transcription machinery and in addition to influence the chromatin

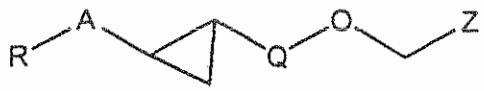
structure of a target cell. Several proteins like SRC-1, ACTR, and Grip1 interact with NRs in a ligand enhanced manner (D. M. Heery et al., Nature 1997, 387, 733; T. Heinzel et al., Nature 1997, 387, 43; K. W. Nettles and G. L. Greene, Annu. Rev. Physiol. 2005, 67, 309).

Nuclear receptor modulators like steroid hormones affect the growth and function of specific cells by binding to intracellular receptors and forming nuclear receptor-ligand complexes. Nuclear receptor-hormone complexes then interact with a HRE in the control region of specific genes and alter specific gene expression (A. Aranda and A. Pascual, Physiol. Rev. 2001, 81, 1269).

The Farnesoid X Receptor alpha (hereinafter also often referred to as NR1H4 when referring to the human receptor) is a prototypical type 2 nuclear receptor which activates genes upon binding to promoter region of target genes in a heterodimeric fashion with Retinoid X Receptor (B. M. Forman et al., Cell 1995, 81, 687). The relevant physiological ligands of NR1H4 are bile acids (D. J. Parks et al., Science 1999, 284, 1365; M. Makishima et al., Science 1999, 284, 1362). The most potent one is chenodeoxycholic acid (CDCA), which regulates the expression of several genes that participate in bile acid homeostasis. Farnesol and derivatives, together called farnesoids, are originally described to activate the rat orthologue at high concentration but they do not activate the human or mouse receptor. FXR is expressed in the liver, throughout the entire gastrointestinal tract including the esophagus, stomach, duodenum, small intestine, colon, ovary, adrenal gland and kidney. Beyond controlling intracellular gene expression, FXR seems to be also involved in paracrine and endocrine signalling by upregulating the expression of the cytokine Fibroblast Growth Factor 15 (rodents) or 19 (monkeys, humans, J. A. Holt et al., Genes Dev. 2003, 17, 1581; T. Inagaki et al., Cell Metab. 2005, 2, 217).

Small molecule compounds which act as FXR modulators have been disclosed in the following publications: WO 2000/037077, WO 2003/015771, WO 2004/048349, WO 2007/076260, WO 2007/092751, WO 2007/140174, WO 2007/140183, WO 2008/051942, WO 2008/157270, WO 2009/005998, WO 2009/012125, WO 2008/025539 and WO 2008/025540. Further small molecule FXR modulators have been recently reviewed (M. L. Crawley, Expert Opin Ther. Pat. 2010, 20,1047; D. Merk et al., Future Med. Chem. 2012, 4, 1015).

In WO 2011/020615 we disclosed chiral cyclopropylidene compounds of the following general formula

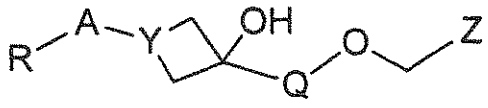


wherein the variables are defined similar as in this application.

The problem underlying the present invention is to generate FXR-agonists with improved physicochemical properties in general, and reduced hydrophobicity, improved aqueous solubility

and better membrane permeability, in particular, compared to compounds claimed in WO 2011/020615.

Said problem has been solved by a compound according to the following Formula (**1**), an enantiomer, diastereomer, tautomer, solvate, prodrug or pharmaceutical acceptable salt thereof



(1)

wherein

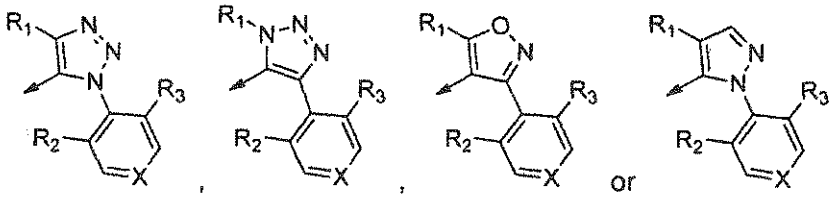
R is selected from the group consisting of $COOR_6$, $CONR_7R_8$, tetrazolyl, $SO_2NR_7R_8$, C_{1-6} alkyl, SO_2-C_{1-6} alkyl and H, with R_6 independently selected from the group consisting of H or C_{1-6} alkyl, and R_7 and R_8 independently from each other selected from the group consisting of H, C_{1-6} alkyl, halo-C_{1-6} alkyl, C_{1-6} alkylene-R_9, SO_2-C_{1-6} alkyl, wherein R_9 is selected from the group consisting of COOH, OH and SO_3H;

A is selected from the group consisting of phenyl, pyridyl, pyrimidyl, pyrazolyl, indolyl, thienyl, benzothienyl, indazolyl, benzisoxazolyl, benzofuranyl, benzotriazolyl, furanyl, benzothiazolyl, thiazolyl, oxadiazolyl, each optionally substituted with one or two groups independently selected from the group consisting of OH, O-C_{1-6} alkyl, O-halo-C_{1-6} alkyl, C_{1-6} alkyl, halo-C_{1-6} alkyl, C_{3-6} cycloalkyl and halogen;

Q is selected from the group consisting of phenyl, pyridyl, thiazolyl, thiophenyl, pyrimidyl, each optionally substituted with one or two groups independently selected from the group consisting of C_{1-6} alkyl, halo-C_{1-6} alkyl, halogen and CF_3;

Y is selected from N or CH;

Z is selected from



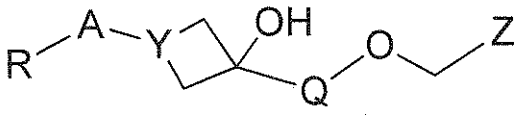
wherein

X = CH, N, NO;

R_1 is selected from the group consisting of hydrogen, C_{1-3} alkyl, C_{3-6} cylcoalkyl, C_{4-5} alkylcycloalkyl, wherein C_{1-3} alkyl is optionally substituted with 1 to 3 substituents independently selected from halogen, hydroxy or C_{1-6} alkoxy;

R_2 and R_3 are independently selected from the group consisting of hydrogen, C_{1-3} alkyl, C_{1-3} haloalkyl, C_{1-3} alkoxy, C_{1-3} haloalkoxy and halogen.

In one aspect, there is provided a compound according to the following Formula (**1**), an enantiomer, diastereomer, tautomer or pharmaceutical acceptable salt thereof



(**1**)

wherein

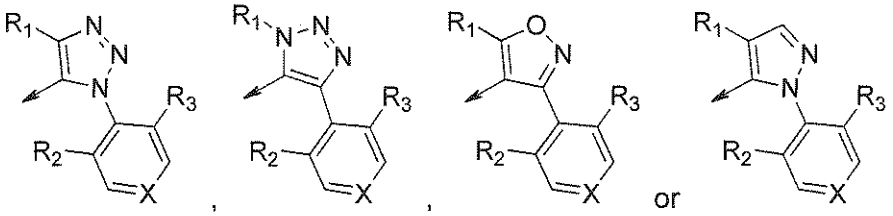
R is selected from the group consisting of $COOR_6$, $CONR_7R_8$, tetrazolyl, $SO_2NR_7R_8$, C_{1-6} alkyl, SO_2-C_{1-6} alkyl and H, with R_6 independently selected from the group consisting of H or C_{1-6} alkyl, and R_7 and R_8 independently from each other selected from the group consisting of H, C_{1-6} alkyl, halo-C_{1-6} alkyl, C_{1-6} alkylene-R_9, SO_2-C_{1-6} alkyl, wherein R_9 is selected from the group consisting of COOH, OH and SO_3H;

A is selected from the group consisting of phenyl, pyridyl, pyrimidyl, pyrazolyl, indolyl, thienyl, benzothienyl, indazolyl, benzisoxazolyl, benzofuranyl, benzotriazolyl, furanyl, benzothiazolyl, thiazolyl, oxadiazolyl, each optionally substituted with one or two groups independently selected from the group consisting of OH, O-C_{1-6} alkyl, O-halo-C_{1-6} alkyl, C_{1-6} alkyl, halo-C_{1-6} alkyl, C_{3-6} cycloalkyl and halogen;

Q is selected from the group consisting of phenyl, pyridyl, thiazolyl, thiophenyl, pyrimidyl, each optionally substituted with one or two groups independently selected from the group consisting of C_{1-6} alkyl, halo-C_{1-6} alkyl, halogen and CF_3;

Y is selected from N or CH;

Z is selected from



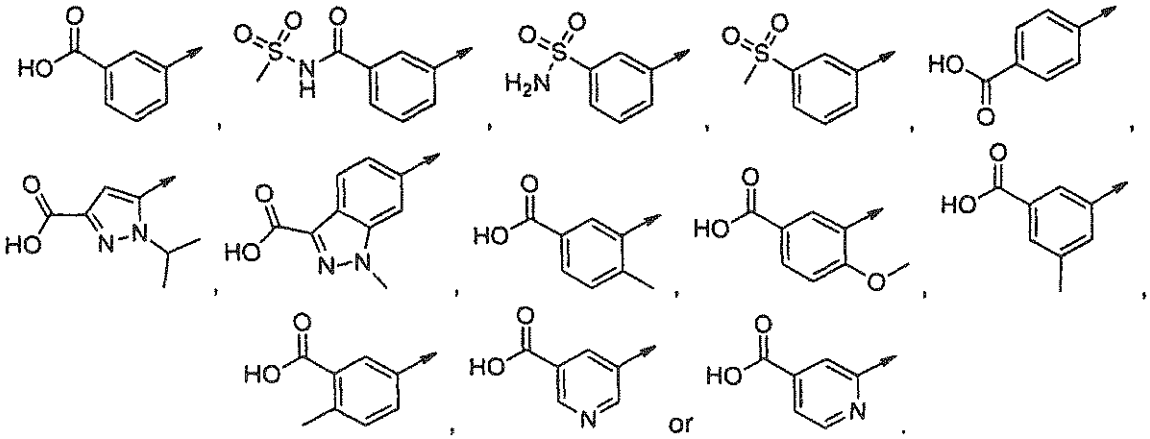
wherein

X = CH, N, NO;

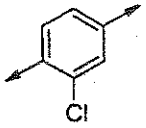
R_1 is selected from the group consisting of hydrogen, C_{1-3} alkyl, C_{3-6} cylcoalkyl, C_{4-5} alkylcycloalkyl, wherein C_{1-3} alkyl is optionally substituted with 1 to 3 substituents independently selected from halogen, hydroxy or C_{1-6} alkoxy;

R_2 and R_3 are independently selected from the group consisting of hydrogen, C_{1-3} alkyl, C_{1-3} haloalkyl, C_{1-3} alkoxy, C_{1-3} haloalkoxy and halogen.

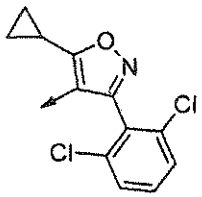
In another embodiment in combination with any of the above or below embodiments, R-A in the compound according to Formula (1) is selected from



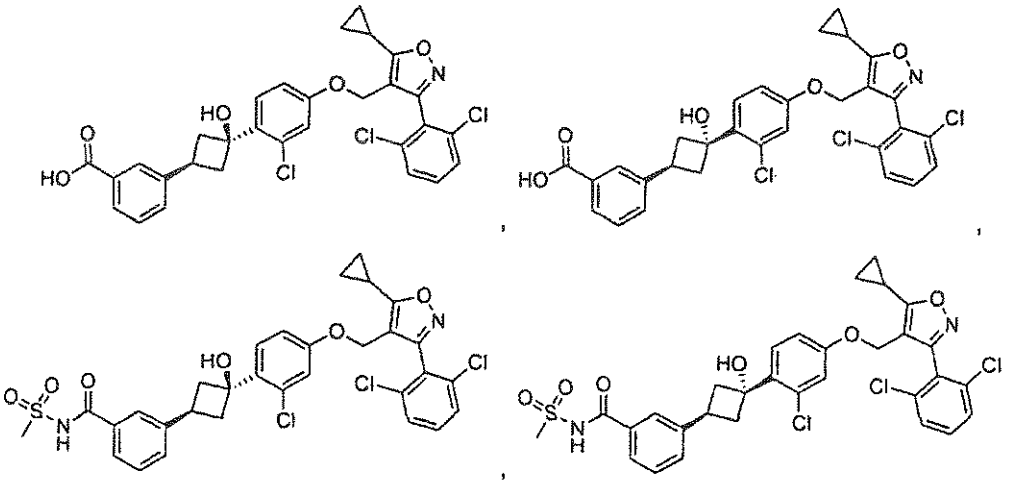
In another embodiment in combination with any of the above or below embodiments, Q in the compound according to Formula (1) is

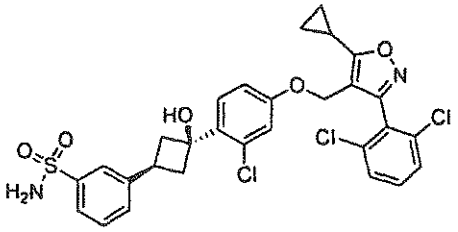
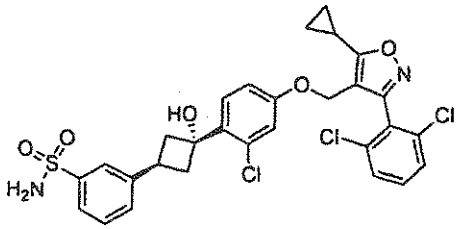
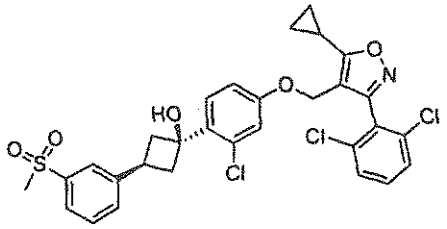
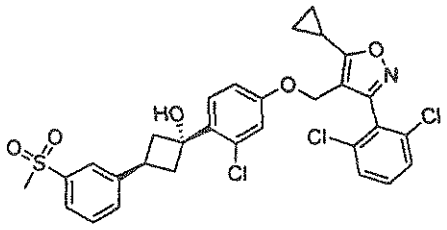
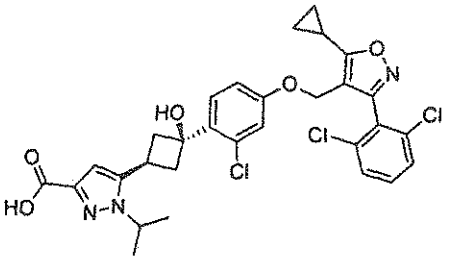
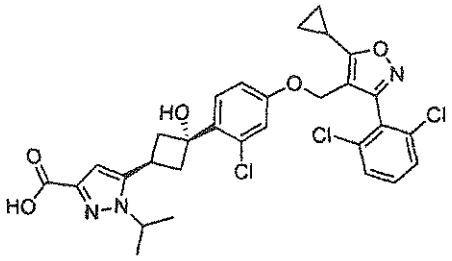
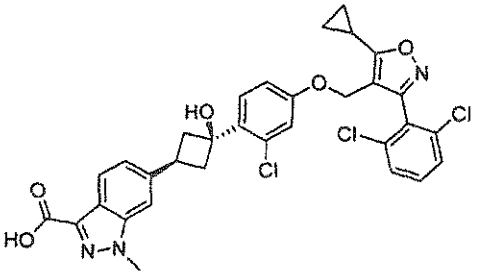
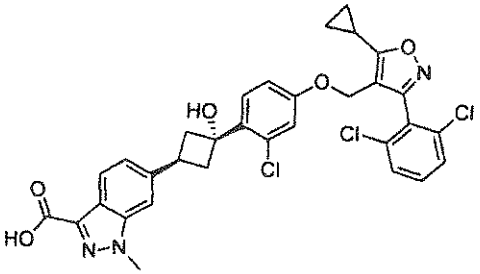
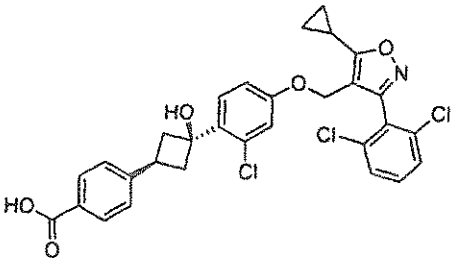
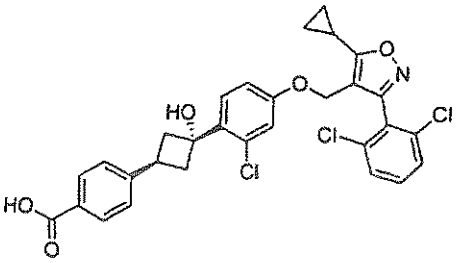
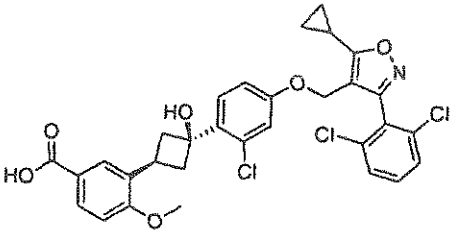
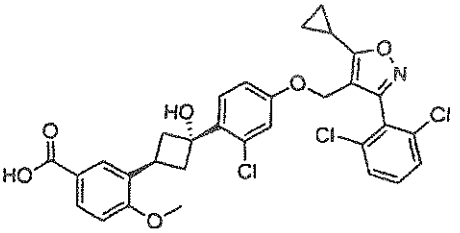


In another embodiment in combination with any of the above or below embodiments, Z in the compound according to Formula (1) is



In another embodiment in combination with any of the above or below embodiments, the compound according to Formula (1) is selected from

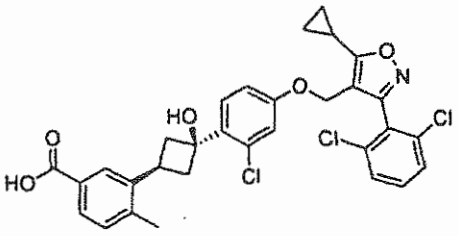
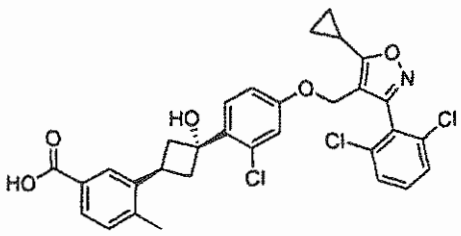
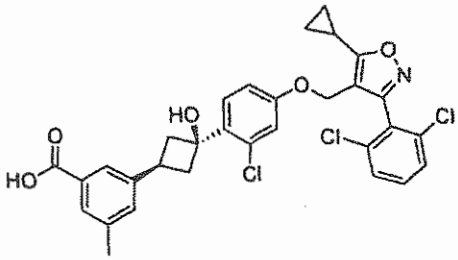
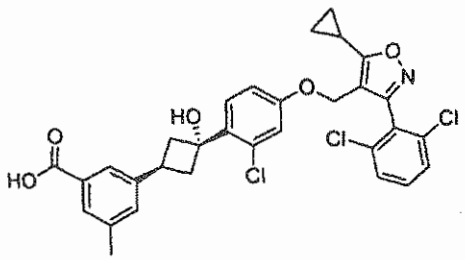
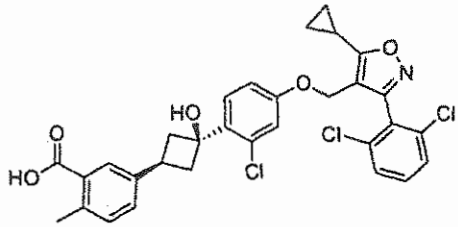
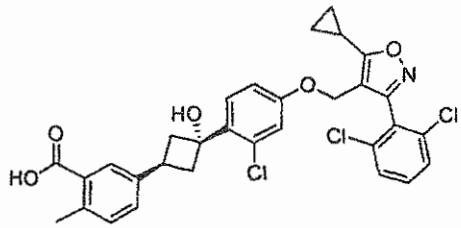
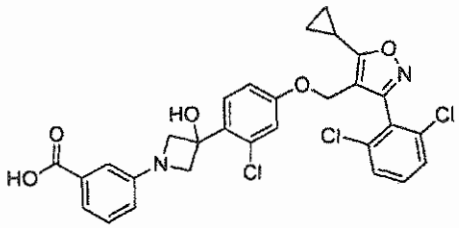
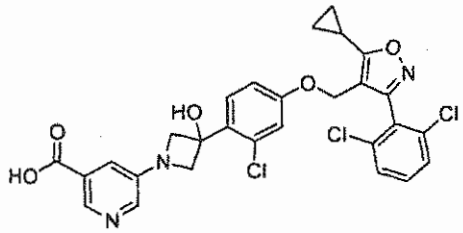
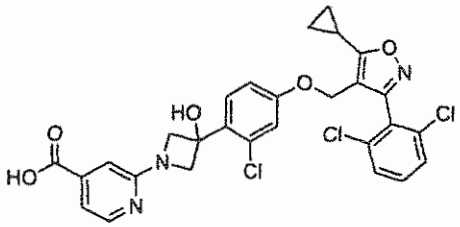



,

,

,

,

,

,

,

,

,

,

,

,

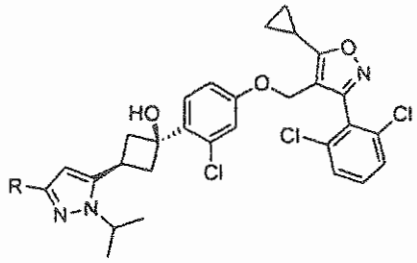
,

,

5


,

,

,

,

,

,

,

,

or .

