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EP 2399914 A1 **WO 2008/054675 A2**
WO 2008/001931 A2 **US 20090258921 A1**

(58) Field of Search:
Other: **BIOSIS