In another embodiment in combination with any of the above or below embodiments, the compound according to Formula (1) is



wherein R is selected from the group consisting of CO_2H, $CONHSO_2Me$, and tetrazolyl.

In another aspect, there is provided a pharmaceutical composition comprising the compound as disclosed above, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

In another embodiment, the present disclosure is directed to a compound according to Formula (**1**) for use as a medicament.

In another aspect, there is provided a compound as disclosed above for use as a medicament.

In another embodiment, the present disclosure is directed to a compound according to Formula (**1**) for use in the prophylaxis and/or treatment of diseases mediated by FXR.

In another embodiment, the present disclosure is directed to the use of a compound according to Formula (**1**) for the preparation of a medicament for the prophylaxis and/or treatment of diseases mediated by FXR.

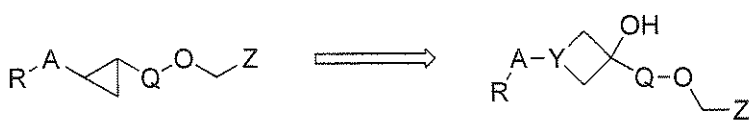
In another aspect, there is provided use of a compound as disclosed above for the preparation of a medicament for the prophylaxis and/or treatment of diseases mediated by FXR.

In another embodiment in combination with any of the above or below embodiments, the disease is selected from chronic intrahepatic or some forms of extrahepatic cholestatic conditions; liver fibrosis; obstructive or chronic inflammatory disorders of the liver; liver cirrhosis; liver steatosis and associated syndromes, cholestatic or fibrotic effects that are associated with alcohol-induced cirrhosis or with viral-borne forms of hepatitis; liver failure or liver ischemia after major liver resection; chemotherapy associated steatohepatitis (CASH); acute liver failure; and/or Inflammatory Bowel Diseases.

In another embodiment in combination with any of the above or below embodiments, the disease is selected from lipid and lipoprotein disorders; Type II Diabetes and clinical complications of Type I and Type II Diabetes, including diabetic nephropathy, diabetic neuropathy, diabetic retinopathy and other observed effects of clinically manifest long term Diabetes; conditions and diseases which result from chronic fatty and fibrotic degeneration of organs due to enforced lipid and specifically triglyceride accumulation and subsequent activation of profibrotic pathways, such as Non-Alcoholic Fatty Liver Disease (NAFLD), or Non-Alcoholic Steatohepatitis (NASH); obesity or metabolic syndrome (combined conditions of dyslipidemia, diabetes or abnormally high body-mass index); and/or cute myocardial infarction, acute stroke or thrombosis which occurs as an endpoint of chronic obstructive atherosclerosis.

In another embodiment in combination with any of the above or below embodiments, the disease is selected from non-malignant hyperproliferative disorders and malignant hyperproliferative disorders, specifically of hepatocellular carcinoma, colon adenoma and polyposis, colon adenocarcinoma, breast cancer, pancreas adenocarcinoma, Barrett's esophagus or other forms of neoplastic diseases of the gastrointestinal tract and the liver.

The improved physico-chemical properties have been achieved by the introduction of a polar hydroxyl group on a 1,3-cyclobutylidene or 1,3-azetidinylidene group replacing the former 1,2-cyclopropylidene ring.



Surprisingly, the resulting compounds maintained their activity on the FXR receptor but demonstrated improved physico-chemical properties, such as higher aqueous solubility and/or membrane permeability.

The compounds of the present invention share a common chemical structure according to Formula (**1**) in claim 1.

In a preferred embodiment in combination with any of the above or below embodiments, the present invention is directed to an enantiomer, diastereomer or pharmaceutically acceptable salt of a compound according to Formula (**1**).

In a preferred embodiment in combination with any of the above or below embodiments, R in Formula (**1**) is selected from the group consisting of $COOR_6$, $CONR_7R_8$, $SO_2NR_7R_8$, and SO_2-C_{1-6} alkyl.

In a preferred embodiment in combination with any of the above or below embodiments, R_6 in Formula (**1**) is H.

In a preferred embodiment in combination with any of the above or below embodiments, R_7 and R_8 in Formula (**1**) are independently from each other selected from the group consisting of H and SO_2-C_{1-6} alkyl.

In a preferred embodiment in combination with any of the above or below embodiments, R_7 in Formula (**1**) is H.

In a preferred embodiment in combination with any of the above or below embodiments, R_8 in Formula (**1**) is SO_2-C_{1-6} alkyl.

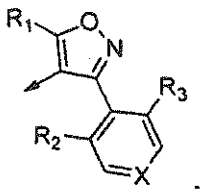
In a preferred embodiment in combination with any of the above or below embodiments, A is selected from the group consisting of phenyl, pyridyl, pyrimidyl, pyrazolyl, indazolyl, and oxadiazolyl.

In a preferred embodiment in combination with any of the above or below embodiments, A is substituted with one or two groups independently selected from C_{1-6} alkyl, more preferably C_{1-3} alkyl. In another preferred embodiment in combination with any of the above or below embodiments, A is unsubstituted.

In a preferred embodiment in combination with any of the above or below embodiments, Q is phenyl.

In a preferred embodiment in combination with any of the above or below embodiments, Q is substituted with one or two groups independently selected from halogen, more preferably one group selected from halogen, in particular Cl.

In a preferred embodiment in combination with any of the above or below embodiments, Z is



In a preferred embodiment in combination with any of the above or below embodiments, X = CH.

In a preferred embodiment in combination with any of the above or below embodiments, R_1 is C_{3-6} cylcoalkyl, in particular cyclopropyl.

In a preferred embodiment in combination with any of the above or below embodiments, R_2 and R_3 are independently selected from halogen, in particular Cl.

The compounds of the present invention can be in the form of a prodrug compound. "Prodrug compound" means a derivative that is converted into a compound according to the present invention by a reaction with an enzyme, gastric acid or the like under a physiological condition in the living body, e.g. by oxidation, reduction, hydrolysis or the like, each of which is carried out enzymatically. Examples of the prodrug are compounds, wherein the amino group in a compound of the present invention is acylated, alkylated or phosphorylated to form, e.g., eicosanoylamino, alanylamino, pivaloyloxymethylamino or wherein the hydroxyl group is acylated, alkylated, phosphorylated or converted into the borate, e.g. acetyloxy, palmitoyloxy, pivaloyloxy, succinyloxy, fumaryloxy, alanyloxy or wherein the carboxyl group is esterified or amidated. These compounds can be produced from compounds of the present invention according to well-known methods. Other examples of the prodrug are compounds, wherein the carboxylate in a compound of the present invention is, for example, converted into an alkyl-, aryl-, choline-, amino, acyloxymethylester, linolenoylester.

Metabolites of compounds of the present invention are also within the scope of the present invention.

Where tautomerism, like e.g. keto-enol tautomerism, of compounds of the present invention or their prodrugs may occur, the individual forms, like e.g. the keto and enol form, are each within the scope of the invention as well as their mixtures in any ratio. Same applies for stereoisomers, like e.g. enantiomers, cis/trans isomers, conformers and the like.

If desired, isomers can be separated by methods well known in the art, e.g. by liquid chromatography. Same applies for enantiomers by using e.g. chiral stationary phases. Additionally, enantiomers may be isolated by converting them into diastereomers, i.e. coupling with an enantiomerically pure auxiliary compound, subsequent separation of the resulting diastereomers and cleavage of the auxiliary residue. Alternatively, any enantiomer of a compound of the present invention may be obtained from stereoselective synthesis using

optically pure starting materials. Another way to obtain pure enantiomers from racemic mixtures would use enantioselective crystallization with chiral counterions.

The compounds of the present invention can be in the form of a pharmaceutically acceptable salt or a solvate. The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids, including inorganic bases or acids and organic bases or acids. In case the compounds of the present invention contain one or more acidic or basic groups, the invention also comprises their corresponding pharmaceutically or toxicologically acceptable salts, in particular their pharmaceutically utilizable salts. Thus, the compounds of the present invention which contain acidic groups can be present on these groups and can be used according to the invention, for example, as alkali metal salts, alkaline earth metal salts or ammonium salts. More precise examples of such salts include sodium salts, potassium salts, calcium salts, magnesium salts or salts with ammonia or organic amines such as, for example, ethylamine, ethanolamine, triethanolamine or amino acids. The compounds of the present invention which contain one or more basic groups, i.e. groups which can be protonated, can be present and can be used according to the invention in the form of their addition salts with inorganic or organic acids. Examples of suitable acids include hydrogen chloride, hydrogen bromide, phosphoric acid, sulfuric acid, nitric acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acids, oxalic acid, acetic acid, tartaric acid, lactic acid, salicylic acid, benzoic acid, formic acid, propionic acid, pivalic acid, diethylacetic acid, malonic acid, succinic acid, pimelic acid, fumaric acid, maleic acid, malic acid, sulfaminic acid, phenylpropionic acid, gluconic acid, ascorbic acid, isonicotinic acid, citric acid, adipic acid, and other acids known to the person skilled in the art. If the compounds of the present invention simultaneously contain acidic and basic groups in the molecule, the invention also includes, in addition to the salt forms mentioned, inner salts or betaines (zwitterions). The respective salts can be obtained by customary methods which are known to the person skilled in the art like, for example, by contacting these with an organic or inorganic acid or base in a solvent or dispersant, or by anion exchange or cation exchange with other salts. The present invention also includes all salts of the compounds of the present invention which, owing to low physiological compatibility, are not directly suitable for use in pharmaceuticals but which can be used, for example, as intermediates for chemical reactions or for the preparation of pharmaceutically acceptable salts.

Further the compounds of the present invention may be present in the form of solvates, such as those which include as solvate water, or pharmaceutically acceptable solvates, such as alcohols, in particular ethanol.

Furthermore, the present invention provides pharmaceutical compositions comprising at least one compound of the present invention, or a prodrug compound thereof, or a pharmaceutically acceptable salt or solvate thereof as active ingredient together with a pharmaceutically acceptable carrier.

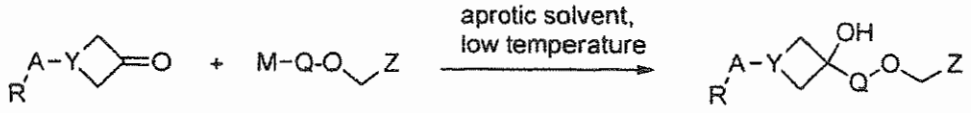
"Pharmaceutical composition" means one or more active ingredients, and one or more inert ingredients that make up the carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing at least one compound of the present invention and a pharmaceutically acceptable carrier.

The pharmaceutical composition of the present invention may additionally comprise one or more other compounds as active ingredients like a prodrug compound or other nuclear receptor modulators.

The compositions are suitable for oral, rectal, topical, parenteral (including subcutaneous, intramuscular, and intravenous), ocular (ophthalmic), pulmonary (nasal or buccal inhalation) or nasal administration, although the most suitable route in any given case will depend on the nature and severity of the conditions being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well-known in the art of pharmacy.

The compounds of the present invention can be prepared by a combination of methods described in Schemes I to III. As depicted in Scheme I a 4-membered cyclic ketone, substituted with substituent A in the 3-position can react with a metalated aromatic or heteroaromatic ring M-Q-O-CH_2Z (M = metal, e.g. Li) in aprotic solvents and preferably at low temperatures to afford a hydroxyl substituted 4-membered ring bearing the substituents A and Q. In the case where Y is CH two isomers can form (A and Q transannular cis or trans to each other). Under optimized conditions the formation of mainly one of the two isomers can be achieved. The two isomers can be separated by appropriate methods known in the art like e.g. silica gel chromatography or preparative RP-HPLC.

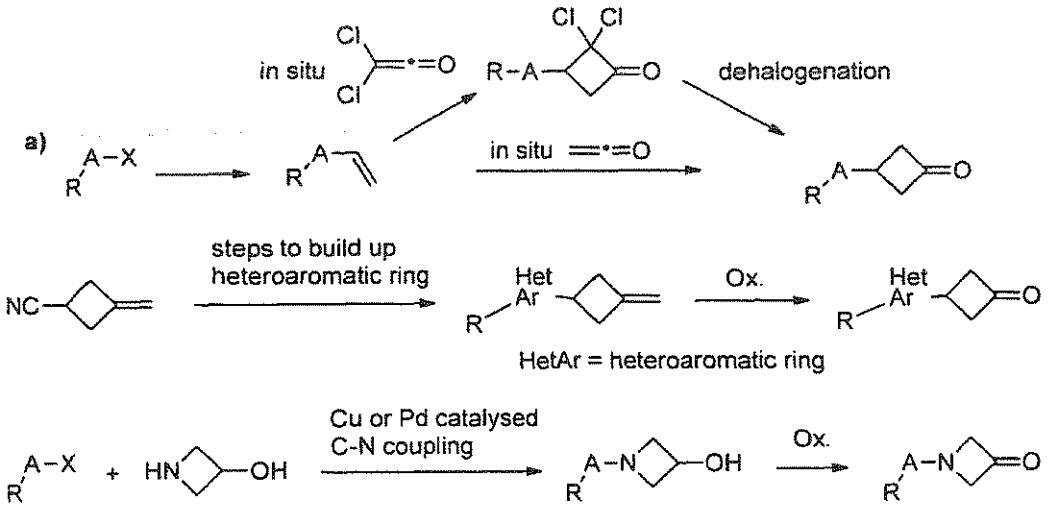
Scheme I



In Scheme II the methods are summarized which are used to prepare the 4-membered cyclic ketones needed for the synthesis of the compounds of this invention. In option a) a vinyl bearing intermediate, e.g. prepared by vinylation of a corresponding halogen-containing starting material R-A-X (X = halogen) can react with in situ formed α,α-dichloro ketene to form a 2,2-dichlorocyclobutanone. After dehalogenation, e.g. with Zn in acetic acid under reflux, the desired 3-substituted cyclobutanones are obtained. Alternatively, the vinyl-intermediates can react with in situ generated unsubtituted ketene to afford in one step the desired cyclobutanone

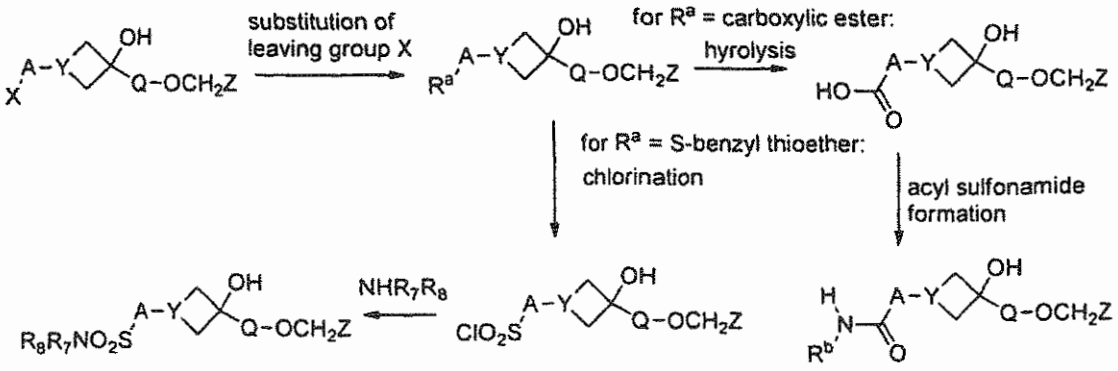
intermediates. In option b) 3-methylenecyclobutanecarbonitrile is used as starting material. Substituted heterocycles can be built up from the cyano group in several steps by methods known to those skilled in the art. The desired cyclobutanones can be obtained by oxidative cleavage of the exocyclic double bond using conditions and reagents known to those skilled in the art, e.g. by the use of OsO_4, ozone or $RhCl_3/NaIO_4$ as oxidants. Option c) shows the methods used to prepare the substituted azetidinones. Cu- or Pd-catalysed C-N cross coupling between 3-hydroxy-azetidine and halo-aromatic or halo-heteroaromatic rings afford the corresponding *N*-substituted 3-hydroxy-azetidines which can be transformed into the desired azetidinones by oxidation.

Scheme II



Scheme III illustrates some possibilities to perform modifications of the substituents at the A group after the formation of the 4-membered hydroxy-bearing rings. For example, a leaving group X (e.g. bromide) can be substituted by a cyano group, a carboxylic ester, methylsulfonyl or thioether by transition metal catalysed cross coupling reactions. The obtained derivatives can be further transformed into other derivatives by methods known to those skilled in the art. For example, the cyano and the ester group can be hydrolysed under basic conditions to the afford a carboxylic acid which in turn can be transformed into acyl-sulfonamides. A benzyl thioether can be chlorinated to afford the chlorosulfonyl intermediate which reacts with ammonia to the corresponding sulfonamides.

Scheme III



As a result, the present invention relates to compounds according to the general Formula (1) which bind to FXR and act as agonists or modulators of FXR.

The invention further relates to the use of said compounds for the treatment and/or prophylaxis of diseases and/or conditions through binding of said nuclear receptor by said compounds. Further the present invention relates to the use of said compounds for the preparation of a medicament for the treatment and/or prophylaxis of diseases and/or conditions through binding of said nuclear receptor by said compounds. Specifically, the present invention relates to the use of compounds according to Formula (1) in the preparation of a medicament for the prophylaxis and/or treatment of chronic intrahepatic or some forms of extrahepatic cholestatic conditions, of liver fibrosis, of acute intraheptic cholestatic conditions, of obstructive or chronic inflammatory disorders that arise out of improper bile composition, of gastrointestinal conditions with a reduced uptake of dietary fat and fat-soluble dietary vitamins, of inflammatory bowel diseases, of lipid and lipoprotein disorders, of Type II Diabetes and clinical complications of Type I and Type II Diabetes, of conditions and diseases which result from chronic fatty and fibrotic degeneration of organs due to enforced lipid and specifically triglyceride accumulation and subsequent activation of profibrotic pathways, of obesity and metabolic syndrome (combined conditions of dyslipidemia, diabetes and abnormally high body-mass index), of acute myocardial infarction, of acute stroke, of thrombosis which occurs as an endpoint of chronic obstructive atherosclerosis, of persistant infections by intracellular bacteria or parasitic protozoae, of non-malignant hyperproliferative disorders, of malignant hyperproliferative disorders, of colon adenocarcinoma and hepatocellular carcinoma in particular, of liver steatosis and associated syndromes, of liver failure or liver malfunction as an outcome of chronic liver diseases or of surgical liver resection, of Hepatitis B infection, of Hepatitis C infection and/or of cholestatic and fibrotic effects that are associated with alcohol-induced cirrhosis or with viral-borne forms of hepatitis.

Medicaments as referred to herein may be prepared by conventional processes, including the combination of a compound according to the present invention and a pharmaceutically acceptable carrier.

FXR is proposed to be a nuclear bile acid sensor. As a result, it modulates both, the synthetic output of bile acids in the liver and their recycling in the intestine (by regulating bile acid binding proteins). But beyond bile acid physiology, FXR seems to be involved in the regulation of many diverse physiological processes which are relevant in the etiology and for the treatment of diseases as diverse as cholesterol gallstones, metabolic disorders such as Type II Diabetes, dyslipidemias or obesity, chronic inflammatory diseases such as Inflammatory Bowel Diseases or chronic intrahepatic forms of cholestasis and many others diseases (T. Claudel et al., Arterioscler. Thromb. Vasc. Biol. 2005, 25, 2020; Y. D. Wang et al., Cell Res. 2008, 18, 1087.

FXR regulates a complex pattern of response genes in the liver and in the gastrointestinal tract. The gene products have impact on diverse physiological processes. In the course of functional analysis of FXR, the first regulatory network that was analyzed was the regulation of bile acid synthesis. While the LXRs induce the key enzyme of the conversion of cholesterol into bile acids, Cyp7A1, via the induction of the regulatory nuclear receptor LRH-1, FXR represses the induction of Cyp7A1 via the upregulation of mRNA encoding SHP, a further nuclear receptor that is dominant repressive over LRH-1. Since FXR binds the end products of this pathway, primary bile acids such as cholic acid (CA) or CDCA, this can be regarded as an example of feedback inhibition on the gene expression level (B. Goodwin et al., Mol. Cell 2000, 6, 517; T. T. Lu et al., Mol. Cell 2000, 6, 507). Parallel to the repression of bile acid synthesis via SHP, FXR induces a range of so-called ABC (for ATP-binding cassette) transporters that are responsible for the export of toxic bile acids from the hepatocyte cytosol into the canaliculi, the small bile duct ramifications where the bile originates. This hepatoprotective function of FXR became first apparent with the analysis of FXR knockout mice (C. J. Sinal et al., Cell 2000, 102, 731). where under- or overexpression of several ABC-transporters in the liver was shown. Further detailed analysis revealed that the major bile salt excretory pump BSEP or ABCB11 (M. Ananthanarayanan et al., J. Biol. Chem. 2001, 276, 28857; J. R. Plass et al., Hepatology 2002, 35, 589) as well as the key enzyme which mediates lipid transfer from lipoproteins to phospholipids, PLTP (N. L. Urizar et al., J. Biol. Chem. 2000, 275, 39313), and the two key canalicular membrane transporters for phospholipids, MRP-2 (ABCC4) (H. R. Kast et al., J. Biol. Chem. 2002, 277, 2908) and MDR-3 (ABCB4); L. Huang et al., J. Biol. Chem. 2003, 278, 51085) are direct targets for ligand-directed transcriptional activation by FXR (summarized in: M. Miyata, J. Pharmacol. Exp. Ther. 2005, 312, 759; G. Rizzo et al., Curr. Drug Targets Immune Endocr. Metabol. Disord. 2005, 5, 289).

The fact that FXR seems to be the major metabolite sensor and regulator for the synthesis, export and re-circulation of bile acids suggested the use of FXR ligands to induce bile flow and change bile acid composition towards more hydrophilic composition. With the development of the first synthetic FXR ligand GW4064 (P. R. Maloney et al., J. Med. Chem. 2000, 43, 2971; T. M. Willson et al., Med. Res. Rev. 2001, 21, 513) as a tool compound and of the semi-synthetic artificial bile acid ligand 6-alpha-ethyl-CDCA, the effects of superstimulation of FXR by potent

agonists could be analyzed. It was shown that both ligands induce bile flow in bile duct ligated animals. Moreover, in addition to choleretic effects, also hepatoprotective effects could be demonstrated (R. Pellicciari et al., J. Med. Chem. 2002, 45, 3569; Y. Liu et al., J. Clin. Invest. 2003, 112, 1678). This hepatoprotective effect was further narrowed down to an anti-fibrotic effect that results from the repression of Tissue Inhibitors of Matrix-Metalloproteinases, TIMP-1 and 2, the induction of collagen-deposit resolving Matrix-Metalloproteinase 2 in hepatic stellate cells and the subsequent reduction of alpha-collagen mRNA and Transforming growth factor beta (TGF-beta) mRNA which are both pro-fibrotic factors by FXR agonists (S. Fiorucci et al., Gastroenterology 2004, 127, 1497; S. Fiorucci et al., J. Pharmacol. Exp. Ther. 2005, 314, 584). Furthermore, anti-cholestatic activity was demonstrated in bile-duct ligated animal models as well as in animal models of estrogen-induced cholestasis (S. Fiorucci et al., J. Pharmacol. Exp. Ther. 2005, 313, 604).

Genetic studies demonstrate that in hereditary forms of cholestasis (Progressive Familiar Intrahepatic Cholestasis = PFIC, Type I – IV) either nuclear localization of FXR itself is reduced as a consequence of a mutation in the FIC1 gene (in PFIC Type I, also called Byler´s Disease) (F. Chen et al., Gastroenterology 2004, 126, 756; L. Alvarez et al., Hum. Mol. Genet. 2004, 13, 2451) or levels of the FXR target gene encoding MDR-3 phospholipid export pump are reduced (in PFIC Type III). Taken together there is a growing body of evidence that FXR binding compounds will demonstrate substantial clinical utility in the therapeutic regimen of chronic cholestatic conditions such as Primary Biliary Cirrhosis (PBC) or Primary Sclerosing Cholangitis (PSC) (reviewed in: G. Rizzo et al., Curr. Drug Targets Immune Endocr. Metabol. Disord. 2005, 5, 289; G. Zollner et al., Mol. Pharm. 2006, 3, 231; S. Y. Cai et al., Expert Opin. Ther. Targets 2006, 10, 409).

The deep impact that FXR activation has on bile acid metabolism and excretion is not only relevant for cholestatic syndromes but even more directly for a therapy against gallstone formation. Cholesterol gallstones form due to low solubility of cholesterol that is actively pumped out of the liver cell into the lumen of the canaliculi. It is the relative percentage of content of the three major components, bile acids, phospholipids and free cholesterol that determines the formation of mixed micelles and hence apparent solubility of free cholesterol in the bile. FXR polymorphisms map as quantitative trait loci as one factor contributing to gallstone disease (H. Wittenburg, Gastroenterology 2003, 125, 868). Using the synthetic FXR tool compound GW4064 it could be demonstrated that activation of FXR leads to an improvement of the Cholesterol Saturation Index (CSI) and directly to an abolishment of gallstone formation in C57L gallstone susceptible mice whereas drug treatment in FXR knockout mice shows no effect on gallstone formation (A. Moschetta et al., Nature Medicine 2004, 10, 1352).

These results qualify FXR as a good target for the development of small molecule agonists that can be used to prevent cholesterol gallstone formation or to prevent re-formation of gallstones

after surgical removal or shockwave lithotripsy (discussed in: S. A. Doggrell, Curr. Opin. Investig. Drugs 2006, 7, 344).

Thus, in one embodiment of the invention, the compound according to Formula (1) and pharmaceutical compositions comprising said compound is used for the prophylaxis and/or treatment of obstructive or chronic inflammatory disorders that arise out of improper bile composition such as cholelithiasis also known as cholesterol gallstones.

Beyond its strong hepatoprotective and choleretic as well as anti-fibrotic effects that FXR shows upon small molecule stimulated activation in the liver, FXR seems to have a role in protecting the intestine from neoplastic transformation and from the development of polyps and their transition into adenocarcinoma in the gut (S. Modica et al., Cancer Res. 2008, 68, 9589 and R. R. Maran et al., J. Pharmacol. Exp. Ther. 2009, 328, 469). Similar to the situation in the intestine absence of FXR leads to a high increase in the formation of Hepatocellular Cacrcinoma (HCC), the most prominent form of liver cancer (I. Kim et al., Carcinogenesis 2007, 28, 940 and F. Yang et al., Cancer Res. 2007, 67, 863). Whereas a functional FXR prevents the formation of colon adenocarcinoma and hepatocellular carcinoma, FXR activation induces liver regeneration after hepatectomy (W. Huang et al., Science 2006, 312, 233).

The combined hepatoprotective, anti-neoplastic and liver regenerative effects associated with FXR activation can be therapeutically exploited for the use of FXR agonists in the treatment of sever liver diseases. In one embodiment, the compounds according to the invention and pharmaceutical compositions comprising said compounds are used in the treatment of liver diseases such as HCC, stimulation of liver regrowth and amelioration of side effects associated with major liver resection, liver cirrhosis independent of the etiology and prevention or treatment of liver ischemia in the course of liver transplantation or major liver surgery.

Since the discovery of the first synthetic FXR agonist and its administration to rodents it became evident that FXR is a key regulator of serum triglycerides (P. Maloney et al., J. Med. Chem. 2000, 43, 2971; T. Willson et al., Med. Res. Rev. 2001, 21, 513). Over the past six years accumulating evidence has been published that activation of FXR by synthetic agonists leads to significant reduction of serum triglycerides, mainly in the form of reduced VLDL, but also to reduced total serum cholesterol (H. R. Kast et al., Mol. Endocrinol. 2001, 15, 1720; N. L. Urizar et al., Science 2002, 296, 1703; G. Lambert et al., J. Biol. Chem. 2003, 278, 2563; M. Watanabe et al., J. Clin. Invest. 2004, 113, 1408; A. Figge et al., J. Biol. Chem. 2004, 279, 2790; S. Bilz et al., Am. J. Physiol. Endocrinol. Metab. 2006, 290, E716).

But the lowering of serum triglycerides is not a stand alone effect. Treatment of db/db or ob/ob mice with synthetic FXR agonist GW4064 resulted in marked and combined reduction of serum triglycerides, total cholesterol, free fatty acids, ketone bodies such as 3-OH Butyrate. Moreover, FXR activation engages with the intracellular insulin signaling pathway in hepatocytes, resulting in reduced output of glucose from liver gluconeogenesis but concomitant increase in liver

glycogen. Insulin sensitivity as well as glucose tolerance were positively impacted by FXR treatment (K. R. Stayrook et al., Endocrinology 2005, 146, 984; Y. Zhang et al., PNAS 2006, 103, 1006; B. Cariou et al., J. Biol. Chem. 2006, 281, 11039; K. Ma et al., J. Clin. Invest. 2006, 116, 1102; D. Duran-Sandoval et al., Biochimie 2005, 87, 93). An effect on reduction of body weight was also recently observed in mice overfed with a high lipid diet (C. Lihong et al., American Diabetes Association (ADA) 66th annual scientific sessions, June 2006, Abstract Number 856-P). This weight loss effect might results from FXR´s induction of FGF-19, a fibroblast growth factor that is known to lead to weight loss and athletic phenotype (J. Holt et al., Genes Dev. 2003, 17, 1581; E. Tomlinson et al., Endocrinology 2002, 143, 1741). In recent patent applications, the effect of FXR agonist on reduction of body weight was demonstrated (WO 2004/087076; WO 2003/080803).

Taken together, these pharmacological effects of FXR agonists can be exploited in different therapeutic ways: FXR binding compounds are thought to be good candidates for the treatment of Type II Diabetes because of their insulin sensitization, glycogenogenic, and lipid lowering effects.

In one embodiment, the compounds according to the invention and pharmaceutical compositions comprising said compounds are used in the prophylaxis and/or treatment of Type II Diabetes which can be overcome by FXR-mediated upregulation of systemic insulin sensitivity and intracellular insulin signalling in liver, increased peripheral glucose uptake and metabolisation, increased glycogen storage in liver, decreased output of glucose into serum from liver-borne gluconeogenesis.

In a further embodiment, said compounds and pharmaceutical compositions are used for the prophylaxis and/or treatment of chronic intrahepatic, such as PBC, PSC, progressive familiar cholestasis (PFIC), alcohol-induced cirrhosis and associated cholestasis, and some forms of extrahepatic cholestatic conditions, or liver fibrosis.

The invention also relates to a compound of Formula (1) or to a pharmaceutical composition comprising said compound for the prophylaxis and/or treatment of gastrointestinal conditions with a reduced uptake of dietary fat and fat-soluble dietary vitamins which can be overcome by increased intestinal levels of bile acids and phospholipids.

In a further embodiment, said compound or pharmaceutical composition is used for preventing and/or treating a disease selected from the group consisting of lipid and lipoprotein disorders such as hypercholesterolemia, hypertriglyceridemia, and atherosclerosis as a clinically manifest condition which can be ameliorated by FXR´s beneficial effect on lowering total plasma cholesterol, lowering serum triglycerides, increasing conversion of liver cholesterol into bile acids and increased clearance and metabolic conversion of VLDL and other lipoproteins in the liver.

In one further embodiment, said compound and pharmaceutical composition are used for the prophylaxis and/or treatment of diseases where the combined lipid lowering, anti-cholestatic and anti-fibrotic effects of FXR-targeted medicaments can be exploited for the treatment of liver steatosis and associated syndromes such as NASH, or for the treatment of cholestatic and fibrotic effects that are associated with alcohol-induced cirrhosis, or with viral-borne forms of hepatitis.

In conjunction with the hypolipidemic effects it was also shown that loss of functional FXR leads to increased atherosclerosis in ApoE knockout mice (E. A. Hanniman et al., J. Lipid Res. 2005, 46, 2595). Therefore, FXR agonists might have clinical utility as anti-atherosclerotic and cardioprotective drugs. The downregulation of Endothelin-1 in Vascular Smooth Muscle Cells might also contribute to such beneficial therapeutic effects (F. He et al., Circ. Res. 2006, 98, 192).

The invention also relates to a compound according to Formula (1) or a pharmaceutical composition comprising said compound for preventive and posttraumatic treatment of cardiovascular disorders such as acute myocardial infarction, acute stroke, or thrombosis which occur as an endpoint of chronic obstructive atherosclerosis.

Beyond controlling intestinal and colonic polyp formation, FXR seems to be expressed in breast cancer tissue and cell lines but not in healthy breast tissue and seems to interact with the Estrogen Receptor in ER positive breast cancer cells (K. E. Swales et al., Cancer Res. 2006, 66, 10120 and F. Journe et al., Breast Cancer Res. Treat. 2009, 115, 523).

This would allow to regard FXR also as a potential target for the treatment of proliferative diseases, especially metastasizing cancer forms that express a small molecule responsive form of FXR.

In a further embodiment, said compounds and pharmaceutical compositions are used for the prophylaxis and/or treatment of malignant hyperproliferative disorders such as different forms of cancer, specifically certain forms of breast, liver or colon cancer where interference with an FXR ligand will have a beneficial impact.

Finally, FXR seems also to be involved in the control of antibacterial defense in the intestine (T. Inagaki et al., PNAS. 2006, 103, 3920) although an exact mechanism is not provided. From these published data, however, one can conclude that treatment with FXR agonists might have a beneficial impact in the therapy of Inflammatory Bowel Disorders (IBD), in particular those forms where the upper (ileal) part of the intestine is affected (e.g. ileal Crohn´s disease) because this seems to be the site of action of FXR´s control on bacterial growth. In IBD the desensitization of the adaptive immune response is somehow impaired in the intestinal immune system. Bacterial overgrowth might then be the causative trigger towards establishment of a chronic inflammatory response. Hence, dampening of bacterial growth by FXR-borne mechanisms might be a key mechanism to prevent acute inflammatory episodes.

Thus, the invention also relates to a compound according to Formula (1) or a pharmaceutical composition comprising said compound for preventing and/or treating a disease related to Inflammatory Bowel Diseases such as Crohn´s disease or Colitis ulcerosa. FXR-mediated restoration of intestinal barrier function and reduction in non-commensal bacterial load is believed to be helpful in reducing the exposure of bacterial antigens to the intestinal immune system and can therefore reduce inflammatory responses.

The invention further relates to a compound or pharmaceutical composition for the prophylaxis and/or treatment of obesity and associated disorders such as metabolic syndrome (combined conditions of dyslipidemias, diabetes and abnormally high body-mass index) which can be overcome by FXR-mediated lowering of serum triglycerides, blood glucose and increased insulin sensitivity and FXR-mediated weight loss.

In a further embodiment, the compounds or pharmaceutical composition of the present invention are useful in preventing and/or treating clinical complications of Type I and Type II Diabetes. Examples of such complications include Diabetic Nephropathy, Diabetic Retinopathy, Diabetic Neuropathies, or Peripheral Arterial Occlusive Disease (PAOD). Other clinical complications of Diabetes are also encompassed by the present invention.

Furthermore, conditions and diseases which result from chronic fatty and fibrotic degeneration of organs due to enforced lipid and specifically triglyceride accumulation and subsequent activation of profibrotic pathways may also be prevented and/or treated by applying the compounds or pharmaceutical composition of the present invention. Such conditions and diseases encompass NASH and chronic cholestatic conditions in the liver, Glomerulosclerosis and Diabetic Nephropathy in the kidney, Macula Degeneration and Diabetic Retinopathy in the eye and Neurodegenerative diseases such as Alzheimer´s Disease in the brain, or Diabetic Neuropathies in the peripheral nervous system.

In practical use, the compounds of the present invention can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral (including intravenous). In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations, such as, for example, suspensions, elixirs and solutions; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations such as, for example, powders, hard and soft capsules and tablets, with the solid oral preparations being preferred over the liquid preparations.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be coated by standard aqueous or non-aqueous techniques. Such compositions and preparations should contain at least 0.1 percent of active compound. The percentage of active compound in these compositions may, of course, be varied and may conveniently be between about 2 percent to about 60 percent of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that an effective dosage will be obtained. The active compounds can also be administered intranasally as, for example, liquid drops or spray.

The tablets, pills, capsules, and the like may also contain a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium 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 dosage unit form is a capsule, it may contain, in addition to 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 form of the dosage unit. For instance, 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 propylparabens as preservatives, a dye and a flavoring such as cherry or orange flavor.

Since the compounds of the present invention mostly represent carboxylic acids or similar anionic isosters thereof, and since it is well known that salt forms of ionic drug compounds can substantially affect the bioavailability of drug compounds, the compounds of the present invention may also be used as salts with various countercations to yield an orally available formulation. Such pharmaceutically acceptable cations may be amongst others mono- or bivalent ions such as ammonium, the alkaline metals sodium or potassium or the alkaline earth metals magnesium or calcium, certain pharmaceutically acceptable amines such as tris(hydroxymethyl)aminomethane, ethylendiamine, diethylamine, piperazine or others, or certain cationic amino acids such as lysine or arginine.

The compounds of the present invention may also be administered parenterally. Solutions or suspensions of these active compounds can be prepared in water suitably mixed with a surfactant such as hydroxy-propylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria

and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

Any suitable route of administration may be employed for providing a mammal, especially a human, with an effective dose of a compound of the present invention. For example, oral, rectal, topical, parenteral, ocular, pulmonary, nasal, and the like may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, and the like. Preferably compounds of the present invention are administered orally.

The effective dosage of active ingredient employed may vary depending on the particular compound employed, the mode of administration, the condition being treated and the severity of the condition being treated. Such dosage may be ascertained readily by a person skilled in the art.

When treating or preventing FXR mediated conditions for which compounds of the present invention are indicated, generally satisfactory results are obtained when the compounds of the present invention are administered at a daily dosage of from about 0.1 milligram to about 100 milligram per kilogram of animal body weight, preferably given as a single daily dose or in divided doses two to six times a day, or in sustained release form. For most large mammals, the total daily dosage is from about 1.0 milligrams to about 1000 milligrams, preferably from about 1 milligram to about 50 milligrams. In the case of a 70 kg adult human, the total daily dose will generally be from about 7 milligrams to about 350 milligrams. This dosage regimen may be adjusted to provide the optimal therapeutic response.

The compounds of the present invention can be prepared according to the procedures of the following Schemes and Examples, using appropriate materials and are further exemplified by the following specific examples. Moreover, by utilizing the procedures described herein, in conjunction with ordinary skills in the art, additional compounds of the present invention claimed herein can be readily prepared. The compounds illustrated in the examples are not, however, to be construed as forming the only genus that is considered as the invention. The examples further illustrate details for the preparation of the compounds of the present invention. Those skilled in the art will readily understand that known variations of the conditions and processes of the following preparative procedures can be used to prepare these compounds. The instant compounds are generally isolated in the form of their pharmaceutically acceptable salts, such as those described above.

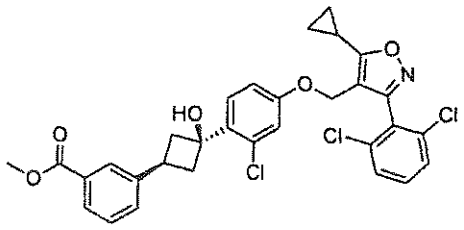
The amine-free bases corresponding to the isolated salts can be generated by neutralization with a suitable base, such as aqueous sodium hydrogen carbonate, sodium carbonate, sodium hydroxide and potassium hydroxide, and extraction of the liberated amine-free base into an organic solvent, followed by evaporation. The amine-free base, isolated in this manner, can be further converted into another pharmaceutically acceptable salt by dissolution in an organic

solvent, followed by addition of the appropriate acid and subsequent evaporation, precipitation or crystallization. The carboxylic free acids corresponding to the isolated salts can be generated by neutralization with a suitable acid, such as aqueous hydrochloric acid, sodium hydrogen sulfate, sodium dihydrogen phosphate, and extraction of the liberated carboxylic-free acid into an organic solvent, followed by evaporation. The carboxylic acid, isolated in this manner, can be further converted into another pharmaceutically acceptable salt by dissolution in an organic solvent, followed by addition of the appropriate base and subsequent evaporation, precipitation or crystallization.

An illustration of the preparation of compounds of the present invention is shown below. Unless otherwise indicated in the schemes, the variables have the same meaning as described above. The examples presented below are intended to illustrate particular embodiments of the invention. Suitable starting materials, building blocks and reagents employed in the synthesis as described below are commercially available from Sigma-Aldrich or Acros Organics, for example, or can be routinely prepared by procedures described in the literature, for example in "March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure", 5th Edition; John Wiley & Sons or T. Eicher, S. Hauptmann "The Chemistry of Heterocycles; Structures, Reactions, Synthesis and Application", 2nd edition, Wiley-VCH 2003; Fieser et al. "Fiesers´ Reagents for organic Synthesis" John Wiley & Sons 2000.

Examples

Example 1: Methyl 3-((1s,3s)-3-(2-chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)benzoate (1)



Step 1: 4-((4-Bromo-3-chlorophenoxy)methyl)-5-cyclopropyl-3-(2,6-dichlorophenyl)-isoxazole (1a)

To a solution of (5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methanol (13 g, 45.8 mmol) in CH_2Cl_2 (DCM) (200 mL) was added dropwise $SOCl_2$ (40 mL, 336 mmol). The resulting mixture was stirred at rt for 2 h and the solvents were removed under reduced pressure. The residue was dissolved in *N,N*-dimethylformamide (DMF) (200 ml) and 4-bromo-3-chlorophenol (9.7 g, 47 mmol), K_2CO_3 (40 g, 290 mmol) and NaI (12 g, 80 mmol) were added to this solution. The mixture was stirred at 60°C overnight, then cooled to rt, diluted with water (1000 mL) and extracted with ethyl acetate (EA) (500 mL x 3). The combined organic phases were washed

with brine (500 mL x 3), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (CC) to give the title compound **1a** (19 g, 88%) as a white solid.

Step 1: Methyl 3-(2,2-dichloro-3-oxocyclobutyl)benzoate (**1b**)

To a 3-necked round bottomed flask, under a nitrogen atmosphere, fitted with a condenser, an overhead stirrer and pressure equalised dropping funnel was dissolved methyl 3-vinylbenzoate (5 g, 31 mmol) in dry Et_2O (150 mL). To this flask was added zinc dust (6 g, 3 eq) and the reaction was sonicated for 30 min. After this time a solution of trichloroacetylchloride (8.7 mL, 2.5 eq) in dry Et_2O (50mL) was added dropwise whilst continuing the sonication over the next 30 min. During the process the reaction mixture was heated to 35°C. The sonication was continued for 2.5 h at reflux and the reaction appeared to be complete by ^1H NMR analysis. The reaction was allowed to cool to rt and quenched with water (~50 mL). This was done in a dropwise manner interspersed sveral times by a few minutes since a delayed exothermic reaction occurred. After 20 min stirring in water the reaction mixture was filtered through a pad of celite and rinsed through with Et_2O. The organic layer was washed with portions of water (2 x 250 mL), saturated sodium bicarbonate (2 x 250 mL) and brine (1 x 250 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure to afford the crude product **1b** as a dark yellow thick oil (crude 8.7 g).

Step 2: Methyl 3-(3-oxocyclobutyl)benzoate (**1c**)

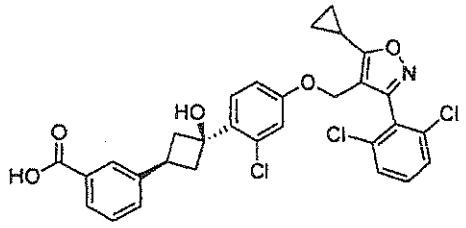
Crude compound **1b** (8.7 g) was dissolved in glacial acetic acid (55 mL) in a round bottomed flask under a nitrogen atmosphere. To this flask was added zinc dust (4.6 g, 2.2 eq) and the reaction was stirred and heated to 120°C for 3 h. After cooling to rt the mixture was filtered though a pad of celite, this was washed with portions of EA. The combined solution was concentrated under reduced pressure before being dissolved in EA (500 mL), washed with brine (150 mL x 2) and then dried over sodium sulfate, filtered and concentrated again. The crude mixture was stirred for 5 min in chloroform (250 mL) and filtered through a sintered funnel. The filtrate was concentrated to give the crude product as a pale yellow oil. The crude product was purified by CC in (PE/EA = 9:1, PE = petroleum ether) to give the desired product **1c** (2.5 g, 38% for 2 steps) as a pale yellow oil.

Step 3: Methyl 3-(3-(2-chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)benzoate (**1**)

To a stirring solution of compound **1a** (1.67 g, 3.5 mmol) in dry THF (30 mL) was added *n*-BuLi (2.5 M in hexane, 1.2 eq, 1.69 mL) dropwise over 10 min at −78°C under a nitrogen atmosphere. This was stirred for 1 h at this temperature before adding a solution of compound **1c** (0.72 g, 1 eq) in dry THF (10 mL) dropwise and stirred for 1 h at this temperature. The reaction mixture was allowed to warm to rt slowly and left stirring overnight. The reaction was quenched with a solution of saturated ammonium chloride solution (50 mL) and EA (250 mL).

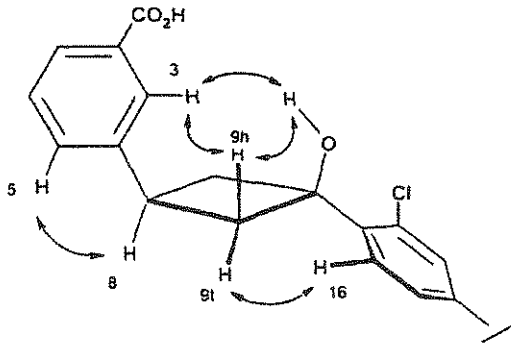
The organic layer was separated and the aq. layer was washed with EA (2 x 100 mL). The combined organic extracts were dried over sodium sulfate, filtered and concentrated to give the crude product as a brown oil. The product was isolated following CC with PE/EA (19:1 to 3:1). The reaction and purification was repeated twice on the same scale and the combined product (3.13 g) was repurified under the same conditions to afford the final product **1** (1.7 g, 19%). ^{1}H NMR ($CDCl_3$): 7.93 (m, 1H), 7.90-7.85 (m, 1H), 7.50-7.30 (m, 5H), 6.88 (s, 1H), 6.75-6.72 (m, 1H), 4.80 (s, 2H), 3.88 (s, 3H), 3.20-3.10 (m, 1H), 3.00-2.91 (m, 2H), 2.60-2.49 (m, 2H), 2.15-2.08 (m, 1H), 1.30-1.25 (m, 2H), 1.15-1.10 (m, 2H).

Example 2: 3-((1s,3s)-3-(2-Chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)benzoic acid (2)



Compound **1** (1.7 g, 2.84 mmol) was dissolved in THF (100 mL) at rt. A solution of LiOH (285 mg, 4.2 eq) in water (20 mL) was added and the solution was stirred and warmed to 35°C for three days. After this time the THF was removed under reduced pressure. The remaining aq. solution was diluted with water (25 mL) and washed with Et_2O (2 x 50 mL). The aq. layer was then transferred to a round bottomed flask and acidified to pH 6 using 1N HCl. The formed white precipitated was filtered off and dried under reduced pressure at 50°C to give title compound **2** (1.3 g, 78%, single isomer by ^{1}H-NMR and LC-MS) as white solid. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.98 (s, 1H), 7.86 (d, J = 7.6 Hz, 1H), 7.58-7.46 (m, 5H), 7.41 (t, J = 7.6 Hz, 1H), 6.91 (d, J = 2.4 Hz, 1H), 6.80 (dd, J = 8.8, 2.4 Hz, 1H), 4.95 (s, 2H), 3.29-3.25 (m, 2H), 2.96 (m, 1H), 2.55-2.49 (m, 2H), 2.37 (m, 1H), 1.24-1.22 (m, 4H). MS (ESI^-) m/z: 584 (582) $[M-1]^-$.

Relevant intensive NOEs (obtained from the ROESY spectra; arrows below) indicate that the two aromatic moieties are 1,3-trans oriented in Example **2**.



Alternative route to Example 2

Step 1: 3-(3-Bromophenyl)cyclobutanone (**2a**)

N,N-Dimethylacetamide (9.0 g, 103 mmol) was dissolved in 1,2-dichloroethane (200 mL). The solution was cooled to 0°C before trifluoromethanesulfonic anhydride (63 g, 223 mmol) was added. The reaction was stirred for an additional 60 min at 0°C. Then 1-bromo-3-vinylbenzene (15 g, 81.9 mmol) and 2,4,6-collidine (10.5 g, 86.6 mmol) were added. The reaction was heated to reflux overnight, quenched by addition of water (300 mL) and stirred for 2 hr at rt. The mixture was extracted with DCM (300 mL × 3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Purification by CC (EA/PE = 1:20) gave the title compound **2a** (5.0 g, 27%) as a pale yellow solid.

Step 2: 3-(3-Bromophenyl)-1-(2-chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)cyclobutanol (**2b**)

To a solution of compound **1a** (14 g, 29.6 mmol) in dry THF (500 ml) at –78°C was added dropwise n-BuLi (18.5 mL, 1.6 M in hexane, 29.6 mmol). The mixture was stirred for an additional 1 h at –78°C and a solution of compound **2a** (6.5 g, 28.9 mmol) in dry THF (50 mL) was added dropwise. The resulting mixture was stirred at –78°C for 1 h and then warmed to rt and quenched with saturated aq. NH_4Cl (500 mL). The mixture was extracted with EA (500 mL × 2), the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by CC (EA/PE = 1:5) to give the title compound **2b** (6.5 g, 37%) as a white solid.

Step 3: 3-(3-Cyanophenyl)-1-(2-chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)cyclobutanol (**2c**)

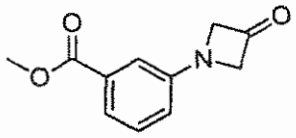
To a solution of compound **2b** (3.1 g, 5 mmol) in DMF (50 mL) were added under argon atmosphere $Zn(CN)_2$ (500 mg, 4.3 mmol), $Pd_2(dba)_3$ (300 mg, 0. 33 mmol) and Xantphos (150 mg, 0.31 mmol). The mixture was stirred for 10 h at 115°C under microwave irradiation. After cooling to rt the reaction mixture was diluted with water (250 mL) and extracted with EA (250 mL × 2). The combined organic layers were washed with brine (100 mL × 3) and dried over Na_2SO_4.

The residue was purified by CC (EA/PE) to give the title compound **2c** (1.2 g, 42%) as a pale yellow solid.

Step 4: 3-((1s,3s)-3-(2-Chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)benzoic acid (2)

To a solution of compound **2c** (15 g, 24.2 mmol) in EtOH (750 mL) was added aq. NaOH (40 g in 100 mL of water). The resulting mixture was heated to reflux overnight and then cooled to rt. The reaction was concentrated in vacuo to remove the volatile solvent, diluted with water (1000 mL) and the pH was adjusted to 2 with diluted aq. HCl (1N). The formed precipitate was collected by filtration to give the crude product as a yellow solid (13.8 g). Purification by preparative preversed phase HPLC (RP-HPLC) afforded the title compound **2** (8.0 g, 56%, single isomer by ^{1}H-NMR) as a white solid.

Preparative Example 3



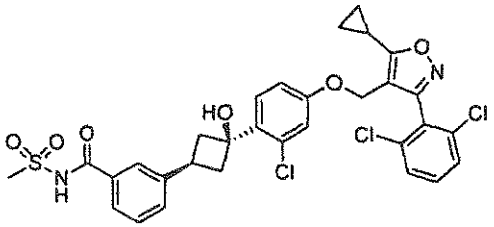
Step 1: Methyl 3-(3-hydroxyazetidin-1-yl)benzoate (**3a**)

To a solution of methyl 3-iodobenzoate (4.5 g, 17.2 mmol) in DMSO (30 mL) was added 3-azetidin-3-ol hydrogen chloride salt (1.3 g, 11.8 mmol), Cs_2CO_3 (9.5 g, 29.2 mmol), CuI (446 mg, 2.3 mmol) and *L*-proline (540 mg, 4.7 mmol) and then the mixture was heated at 90°C for 18 h under argon atmosphere. The solution was diluted with EA and water and the organic layer was washed with brine three times, concentrated under reduced pressure and purified by CC (PE/EA = 2:1) to give compound **3a** (1.6 g, 66%) as a yellow solid.

Step 2: Methyl 3-(3-oxoazetidin-1-yl)benzoate (**3**)

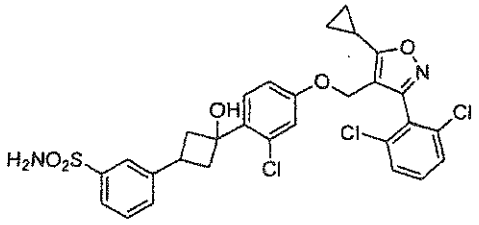
To a solution of compound **3a** (1.60 g, 7.7 mmol) in dry DCM (30 mL) was added Dess-Martin periodinane (6.5 g, 15.4 mmol) at 0°C and the mixture was stirred at rt for 2 h under N_2 atmosphere. The mixture was quenched with saturated sodium bicarbonate solution and diluted with EA. The organic portion was washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure and purified by CC (PE/EA = 4:1) to give compound **3** (1.2 g, 75%) as a white solid.

Example 4: 3-((1s,3s)-3-(2-Chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)-N-(methylsulfonyl)benzamide (4)



To the solution of compound **2** (100 mg, 0.17 mmol) in DCM (5 mL) were added EDCI·HCl (100 mg, 0.52 mmol), DMAP (100 mg, 0.81 mmol) and $MeSO_2NH_2$ (40 mg, 0.42 mmol). The mixture was stirred at 30°C overnight and then diluted with EA and washed by H_2O, brine and dried over Na_2SO_4. Concentration in vacuo and purification by prep-TLC gave crude target compound as a light yellow solid. RP-HPLC purification afforded the title compound **4** (38 mg, 33%) as a white solid. ^1H NMR (400 MHz, CD_3OD) δ: 7.87 (s, 1H), 7.74 (d, J = 7.6 Hz, 1H), 7.61-7.53 (m, 4H), 7.50-7.46 (m, 2H), 6.91 (d, J = 2.4 Hz, 1H), 6.80 (dd, J = 8.8, 2.4 Hz, 1H), 4.95 (s, 2H), 3.38(s, 3H), 3.30-3.26 (m, 2H), 3.01 (m, 1H), 2.57-2.51 (m, 2H), 2.37 (m, 1H), 1.25-1.23 (m, 4H). MS (ESI^-) m/z: 659 $[M-1]^-$.

Example 5: 3-(3-(2-Chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)benzenesulfonamide (**5**)



Step 1: 3-(3-(Benzylthio)phenyl)-1-(2-chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)cyclobutanol (**5a**)

To a solution of compound **2b** (619 mg, 1 mmol) in toluene (20 mL) under argon atmosphere were added K_2CO_3 (276 mg, 2 mmol), phenylmethanethiol (125 mg, 1 mmol), $Pd_2(dba)_3$ (200 mg, 0. 22 mmol) and Xantphos (75 mg, 0.16 mmol). Then the mixture was stirred at 115°C for 4 h. After being cooled to rt, the reaction was diluted with water (100 mL) and extracted with EA (100 mL × 2). The combined organic layers were washed with brine (100 mL × 2), dried over Na_2SO_4 and concentrated to dryness. Purification by CC gave compound the compound **5a** (200 mg; 30%) as a pale yellow solid. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.36-7.32 (m, 3H), 7.28-7.07 (m, 9H), 7.01 (d, J = 7.2 Hz, 1H), 6.82 (d, J = 2.0 Hz, 1H), 6.66 (dd, J = 8.8, 2.0 Hz, 1H), 4.75 (s, 2H), 4.04 (s, 2H), 3.06-3.00 (m, 2H), 2.84-2.78 (m, 2H), 2.44-2.38 (m, 3H), 2.09 (m, 1H), 1.24-1.18 (m, 2H), 1.11-1.08 (m, 2H). MS (ESI^+) m/z: 662 $[M+1]^+$.

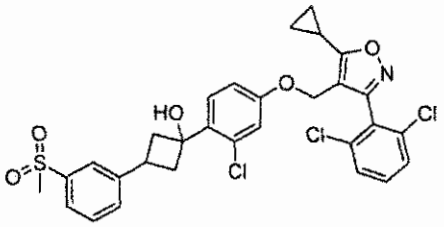
Step 2: 3-(3-(2-Chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)benzene-1-sulfonyl chloride (**5b**)

To a solution of compound **5a** (34 mg, 0.05 mmol) in $CH_3CN/HOAc/H_2O$ (1 mL/37 µL/25 µL) was added 2,4-dichloro-5,5-dimethylhydantion (20 mg, 0.1 mmol). The mixture was stirred at 0-5°C for 2 h. The reaction was diluted with water and extracted with CH_2Cl_2. The combined organic layers were washed with a 5% $NaHCO_3$ solution, brine and dried over Na_2SO_4. Concentration to dryness afforded the crude product **5b** (30 mg) as a colorless oil, which was used directly in the next step.

Step 3: 3-(3-(2-Chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)benzenesulfonamide (5)

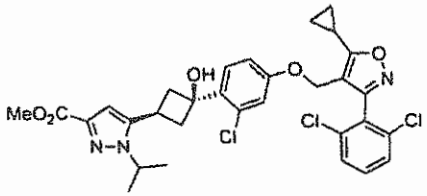
To the solution of compound **5b** (30 mg) in CH_3CN (2 mL) was added NH_4OH (0.3 mL). The mixture was stirred at rt for 1 h. Concentration to dryness and purification by prep. RP-HPLC gave the title compound **5** (3.5 mg, 10% for two steps) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.85 (s, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.54-7.41 (m, 5H), 7.35 (d, J = 8.4 Hz, 1H), 6.90 (s, 1H), 6.75 (d, J = 8.4 Hz, 1H), 4.83 (s, 2H), 4.77 (s, broad, 2H), 3.20 (t, J = 10.4 Hz, 2H), 3.04 (m, 1H), 2.58 (t, J = 10.6 Hz, 2H), 2.17 (m, 1H), 1.31-1.30 (m, 2H), 1.20-1.16 (m, 2H). MS (ESI^-) m/z: 617 $[M-1]^-$.

Example 6: 1-(2-Chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-(3-(methylsulfonyl)phenyl)cyclobutanol (**6**)



To the solution of compound **2b** (200 mg, 0.32 mmol) in DMSO, sodium methanesulfinate (50 mg, 0.46 mmol), CuI (20 mg, 0.1 mmol), *L*-proline (37 mg, 0.32 mmol) and diisopropylethylamine (DIEA) (41 mg, 0.32 mmol) was added. The mixture was stirred at 95°C overnight and then diluted with water and extracted with EA. The combined organic layers were washed with water and dried over Na_2SO_4. Concentration to dryness under reduced pressure and purification by prep. RP-HPLC gave the title compound **6** as a white solid (35 mg, 21%, single isomer by ^1H NMR and LC-MS). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.84 (s, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.53 (t, J = 7.6 Hz, 1H), 7.44-7.41 (m, 3H), 7.34 (t, J = 7.2 Hz, 1H), 6.90 (d, J = 2.8 Hz, 1H), 6.75 (dd, J = 8.4, 2.0 Hz, 1H), 4.83 (s, 2H), 3.24-3.19 (m, 2H), 3.08-3.04 (m, 4H), 2.62-2.56 (m, 2H), 2.17 (m, 1H), 1.31-1.29 (m, 2H), 1.20-1.16 (m, 2H). MS (ESI+) m/z: 618 (620) $[M+1]^+$, 600 (602) $[M-H_2O+1]^+$.

Example 7: Methyl 5-((1s,3s)-3-(2-chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)-1-isopropyl-1H-pyrazole-3-carboxylate (7)



Step 1: Methyl 1-isopropyl-5-vinyl-1*H*-pyrazole-3-carboxylate (7a)

A suspension of methyltriphenylphosphonium bromide (2.69 g, 7.52 mmol) in dry THF (40 mL) was cooled to –78°C and *n*-butyllithium (1.6 M solution in hexane, 3.7 mL, 5.91 mmol) was added dropwise. The yellow-orange suspension was stirred at –78°C for 50 min and then a solution of methyl 5-formyl-1-isopropyl-1*H*-pyrazole-3-carboxylate (prepared as described in WO 2011/020615, 1.05 g, 5.37 mmol) in dry THF (10 mL) was added dropwise. The mixture was stirred at –78°C for 1.75 h, the cooling bath was removed and the mixture (off-white suspension) was stirred at rt for 1 h. The mixture was then partitioned between diluted aq. $NaHCO_3$ solution (150 mL) and EA (150 mL). The aq. layer was extracted twice with EA (50 mL each) and the combined organic layer was washed twice with water (50 mL each) and concentrated without drying to give 2.74 g of a yellow oil which slowly crystallized. The crude product was purified by CC (preadsorption with CH_2Cl_2, hexane/EA 4:1) to give alkene **7a** (590 mg, 57%) as a colorless oil. ^{1}H NMR (DMSO-d_6) δ: 7.02 (s, 1H), 6.87 (dd, J = 17.3, 11.2 Hz, 1H), 5.94 (dd, J = 17.3, 1.3 Hz, 1H), 5.45 (dd, J = 11.2, 1.3 Hz, 1H), 4.80 (sept, J = 6.6 Hz, 1H), 3.79 (s, 3H), 1.38 (d, J = 6.6 Hz, 6H). $C_{10}H_{14}N_2O_2$ (194.23). LC-MS (ESI): 195 $[M+H]^+$.

Step 2: Methyl 1-isopropyl-5-(3-oxocyclobutyl)-1*H*-pyrazole-3-carboxylate (7b)

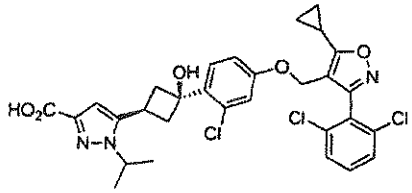
The reaction was performed in two dry sealed tubes (two batches of equal quantity). The batches were combined for workup and purification. Single batch procedure: To a solution of *N,N*-dimethylacetamide (0.22 mL, 2.34 mmol) in 1,2-dichloroethane (12 mL) under nitrogen at –15 to –20°C was added dropwise trifluoromethanesulfonic anhydride (0.43 mL, 2.57 mmol), forming an opaque suspension. The mixture was stirred at –15°C for 10 min, and a solution of alkene **7a** (151 mg, 0.78 mmol) and sym.-collidine (0.42 mL, 3.12 mmol) in 1,2-dichloroethane (3 mL) was added dropwise (yellow solution formed). Upon completion of the addition the cooling was bath removed, the mixture was allowed to warm to rt (orange turbid solution) and the tube was sealed. The mixture was then stirred at 90°C for 15 h (brown mixtures). Water (5 mL) was added at rt and the mixtures were stirred at 100°C for 2 h (turbid two-phase solutions). After cooling to rt, the mixtures were combined and partitioned between diluted aq. $NaHCO_3$ solution and CH_2Cl_2 and the aq. layer was extracted three times with CH_2Cl_2 (30 mL each). The combined organic layer was dried (Na_2SO_4), filtered and concentrated to give a brown oil (2.2 g). Purification by CC (6x13 cm, preadsorption with CH_2Cl_2, toluene/EA 3:1) gave cyclobutanone **7b** (115.5 mg, 31%) as a yellow oil. ^{1}H NMR (DMSO-d_6) δ: 6.81 (s, 1H), 4.58

(sept, J = 6.5 Hz, 1H), 3.78 (s, 3H), 3.85-3.73 (m, 1H), 3.59-3.45 (m, 2H), 3.37-3.24 (m, 2H, partially overlapped by water signal), 1.39 (d, J = 6.6 Hz, 6H). $C_{12}H_{16}N_2O_3$ (236.27). LC-MS (ESI): 237 $[M+H]^+$.

Step 3: Methyl 5-((1s,3s)-3-(2-chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)-1-isopropyl-1H-pyrazole-3-carboxylate (7)

A solution of bromide **1a** (368 mg, 0.78 mmol) in dry THF (6 mL) was cooled to –78°C and a 1.6M *n*-butyllithium solution in hexanes (0.48 mL, 0.76 mmol) was added dropwise. The mixture was stirred at –78°C for 20 min and a solution of cyclobutanone **7b** (164 mg, 0.69 mmol) in dry THF (4 mL) was added dropwise. The mixture was stirred at –78°C for 2.5 h and saturated aq. NH_4Cl solution (1 mL) was added dropwise at this temperature. The cooling bath was removed and the mixture was allowed to warm to rt and stirred at rt for 0.5 h. The mixture was then added to diluted aq. NH_4Cl solution and extracted three times with EA. The combined organic layer was dried (Na_2SO_4), filtered and concentrated to give 516 mg of an almost colorless oil. Purification by CC (4.5×23 cm, preadsorption with CH_2Cl_2, eluent hexane/acetone = 2:1) afforded recovered cyclobutanone **7b** (31.3 mg, 19%, slightly yellow oil) and impure product (333 mg). Repurification by CC (4×22 cm, hexane/EA = 1:1) or prep-TLC gave pure product **7** (210 mg, 48%) as white foam. ^{1}H NMR (DMSO-d_6) δ: 7.65 (d, J = 2.1 Hz, 1H), 7.62 (s, 1H), 7.59-7.48 (m, 2H), 6.92 (d, J = 2.4 Hz, 1H), 6.76 (dd, J = 8.6, 2.6 Hz, 1H), 6.66 (s, 1H), 5.49 (s, 1H), 4.92 (s, 2H), 4.42 (quint-like m, J = 6.5 Hz, 1H), 3.78 (s, 3H), 3.24-3.11 (m, 2H, partially overlapped by water signal), 3.04-2.90 (m, 1H), 2.54-2.33 (m, 3H, partially overlapped by DMSO signal), 1.32 (d, J = 6.5 Hz, 6H), 1.26-1.08 (m, 4H). $C_{31}H_{30}Cl_3N_3O_5$ (630.95). LC-MS (ESI): 630, 632 $[M+H]^+$.

Example 8: 5-((1s,3s)-3-(2-Chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)-1-isopropyl-1H-pyrazole-3-carboxylic acid (**8**)



Ester **7** (98.3 mg, 0.156 mmol) was dissolved in a mixture of THF (7.5 mL), MeOH (2.5 mL) and water (2.5 mL) and $LiOH \cdot H_2O$ (65 mg, 1.56 mmol) was added at rt. The mixture was stirred at rt for 18 h. The mixture was partitioned between diluted aq. NH_4Cl solution and EA and the organic layer was washed once with water. The combined aq. layer was extracted twice with EA. The combined organic layer was dried (Na_2SO_4), filtered and concentrated to give 103 mg of an almost white solid. The product was purified by CC (3×3.5 cm, EA/EtOH = 10:1 to 1:4) to afford **8** (94.8 mg, 99%) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 7.66-7.60 (m, 1H), 7.62 (s, 1H),

7.59-7.49 (m, 2H), 6.91 (d, J = 2.5 Hz, 1H), 6.76 (dd, J = 8.6, 2.4 Hz, 1H), 6.38 (s, 1H), 5.51 (s, 1H, exchangeable with D_2O), 4.92 (s, 2H), 4.31 (quint-like m, J = 6.5 Hz, 1H), 3.25-3.08 (m, 2H, partially overlapped by water signal), 2.93-2.77 (m, 1H), 2.57-2.43 (m, 1H, hidden by DMSO signal), 2.43-2.29 (m, 2H, partially overlapped by DMSO signal), 1.29 (d, J = 6.5 Hz, 6H), 1.26-1.08 (m, 4H). The CO_2H signal does not appear in the spectrum. $C_{30}H_{28}Cl_3N_3O_5$ (616.92). LC-MS (ESI): 616, 618 $[M+H]^+$.

Alternative route to Example 8

Step 1: 1-(3-Methylenecyclobutyl)ethanone (**8a**)

Methylene cyclobutane carbonitrile (5.0 g, 53.7 mmol) was dissolved in dry diethylether (25 mL), cooled in an ice bath and MeMgBr (26.8 mL, 80.5 mmol, 3 M in ether) was added dropwise. The mixture was left stirring overnight at rt, cooled to 0°C, quenched carefully with 15% $NaHSO_4$ aq. sol. (100 mL). The mixture was stirred at rt for 30 min. and the layers were separated. The aq. phase was extracted with pentane (50 mL) and diethylether (50 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. The solvents were removed under vacuum at rt and the crude product was obtained as a yellowish liquid.

Step 2: Ethyl 4-(3-methylenecyclobutyl)-2,4-dioxobutanoate (**8b**)

Sodium (1.15 g, 49.9 mmol) was dissolved in dry EtOH (30 mL, denaturated with 5% diethylether). Compound **8a** (5.5 g, 49.9 mmol, crude) was dissolved in dry EtOH (45 mL) and the above prepared sodium ethoxide solution was added. This mixture was stirred at rt for 15 min and then diethyl oxalate (6.8 mL, 49.9 mmol) was added dropwise. The reaction mixture was placed in a pre-heated (to 67 °C) oil bath and stirred at this temperature for 4.5 h. The mixture was left at rt overnight. The solvent was removed, EA (100 mL) and 1 M HCl (70 mL) were added and organic phase was separated. The aq. phase was re-extracted with EA (50 mL). The combined organic phases were washed with water, brine and dried over anh. Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified on silica using hexanes/MTBE 9:1 as eluent giving pure product **8b**. Yield: 6.29 g, 56% over two steps. ^{1}H-NMR ($CDCl_3$), δ (ppm): 6.36 (s, 1H), 4.85-4.80 (m, 2H), 4.34 (q, J = 8.0 Hz, 2H), 3.35-3.25 (m, 1H), 3.05-2.85 (m, 4H), 1.36 (t, J = 8.0 Hz, 3H).

Step 3: Ethyl 1-isopropyl-5-(3-methylenecyclobutyl)-1*H*-pyrazole-3-carboxylate (**8c**)

Compound **8b** (6.29 g, 29.9 mmol) was dissolved in dry EtOH (65 mL, denaturated with 5% of MeOH) and isopropyl hydrazine hydrochloride (3.97 g, 35.9 mmol) was added. The reaction mixture was stirred for 3 h at rt. The solvent was removed and to the oily residue were added EA (100 mL), water (50 mL) and sat. $NaHCO_3$ (50 mL) sequentially. The layers were separated and the aq. phase was re-extracted with EA (50 mL). The combined organic phases were washed with brine (70 mL) and dried over anh. Na_2SO_4. The solvent was removed under

vacuum and the residue was dried under reduced pressure. Yield: 7.23 g (contains 3.4% of EtOAc by NMR, recalculated pure yield: 6.98 g, 94%). Crude product **8c** is 98% pure by HPLC and NMR. ^{1}H-NMR ($CDCl_3$), δ (ppm): 6.62 (s, 1H), 4.88-4.82 (m, 2H), 4.42-4.32 (m, 3H), 3.56-3.45 (m, 1H), 3.17-3.07 (m, 2H), 2.88-2.79 (m, 2H), 1.49 (d, J = 8.0 Hz, 6H), 1.37 (t, J = 8.0 Hz, 3H).

Step 4: Ethyl 1-isopropyl-5-(3-oxocyclobutyl)-1*H*-pyrazole-3-carboxylate (**8d**)

Compound **8c** (6.45 g, 26.0 mmol) was dissolved in a mixture of MeCN (77 mL) and water (13 mL) and cooled in an ice-bath. To this solution $RuCl_3$×H2O (0.19 g, 0.86 mmol) was added, followed by portion-wise addition of $NaIO_4$ (19.35 g, 90.9 mmol). An exotherm was observed during this addition. The obtained thick slurry was stirred at rt for 45 min. The reaction mixture was diluted with $Na_2S_2O_3$ aq. sol. (10%, 260 mL), water (50 mL) and DCM (100 mL). The phases were separated and the aq. phase was extracted with DCM (2×70 mL). The combined organic phases were washed with $Na_2S_2O_3$ aq. sol. (10%, 50 mL), water (100 mL), brine (100 mL) and dried over anh. Na_2SO_4.The crude product (6.5 g) was purified on silica, eluting with hexanes/MTBE to give pure product as an oil that solidified upon storage at –20°C. Yield: 5.8 g (78% over two steps). ^{1}H-NMR (DMSO-d_6), δ (ppm): 6.78 (s, 1H), 4.57 (h, J = 8.0 Hz, 1H), 4.26 (q, J = 8.0 Hz, 2H), 3.85-3.75 (m, 1H), 3.58-3.45 (m, 2H), 3.35-3.25 (m, 2H), 1.39 (d, J = 8.0 Hz, 6H), 1.28 (t, J = 8.0 Hz, 3H).

Step 5: 4-((4-Bromo-3-chlorophenoxy)methyl)-5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazole (**8e**)

3-Chloro-4-bromophenol (3.8 g, 18.3 mmol) was mixed with (5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methanol (3.47 g, 12.2 mmol) and triphenylphosphine (6.41 g, 24.4 mmol) in toluene (150 mL). The mixture was cooled in an ice-bath and DIAD (4.8 mL, 24.4 mmol) as a solution in toluene (10 mL) was added drop-wise. The reaction was stirred at rt for 21 h and the solvents were removed on a rotavap leaving a yellow oily residue. This was dissolved in DCM (200 mL), silica (~20 g) was added and the mixture was evaporated to dryness. This material was loaded on the top of a silica column and purified eluting with hexanes/MTBE 9:1. The product containing fractions were pooled and the solvent removed under reduced pressure, leaving pure product **8e** as a colourless oil that crystallized upon drying under vacuum overnight. Yield: 5.07 g (88%).^{1}H-NMR ($CDCl_3$), δ (ppm): 7.45-7.30 (m, 4H), 6.90 (s, 1H), 6.60-6.55 (m, 1H), 2.15-2.07 (m, 1H), 1.32-1.25 (m, 2H), 1.20-1.11 (m, 2H).

Step 6: Ethyl 5-((1s,3s)-3-(2-chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)-1-isopropyl-1*H*-pyrazole-3-carboxylate (**8f**)

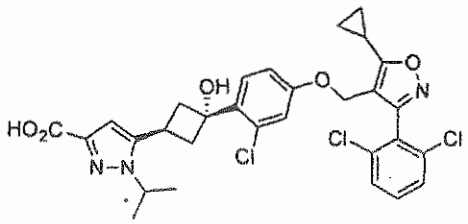
LiCl (0.684 g, 16.15 mmol) was dissolved in THF (20 mL) at rt and *i*PrMgCl (2.0 M in THF, 8.1 mL, 16.15 mmol) was added. The mixture was stirred for 10 min at rt, cooled in an ice-bath and a solution of compound **8e** (2.55 g, 5.38 mmol) in THF (20 mL) was added over 5 min. The cooling bath was removed and the mixture was stirred at rt for 4 h. The mixture was cooled to –

10°C and a solution of compound **8d** (1.48 g, 5.92 mmol) in THF (16 mL) was added rapidly. The mixture was stirred at rt for 90 min. and then 0.5 M $NaHSO_4$ aq. (35 mL) and EA (50 mL) were added. The resulting mixture was stirred for 10 min., the layers were separated and the aq. layer was extracted with EA (30 mL). The combined organic phases were washed with $NaHCO_3$ aq. (50 mL), brine (50 mL) and dried over anh. Na_2SO_4. The crude product (3.79 g) was obtained after removal of the solvent as a white foam. 3.6 g of this crude was purified on silica column, eluting with hexanes/EA 3:2 to give pure product **8f** as a solid foam. Yield: 1.62 g (49%). ^{1}H-NMR (DMSO-d_6), δ (ppm): 7.65-7.47 (m, 4H), 6.93-6.91 (m, 1H), 6.79-6.72 (m, 1H), 6.65 (s, 1H), 5.48 (s, 1H), 4.92 (s, 2H), 4.42 (h, J = 8.0 Hz, 1H), 4.26 (q, J = 8.0 Hz, 2H), 3.32 (s, 2H), 3.22-3.14 (m, 2H), 3.05-2.90 (m, 1H), 2.45-2.35 (m, 2H), 1.35-1.10 (m, 14H).

Step 7: 5-((1s,3s)-3-(2-Chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)-1-isopropyl-1*H*-pyrazole-3-carboxylic acid (**8**)

Compound **8f** (1.60 g, 2.48 mmol) was dissolved in THF (100 mL), then MeOH (50 mL), water (50 mL) and LiOH×H2O (1.04 g, 24.8 mmol) were added sequentially. The mixture was stirred for 4.5 h at rt and then concentrated under reduced pressure to remove MeOH and THF. The remaining aq. solution was acidified by addition of 1 M HCl aq. (24 mL) to reach pH of 4.05 (pH electrode control). Already at approx. pH 7 a precipitate started to form. The formed solid was filtered off, washed on the filter with water and dried under vacuum at rt to give product **8** as a white powder. Yield: 1.40 g (92%). ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.44-7.32 (m, 4H), 6.91 (d, J = 4.0 Hz, 1H), 6.78 (s, 1H), 6.75 (dd, J = 4.0 Hz, J = 8.0 Hz, 1H), 4.83 (s, 2H), 4.35-4.20 (m, 1H), 3.25-3.14 (m, 2H), 3.04-2.90 (m, 1H), 2.62-2.54 (m, 2H), 2.21-2.11 (m, 1H), 1.46 (d, J = 8.0 Hz, 6H), 1.34-1.28 (m, 2H), 1.20-1.14 (m, 2H). ^{13}C-NMR ($CDCl_3$), δ (ppm): 172.7, 164.8, 159.2, 158.4, 147.2, 141.3, 135.8, 134.1, 132.8, 131.3, 128.1, 127.6, 127.3, 117.7, 113.3, 110.0, 106.3, 73.1, 59.8, 51.1, 41.7, 22.6, 22.0, 8.5, 7.8. MS (ESI$^+$) *m/z*: 616.4 $[M+1]^+$.

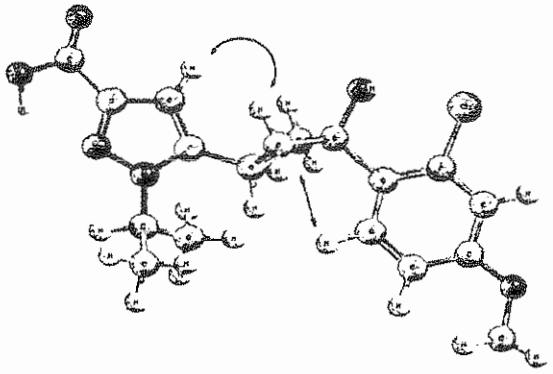
Example 8A: 5-((1r,3r)-3-(2-Chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)-1-isopropyl-1*H*-pyrazole-3-carboxylate (**8A**)



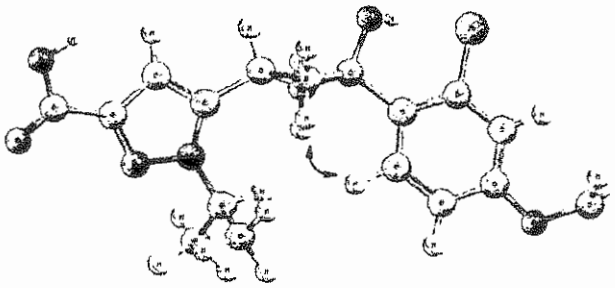
Example **8A** can be prepared by subjecting the crude product **8f** to the ester hydrolysis as described for **8** and isolation from the crude product **8** as a minor isomer by preparative RP-HPLC. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.42-7.30 (m, 2H), 7.11 (d, J = 8.0 Hz, 1H), 6.75-6.65 (m, 1H),

6.57 (s, 1H), 4.79 (s, 2H), 4.50-4.41 (m, 1H), 3.96-3.85 (m, 1H), 2.98-2.90 (m, 2H), 2.67-2.57 (m, 2H), 2.20-2.09 (m, 1H), 1.51 (d, J = 8.0 Hz, 6H), 1.32-1.14 (m, 4H). ^{13}C-NMR ($CDCl_3$), δ (ppm): 172.6, 166.2, 159.2, 158.4, 147.4, 141.2, 135.7, 134.6, 132.8, 131.3, 128.1, 127.7, 127.5, 116.8, 113.5, 110.0, 105.8, 75.1, 59.8, 51.2, 41.8, 25.4, 22.6, 8.5, 7.8. MS (ESI$^+$) m/z: 616.3 $[M+1]^+$.

The transannular configuration of the major isomer (compound **8**) and the minor isomer (compound **8A**) was confirmed by NOE experiments. The detected indicative NOEs between protons are indicated in the following pictures by double arrows:

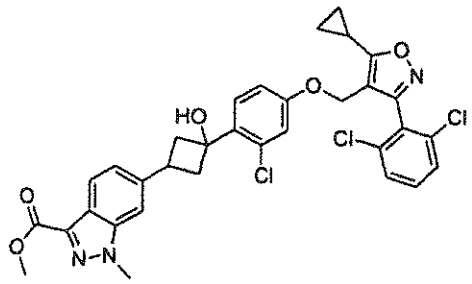


*NOEs detected for example **8** with 1,3-trans transannular configuration of the aromatic moieties*



*NOEs detected for example **8A** with 1,3-cis transannular configuration of the aromatic moieties*

Example 9: Methyl 6-(3-(2-chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)-1-methyl-1*H*-indazole-3-carboxylate (**9**)



Step 1: Methyl 1-methyl-6-vinyl-1*H*-indazole-3-carboxylate (**9a**)

To the solution of methyl 6-bromo-1-methyl-1*H*-indazole-3-carboxylate (60 mg, 0.22 mmol) in DMF (10 mL), tributyl(vinyl)tin (99 µL, 0.34 mmol), $Pd(Ph_3)_4$ (11 mg, 9 µmol) was added. After the addition was completed, the mixture was stirred at 90°C for 4 h under Ar. Then the solvent was removed under reduced pressure. Purification by CC afforded compound **9a** (52 mg, 88%).

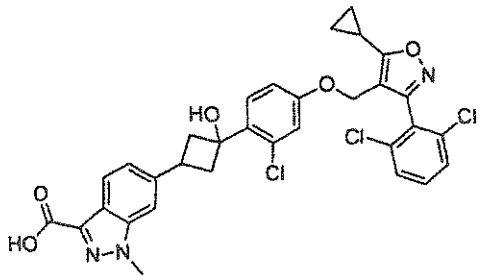
Step 2: Methyl 1-methyl-6-(3-oxocyclobutyl)-1*H*-indazole-3-carboxylate (**9b**)

Following the procedure as described in Example 7/Step 2, compound **9b** was obtained from **9a** in 57% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.14 (d, J = 8.4 Hz, 1H), 7.31 (s, 1H), 7.23 (d, J = 8.8 Hz, 1H), 4.13 (s, 3H), 3.99 (s, 3H), 3.87-3.79 (m, 1H), 3.58-3.51 (m, 2H), 3.33-3.26 (m, 2H). m/z: 259 $[M+1]^+$.

Step 3: Methyl 6-(3-(2-chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)-1-methyl-1*H*-indazole-3-carboxylate (**9**)

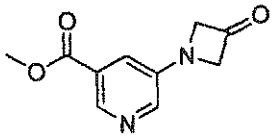
Following the procedure as described in Example 7/Step 3, compound **9** was obtained from **9b** in 40% yield.

Example 10: 6-(3-(2-Chloro-4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)phenyl)-3-hydroxycyclobutyl)-1-methyl-1*H*-indazole-3-carboxylic acid (**10**)



Following the procedure as described in Example 8, compound **10** was obtained from compound **9** in 45% yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.14 (d, J = 8.0 Hz, 1H), 7.48 (d, J = 8.8 Hz, 1H), 7.43-7.32 (m, 4H), 7.29 (m, 1H), 6.92 (d, J = 2.4 Hz, 1H), 6.76 (dd, J = 7.2 Hz, 2.4 Hz, 1H), 4.84 (s, 2H), 4.18 (s, 3H), 3.45-3.40 (m, 1H), 3.28-3.23 (m, 2H), 3.19-3.10 (m, 1H), 2.68-2.63 (m, 2H), 2.21-2.14 (m, 1H), 1.33-1.29 (m, 2H), 1.20-1.15 (m, 2H). m/z: 638 $[M+1]^+$.

Preparative Example 11



Step1: Methyl 5-(3-hydroxyazetidin-1-yl)nicotinate (11a)

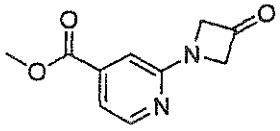
A mixture of methyl 5-bromonicotinate (2.00 g, 9.26 mmol), azetidin-3-ol (1.01 g, 9.26 mmol), Cs_2CO_3 (9.06 g, 27.8 mmol), BINAP (1.15 g, 1.85 mmol) and $Pd(OAc)_2$ (0.44 g, 1.85 mmol) in dry dioxane (115 mL) was heated overnight at 85°C under N_2 atmosphere. The resulting mixture was filtrated, concentrated under reduced pressure and purified by prep-HPLC to give compound **11a** (250 mg, 13%) of as a yellow solid.

Step 2: Methyl 5-(3-oxoazetidin-1-yl)nicotinate (11)

To a solution of compound **11a** (250 mg, 1.20 mmol) in dry DCM (15 mL) was added Dess-Martin periodinane (1.014 g, 2.40 mmol) at 0°C under N_2 atmosphere and the solution was stirred at rt for 2 h. The resulting solution was quenched with saturated sodium bicarbonate solution and diluted with EA. The organic portion was washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure and purified by CC (DCM/MeOH = 150:1) to give compound **11** (140 mg, 57%) of as a yellow solid.

Preparative Example 12

Using a similar procedure as that described in Preparative Example **11** the following compound has been prepared:

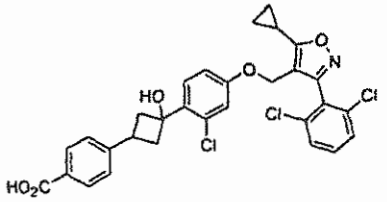
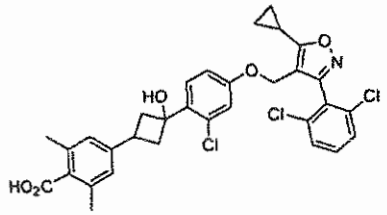
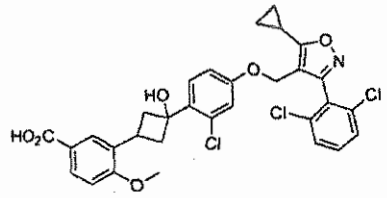
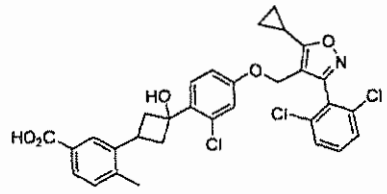
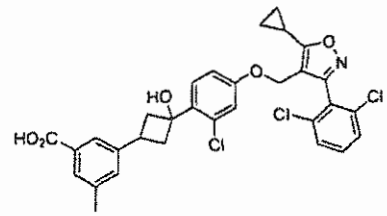
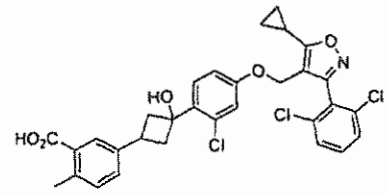
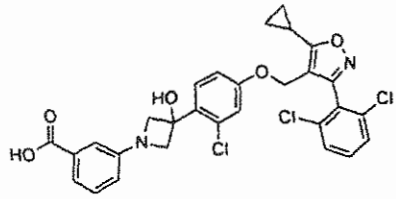


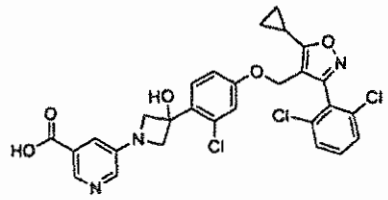
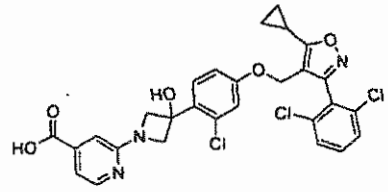
12

Example 13/1 to 13/9

The following table lists further examples prepared according the above mentioned preparative examples and examples. All listed compounds were prepared as single isomers.

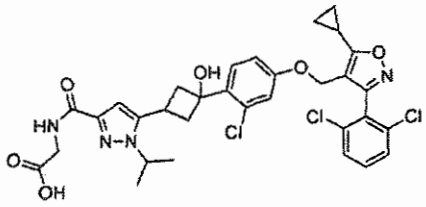
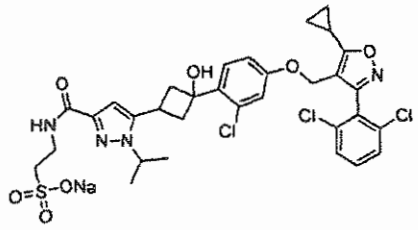
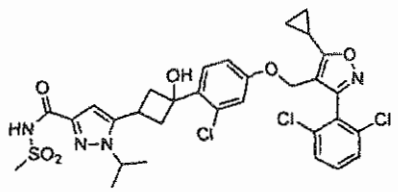
#	Structure	Analytical data

#	Structure	Analytical data
13/1		^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.15-1.25 (m, 4H), 2.35-2.50 (m, 5H, partially under solvent signal), 2.80-2.91 (m, 1H), 3.11-3.20 (m, 2H), 4.93 (s, 2H), 6.72-6.81 (m, 1H), 6, 93 (s, 1H), 7.40-7.51 (m, 2H), 7.52-7.60 (m, 2H), 7.62-7.66 (m, 2H), 7.85-7.90 (m, 2H). MS Calcd.: 583; MS Found: 584 $[M+H]^+$.
13/2		^{1}H NMR (400 MHz, CD_3OD) δ: 1.05-1.12 (m, 4H), 2.18-2.34 (m, 9H), 2.62-2.71 (m, 1H), 2.99-3.09 (m, 2H), 4.78 (s, 2H), 6.60-6.64 (m, 1H), 6.72-6.78 (m, 1H), 6.85 (s, 2H), 7.30-7.42 (m, 4H). MS Calcd.: 611; MS Found: 612 $[M+H]^+$.
13/3		^{1}H NMR (400 MHz, CD_3OD) δ: 1.10-1.23 (m, 4H), 2.36-2.49 (m, 3H), 3.00-3.12 (m, 1H), 3.15-3.25 (m, 2H), 3.87 (s, 3H), 4.95 (s, 2H), 6.72-6.80 (m, 1H), 6.89 (s, 1H), 6.90-7.00 (m, 1H), 7.42-7.60 (m, 4H), 7.89-7.93 (m, 1H), 7.98 (s, 1H). MS Calcd.: 613; MS Found: 612 $[M-H]^-$.
13/4		^{1}H NMR (400 MHz, CD_3OD) δ: 1.20-1.30 (m, 4H), 2.27 (s, 3H), 2.30-2.55 (m, 3H), 2.98-3.10 (m, 1H), 3.25-3.40 (m, 2H, partially under solvent signal), 4.95 (s, 2H), 6.76-6.84 (m, 1H), 6.91 (s, 1H), 7.20-7.25 (m, 1H), 7.43-7.63 (m, 4H), 7.75-7.82 (m, 1H), 8.03 (s, 1H). MS Calcd.: 597; MS Found: 596 $[M-H]^-$.
13/5		^{1}H NMR (400 MHz, CD_3OD) δ: 1.20-1.25 (m, 4H), 2.33-2.43 (m, 4H), 2.46-2.56 (m, 2H), 2.88-2.97 (m, 1H) 3.22-3.30 (m, 2H), 4.94 (s, 2H), 6.78-6.82 (m, 1H), 6.90 (s, 1H), 7.37 (s, 1H), 7.43-7.60 (m, 4H), 7.69 (s, 1H), 7.78 (s, 1H). MS Calcd.: 597; MS Found: 596 $[M-H]^-$.
13/6		^{1}H NMR (400 MHz, CD_3OD) δ: 1.17-1.23 (m, 4H), 2.31-2.40 (m, 4H), 2.42-2.50 (m, 2H), 2.83-2.92 (m, 1H) 3.19-3.26 (m, 2H), 4.92 (s, 2H), 6.74-6.80 (m, 1H), 6.88 (s, 1H), 7.19-7.22 (m, 1H), 7.43-7.57 (m, 4H), 7.83 (s, 1H). MS Calcd.: 597; MS Found: 598 $[M+H]^+$.
13/7		^{1}H NMR (400 MHz, CD_3OD) δ: 1.09-1.11 (m, 4H), 2.19-2.26 (m, 1H), 4.08-4.10 (m, 2H), 4.19-4.21 (m, 2H), 4.80 (s, 2H), 6.64-6.67 (m, 2H), 6.75 (s, 1H), 7.16-7.21 (m, 2H), 7.28-7.39 (m, 6H); MS Calcd.: 584; MS Found: 585 (M+1).

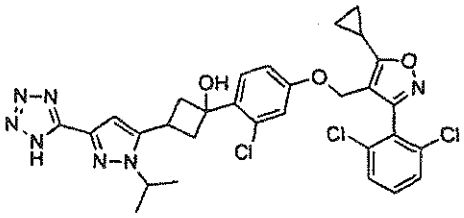
#	Structure	Analytical data
13/8		^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.11 (m, 2H), 1.24 (m, 2H), 2.11 (m, 1H), 4.33 (m, 2H), 4.46 (m, 2H), 4.78 (s, 2H), 6.67 (dd, J = 1.2Hz, 8.4 Hz, 1H), 6.77 (d, J = 2.4 Hz, 1H), 6.67 (d, J = 8.4 Hz, 1H), 7.28-7.35 (m, 3H), 7.55 (d, J = 1.2 Hz, 1H), 7.94 (s, 1H), 8.56 (d, J = 3.6 Hz, 1H); MS Calcd.: 585; MS Found: 586 (M+1).
13/9		^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.13-1.23 (m, 4H), 2.50 (m, 1H), 4.23 (d, J = 8.4 Hz, 2H), 4.51 (d, J = 9.3 Hz, 2H), 4.96 (s, 2H), 6.24 (s, 1H), 6.80 (d, J = 7.5 Hz, 1H), 6.88 (s, 1H), 6.97 (s, 1H), 7.07 (s, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.58-7.66 (m, 3H), 8.25 (s, 1H); MS Calcd.: 585; MS Found: 586 (M+1).

Example 14/1 and 14/2

Using a similar procedure as described in the Examples 1 to 13 and Schemes above, the following compounds were obtained by using the appropriate building blocks.

#	Structure	Analytical data
14/1		^{1}H NMR (400 MHz, DMSO-d_6): δ 1.13~1.23 (m, 4H), 1.33 (d, J = 6.4 Hz, 6H), 2.37~2.47 (m, 3H), 2.90-2.95 (m, 1H), 3.14-3.19 (t, J = 8.8 Hz, 2H), 3.57 (d, J = 4.0 Hz, 2H), 4.38 (m, 1H), 4.92 (s, 2H), 5.51 (s, 1H), 6.51 (s, 1H), 6.76 (dd, J = 2.4 Hz, J = 8.4 Hz, 1H), 6.91 (d, J = 2.4 Hz, 1H), 7.51-7.58 (m, 2H), 7.62-7.65 (m, 2H), 7.69 (s, 1H); MS Calcd.: 672; MS Found: 673 $[M+H]^+$.
14/2		^{1}H NMR (400 MHz, DMSO-d_6): δ 1.13~1.20 (m, 4H), 1.31 (d, J = 6.0 Hz, 6H), 2.36~2.47 (m, 3H), 2.59-2.63 (m, 2H), 2.89-2.93 (m, 1H), 3.17-3.19 (m, 2H), 3.47-3.52 (m, 2H), 4.33-4.39 (m, 1H), 4.92 (s, 2H), 5.51 (s, 1H), 6.50 (s, 1H), 6.76 (dd, J = 2.4 Hz, J = 8.4 Hz, 1H), 6.91 (d, J = 2.4 Hz, 1H), 7.51-7.58 (m, 2H), 7.62-7.65 (m, 2H), 8.18-8.20 (m, 1H); MS Calcd.: 744; MS Found: 721 $[M-Na]^-$.
14/3		^{1}H NMR (400 MHz, DMSO-d_6): δ 1.10~1.25 (m, 4H), 1.34 (d, J = 6.4 Hz, 6H), 3.03-2.95 (m, 1H), 2.50-2.30 (m, 3H), 3.20-3.10 (m, 2H), 4.50-4.35 (m, 1H), 4.92 (s, 2H), 5.5 (s, 1H), 6.92 (s, 1H), 6.78-6.70 (m, 2H), 7.70-7.49 (m, 4H), 11.44 (s, 1H); MS Calcd.: 694; MS Found: 695 $[M+H]^+$.

The following compound can be prepared in the same manner by using similar procedures as described above:



Assays

FRET activity assay

Determination of a ligand mediated cofactor peptide interaction to quantify ligand binding to the nuclear receptor FXR was performed as follows: Preparation of human FXR alpha ligand binding domain: The human FXRalpha LBD was expressed in *E. coli* strain BL21(DE3) as an N-terminally GST tagged fusion protein. The DNA encoding the FXR ligand binding domain was cloned into vector pDEST15 (Invitrogen). Expression was under control of an IPTG inducible T7 promoter. The amino acid boundaries of the ligand binding domain were amino acids 187–472 of Database entry NM_005123 (RefSeq). Expression and purification of the FXR-LBD: An overnight preculture of a transformed *E.coli* strain was diluted 1:20 in LB-Ampicillin medium and grown at 30°C to an optical density of OD_{600}=0.4–0.6. Gene expression was then induced by addition of 0.5 mM IPTG. Cells were incubated an additional 6 h at 30°C, 180 rpm. Cells were collected by centrifugation (7000 x g, 7 min, rt). Per liter of original cell culture, cells were resuspended in 10 mL lysis buffer (50 mM Glucose, 50 mM Tris pH 7.9, 1 mM EDTA and 4 mg/mL lysozyme) and left on ice for 30 min. Cells were then subjected to sonication and cell debris removed via centrifugation (22000 x g, 30 min, 4°C). Per 10 mL of supernatant 0.5 mL prewashed Glutathione 4B sepharose slurry (Qiagen) was added and the suspension kept slowly rotating for 1 h at 4°C. Glutathione 4B sepharose beads were pelleted by centrifugation (2000 x g, 15 sec, 4°C) and washed twice in wash buffer (25 mM Tris, 50 mM KCl, 4 mM $MgCl_2$ and 1M NaCl). The pellet was resuspended in 3 mL elution buffer per liter of original culture (elution buffer: 20 mM Tris, 60 mM KCl, 5 mM $MgCl_2$ and 80 mM glutathione added immediately prior to use as powder). The suspension was left rotating for 15 min at 4°C, the beads pelleted and eluted again with half the volume of elution buffer than the first time. The eluates were pooled and dialysed overnight in 20 mM Hepes buffer (pH 7.5) containing 60 mM KCl, 5 mM $MgCl_2$ as well as 1 mM dithiothreitol and 10% (v/v) glycerol. The protein was analysed by SDS-Page.

The method measures the ability of putative ligands to modulate the interaction between the purified bacterial expressed FXR ligand binding domain (LBD) and a synthetic biotinylated

peptide based on residues 676–700 of SRC-1 (LCD2, 676–700). The sequence of the peptide used was B-CPSSHSSLTERHKILHRLLQEGSPS-COOH where the N-terminus was biotinylated (B). The ligand binding domain (LBD) of FXR was expressed as fusion protein with GST in BL-21 cells using the vector pDEST15. Cells were lysed by sonication, and the fusion proteins purified over glutathione sepharose (Pharmacia) according to the manufacturers instructions. For screening of compounds for their influence on the FXR-peptide interaction, the Perkin Elmer LANCE technology was applied. This method relies on the binding dependent energy transfer from a donor to an acceptor fluorophor attached to the binding partner of interest. For ease of handling and reduction of background from compound fluorescence LANCE technology makes use of generic fluorophore labels and time resolved detection Assays were done in a final volume of 25 µL in a 384 well plate, in a Tris-based buffer (20 mM Tris-HCl pH 7.5; 60 mM KCl, 5 mM $MgCl_2$; 35 ng/µL BSA), containing 20–60 ng/well recombinantly expressed FXR-LBD fused to GST, 200–600 nM N-terminally biotinylated peptide, representing SRC1 aminoacids 676–700, 200 ng/well Streptavidin-xlAPC conjugate(Prozyme) and 6–10 ng/well Eu W1024 – antiGST (Perkin Elmer). DMSO content of the samples was kept at 1%. After generation of the assay mix and diluting the potentially FXR modulating ligands, the assay was equilibrated for 1 h in the dark at rt in FIA-plates black 384 well (Greiner). The LANCE signal was detected by a Perkin Elmer VICTOR2VTM Multilabel Counter. The results were visualized by plotting the ratio between the emitted light at 665 and 615 nm. A basal level of FXR-peptide formation is observed in the absence of added ligand. Ligands that promote the complex formation induce a concentration-dependent increase in time-resolved fluorescent signal. Compounds which bind equally well to both monomeric FXR and to the FXR-peptide complex would be expected to give no change in signal, whereas ligands which bind preferentially to the monomeric receptor would be expected to induce a concentration-dependent decrease in the observed signal.

To assess the inhibitory potential of the compounds, EC_{50}-values were determined for example compounds as listed below in Table 1 (A = EC_{50} < 25 nM; B = 25 ≤ EC_{50} < 100 nM; C = EC_{50} ≥ 100 nM).

Table 1

Group	Example #
A	4, 8, 10, 13/8, 13/9, 14/1, 14/2
B	1, 2, 5, 6, 8A, 13/1, 13/3, 13/4, 13/5, 13/7
C	13/2, 13/6

Mammalian one hybrid (M1H) assay

Determination of a ligand mediated Gal4 promoter driven transactivation to quantify ligand binding mediated activation of FXR was performed as follows: The cDNA part encoding the FXR ligand binding domain was cloned into vector pCMV-BD (Stratagene) as a fusion to the yeast GAL4 DNA binding domain under the control of the CMV promoter. The amino acid boundaries of the ligand binding domain were amino acids 187–472 of Database entry NM_005123 (RefSeq). The plasmid pFR-Luc (Stratagene) was used as the reporter plasmid, containing a synthetic promoter with five tandem repeats of the yeast GAL4 binding sites, driving the expression of the Photinus pyralis (American firefly) luciferase gene as the reporter gene. In order to improve experimental accuracy the plasmid pRL-CMV (Promega) was cotransfected. pRL-CMV contains the constitutive CMV promoter, controlling the expression of the Renilla reniformis luciferase. All Gal4 reporter gene assays were done in HEK293 cells (obtained from DSMZ, Braunschweig, Germany) grown in MEM with L-Glutamine and Earle's BSS supplemented with 10% fetal bovine serum, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, and 100 units Penicilin/Streptavidin per mL at 37°C in 5% CO_2. Medium and supplements were obtained from Invitrogen. For the assay, 5 x 10^5 cells were plated per well in 96well plates in 100 µL per well MEM without Phenol Red and L-Glutamine and with Earle's BSS supplemented with 10% charcoal/dextran treated FBS (HyClone, South Logan, Utah), 0.1 mM nonessential amino acids, 2 mM glutamine, 1 mM sodium pyruvate, and 100 units Penicilin/ Streptavidin per mL, incubated at 37 °C in 5% CO_2. The following day the cells were >90% confluence. Medium was removed and cells were transiently transfected using 20 µL per well of a OptiMEM - polyethylene-imine-based transfection-reagent (OptiMEM, Invitrogen; Polyethyleneimine, Aldrich Cat No. 40,827-7) including the three plasmids described above. MEM with the same composition as used for plating cells was added 2–4 h after addition of transfection mixture. Then compound stocks, prediluted in MEM were added (final vehicle concentration not exceeding 0.1%). Cells were incubated for additional 16 h before firefly and renilla luciferase activities were measured sequentially in the same cell extract using a Dual-Light-Luciferase-Assay system (Dyer et al., Anal. Biochem. 2000, 282, 158–161). All experiments were done in triplicates.

To assess the FXR agonistic potency of the example compounds, potency ranges were determined in the M1H assay as listed below in Table 2 (A = EC_{50} < 25 nM; B = 25 ≤ EC_{50} < 100 nM; C = EC_{50} ≥ 100 nM).

Table 2

Group	Example #
A	13/4, 13/5, 13/6
B	2, 8, 8A, 10, 13/1, 13/3, 13/7

C	1, 4, 5, 6, 13/2, 13/8, 13/9, 14/1

Aqueous solubility assay

The aq. solubility in PBS, pH 7.4 was determined as follows. A 10 mM compound stock solution in DMSO was added to PBS (pH 7.4) to reach a theoretical final concentration of 200 µM. The resulting solution/suspension was shaken at 1250 rpm for 1 h and then stored in the dark at rt for 23 h. At this time any precipitate is separated from the solution by centrifugation at 3900 rpm for 30 min. The aq. solubility was determined by comparing the peak area of the principle peak in a calibration standard (200 µM) in an organic solvent (methanol/water 60:40, v/v) with the peak area of the corresponding peak in the buffer sample. As detection method was used HPLC-UV/VIS at 230 *nm*.

Parallel Artificial Membrane Permeation Assay (PAMPA)

For the PAMPA, 5 mM stock solutions of test items were prepared in DMSO. 5 mM stock solutions of reference items were prepared in EtOH (carbamazepine, guanabenz) or in EtOH:H_2O 1:1 (v/v) (ceftriaxone), respectively. Compounds were diluted in PBS (pH 7.4) to obtain the starting solutions containing 5% of the respective organic solvent and 250 µM reference compounds or 10 µM test items, respectively. For the assay, a modified procedure of the PAMPA as described by Kansy et al. Kansy et al. (J. Med. Chem. 1998, 41, 1007) was used. The reference compounds for low (ceftriaxone), medium (guanabenz) and high permeation (carbamazepine) were included as internal controls.

Permeation experiments were carried out in a Multiscreen 96 well tray (donor) covered by a 96-well Multiscreen Immobilon (acceptor). The hydrophobic filter material of the Immobilon plate was pre-wetted with 70% ethanol and treated with a solution of lipids (lecithin dissolved in dodecane). The donor plate was filled with test compounds and reference compounds and both plates were inserted into each other and placed onto an orbital shaker for 15 min at 100 rpm. The transport study was started by applying 150 µL PBS-buffer containing the test and reference compounds to the donor plate. After 15 – 16 h of diffusion at rt, the contents of the acceptor and donor plate were collected and quantified using LC/MS-detection (test items) or by UV spectroscopy using a Spectramax Plus^{384} (Molecular Devices) (reference items). The absorption maxima for the reference items ceftriaxone, guanabenz and carbamazepine were 240 nm, 270 nm and 286 nm, respectively. Recovery samples were prepared as described for the permeation assay samples and were incubated in representative vials during the permeation period under the same conditions.

For LC/MS analysis of the test items, 100 µL incubate were removed from acceptor and donor compartment and processed for acetonitrile (ACN) precipitation as described below. Additionally, test item samples from the lipid layer were extracted by flushing each well two

times with 150 µL EA. The solutions were collected in 1.5 mL reaction tubes and the solvent was evaporated. The dried residues were resuspended in a PBS/DMSO/ACN mixture reflecting the composition of the acceptor and donor samples (i.e. 100 µL buffer supplemented with 5% DMSO, 200 µL ACN+ISTD). The final solvent content of each sample was 66% ACN.

Samples from donor and acceptor compartments and calibration standards were precipitated by addition of 200 µL ACN/ISTD or 400 µL ACN/ISTD, respectively. After vigorous shaking (10 seconds) and centrifugation (5 min at 4800 x g, rt), the particle free supernatants were subjected to LC-MS/MS. Membrane compartments were extracted as described above. After reconstitution, samples were vigorously shaken (10 seconds) and spun down (5 min at 4800 x g, rt). The particle free supernatants were subjected to LC-MS/MS.

For analysis of compounds under the present invention, the HPLC system consisted of an Accela U-HPLC pump and an Accela auto sampler (Thermo Fisher Scientific, USA). Mass spectrometry was performed on an Exactive mass spectrometer (orbitrap technology with accurate mass) equipped with an heated electrospray (H-ESI2) interface (Thermo Fisher Scientific, USA) connected to a PC running the standard software Xcalibur 2.1.

The LC was performed in the gradient mode (Table 3) using ACN/0.1% formic acid as organic phase (A) and 10 mM ammonium formate/0.1% formic acid as aq. phase (B); and the pump flow rate was set to 500 µL/min. Separation was performed on a Gemini C6-Phenyl, 3 µm, 50x2.0 mm (Phenomenex, Germany) analytical column with a pre-column (Gemini C6-Phenyl, 3 µm, 4x2.0 mm).

Table 3: HPLC gradients

Mobile phase	0 min	0.1 min	1.2 min	2.6 min	2.7 min	3.5 min
A (%)	5	5	97	97	5	5
B (%)	95	95	3	3	95	95

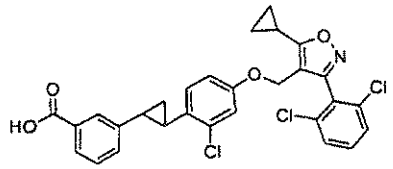
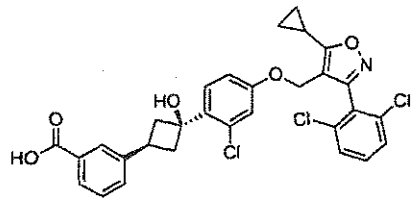
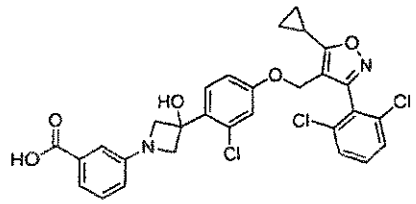
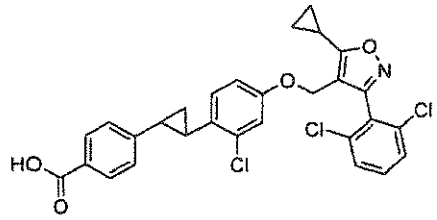
As MS tune file a generic tune file was used for all analytes applying the positive or negative ion mode. As lock mass for internal mass calibration the $[M+H]^+$ ion of diisooctyl phthalate(m/z 391.28429), which is ubiquitously present in the solvent system, was used.

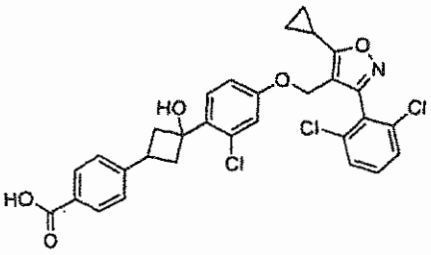
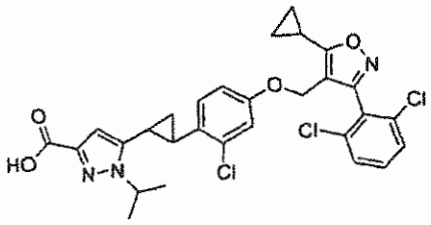
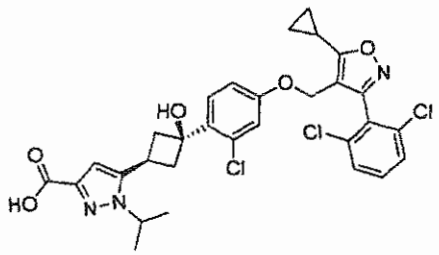
Analyte was acquired by scanning ±1 Thomson around the expected mass of the monoisotopic $[M+H]^+$ or $[M-H]^-$ ion. The mass resolution of the Orbitrap was set to 50,000. The accurate mass of each analyte was used for peak integration. Further instruments settings were as follows: HCD-Gas off, AGC high dynamic range, max. trap injection time 100 ms, sheath gas 30, aux gas 8, sweep gas 2, spray voltage 4 kV, capillary temperature 250°C, ESI 2 heater temperature 250°C.

The objective of the present invention was to generate FXR-agonists with improved physico-chemical properties compared to compounds claimed in WO 2011/020615. This was achieved by the introduction of a polar hydroxyl group on a 1,3-cyclobutylidene or 1,3-azetidinylidene group replacing the former 1,2-cyclopropylidene ring.

Surprisingly, the resulting compounds maintained their activity on the FXR receptor but demonstrated improved physico-chemical properties, such as higher aq. solubility and/or membrane permeability. A direct comparison of the corresponding compounds of the two series is given in Table 4.

Table 4

Structure	Aqueous solubility (PBS, pH 7.4)	PAMPA, Membrane permeability in % Flux *	clogD (ChemAxon software)
	20 µM	13.6	5.1
	192 µM	24.0	4.4
	195 µM	n.d.**	4.4
	72 µM	20.7	5.2

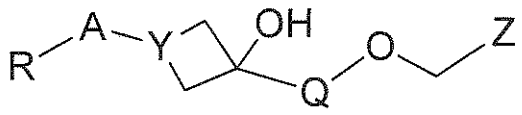
Structure	Aqueous solubility (PBS, pH 7.4)	PAMPA, Membrane permeability in % Flux *	clogD (ChemAxon software)
	192 µM	21.1	4.5
	158 µM	28.9	4.2
	171 µM	46.1	3.5

* Flux (%) = (*c* acceptor well) / sum (*c* donor well + *c* acceptor well) × 100 × 2
** n.d. = not determined

In each case either the aqueous solubility or the PAMPA membrane permeability or both are significantly improved by the introduction of the hydroxy-cyclobutyl or hydroxy-azetidyl moiety. As most nuclear receptor active molecules, FXR agonists are generally very lipophilic (M. L. Crawley, Expert Opin. Ther. Patents 2010, 20, 1047). Therefore, better aqeous solubility and membrane permeability are supposed to result in a higher oral bioavailability and in general in a better suitability for clinical development of those compounds as drugs (L. Huang, J. Dong, S. Karki in Evaluation of drug candidates for preclinical development (Eds. C. Han, C. B. Davis, B. Wang), Wiley & Sons, Hoboken 2010, 187-217).

Claims

1. A compound according to the following Formula (**1**), an enantiomer, diastereomer, tautomer or pharmaceutical acceptable salt thereof



(**1**)

wherein

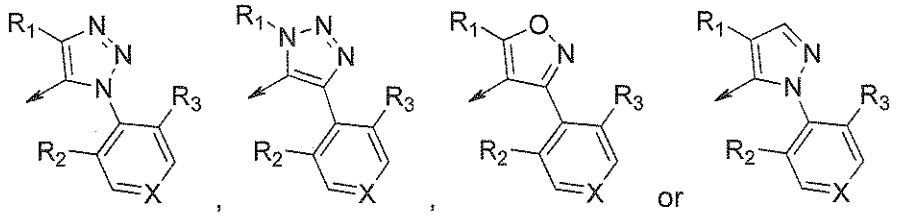
R is selected from the group consisting of $COOR_6$, $CONR_7R_8$, tetrazolyl, $SO_2NR_7R_8$, C_{1-6} alkyl, SO_2-C_{1-6} alkyl and H, with R_6 independently selected from the group consisting of H or C_{1-6} alkyl, and R_7 and R_8 independently from each other selected from the group consisting of H, C_{1-6} alkyl, halo-C_{1-6} alkyl, C_{1-6} alkylene-R_9, SO_2-C_{1-6} alkyl, wherein R_9 is selected from the group consisting of COOH, OH and SO_3H;

A is selected from the group consisting of phenyl, pyridyl, pyrimidyl, pyrazolyl, indolyl, thienyl, benzothienyl, indazolyl, benzisoxazolyl, benzofuranyl, benzotriazolyl, furanyl, benzothiazolyl, thiazolyl, oxadiazolyl, each optionally substituted with one or two groups independently selected from the group consisting of OH, O-C_{1-6} alkyl, O-halo-C_{1-6} alkyl, C_{1-6} alkyl, halo-C_{1-6} alkyl, C_{3-6} cycloalkyl and halogen;

Q is selected from the group consisting of phenyl, pyridyl, thiazolyl, thiophenyl, pyrimidyl, each optionally substituted with one or two groups independently selected from the group consisting of C_{1-6} alkyl, halo-C_{1-6} alkyl, halogen and CF_3;

Y is selected from N or CH;

Z is selected from



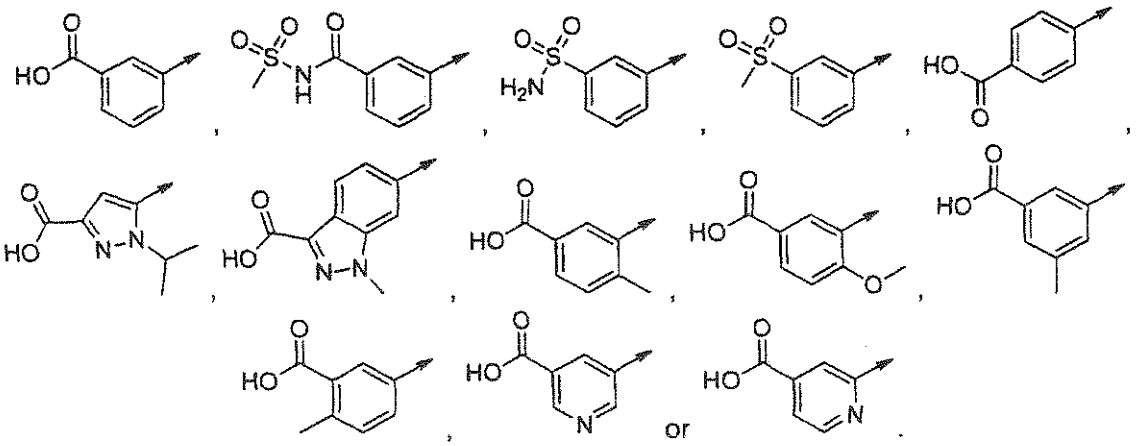
wherein

X = CH, N, NO;

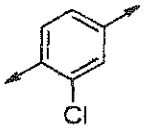
R_1 is selected from the group consisting of hydrogen, C_{1-3} alkyl, C_{3-6} cylcoalkyl, C_{4-5} alkylcycloalkyl, wherein C_{1-3} alkyl is optionally substituted with 1 to 3 substituents independently selected from halogen, hydroxy or C_{1-6} alkoxy;

R_2 and R_3 are independently selected from the group consisting of hydrogen, C_{1-3} alkyl, C_{1-3} haloalkyl, C_{1-3} alkoxy, C_{1-3} haloalkoxy and halogen.

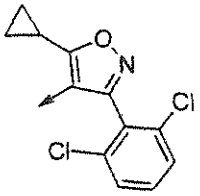
2. The compound according to claim 1 wherein R-A is selected from



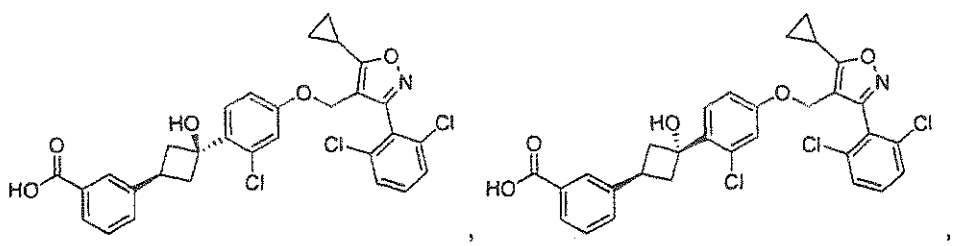
3. The compound according to claim 1 or 2 wherein Q is

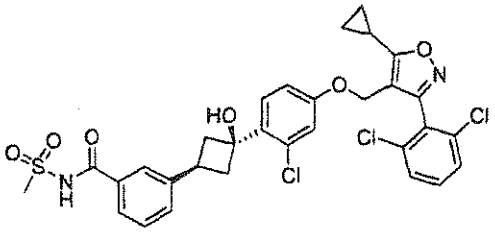
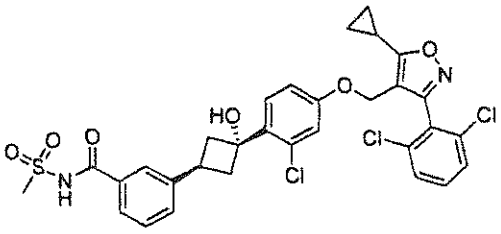
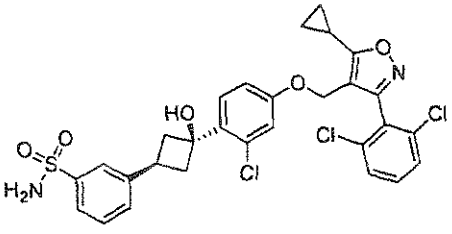


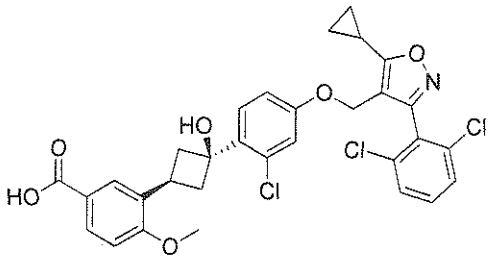
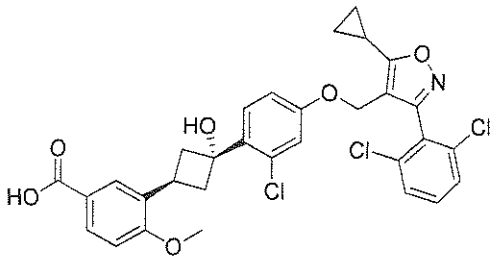
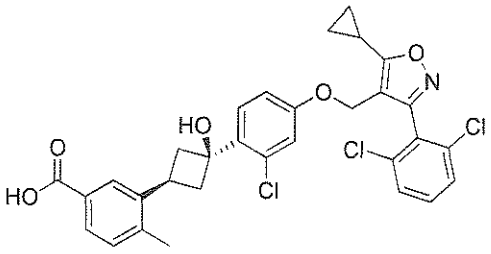
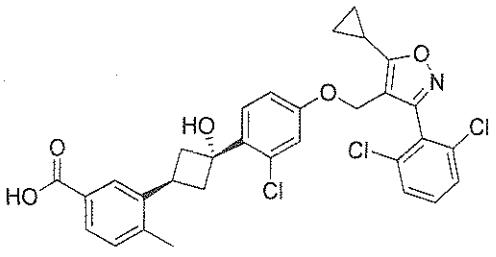
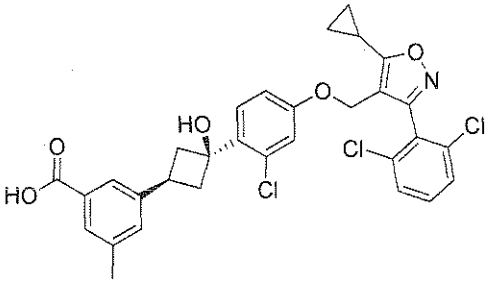
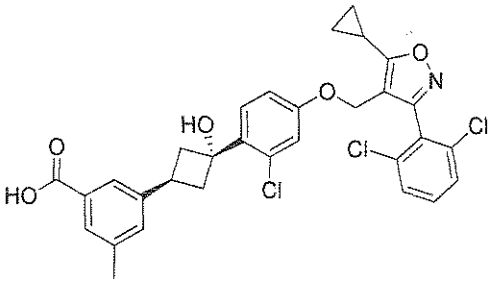
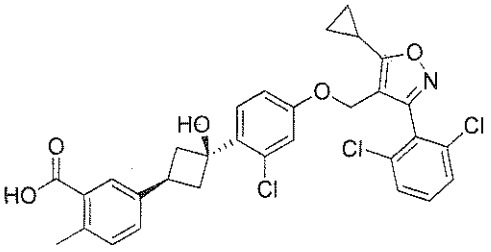
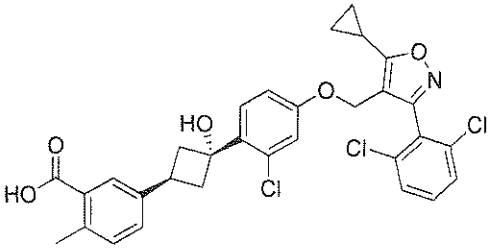
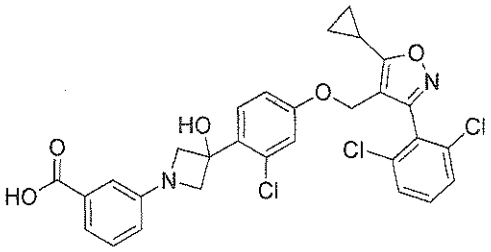
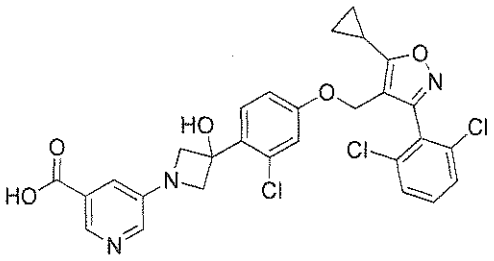
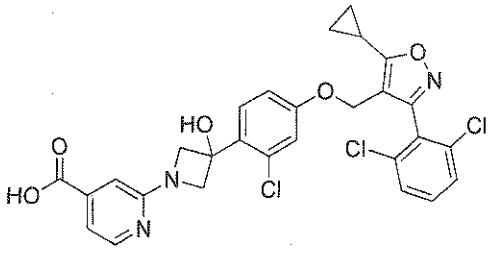
4. The compound according to any of claims 1 to 3 wherein Z is



5. The compound according to any of claims 1 to 4 selected from

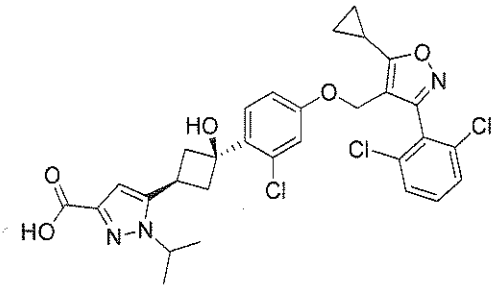



,

,

,


,

,

,

,

,

,

,

,

,

,

or

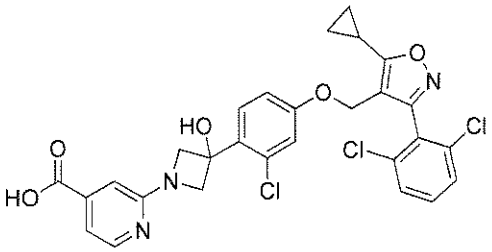
or a pharmaceutically acceptable salt thereof.

6. The compound according to any of claims 1 to 5 having the following structure



or a pharmaceutically acceptable salt thereof.

7. The compound according to any of claims 1 to 5 having the following structure



or a pharmaceutically acceptable salt thereof.

8. A pharmaceutical composition comprising the compound according to any of claims 1 to 7, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

9. A compound according to any of claims 1 to 7 for use as a medicament.

10. Use of a compound according to any of claims 1 to 7 for the preparation of a medicament for the prophylaxis and/or treatment of diseases mediated by FXR.

11. The use according to claim 10 wherein the disease is selected from

chronic intrahepatic or some forms of extrahepatic cholestatic conditions;

liver fibrosis;

obstructive or chronic inflammatory disorders of the liver;

liver cirrhosis;

liver steatosis and associated syndromes, cholestatic or fibrotic effects that are associated with alcohol-induced cirrhosis or with viral-borne forms of hepatitis;

liver failure or liver ischemia after major liver resection;

chemotherapy associated steatohepatitis (CASH);

acute liver failure; and/or

Inflammatory Bowel Diseases.

12. The use according to claim 10 wherein the disease is selected from

lipid and lipoprotein disorders;

Type II Diabetes and clinical complications of Type I and Type II Diabetes, including diabetic nephropathy, diabetic neuropathy, diabetic retinopathy and other observed effects of clinically manifest long term Diabetes;

conditions and diseases which result from chronic fatty and fibrotic degeneration of organs due to enforced lipid and specifically triglyceride accumulation and subsequent activation of profibrotic pathways; such as Non-Alcoholic Fatty Liver Disease (NAFLD) or Non-Alcoholic Steatohepatitis (NASH);

obesity or metabolic syndrome (combined conditions of dyslipidemia, diabetes or abnormally high body-mass index); and/or

acute myocardial infarction, acute stroke or thrombosis which occurs as an endpoint of chronic obstructive atherosclerosis.

13. The use according to claim 10 wherein the disease is selected from

non-malignant hyperproliferative disorders and malignant hyperproliferative disorders, specifically of hepatocellular carcinoma, colon adenoma and polyposis, colon adenocarcinoma, breast cancer, pancreas adenocarcinoma, Barrett's esophagus or other forms of neoplastic diseases of the gastrointestinal tract and the liver.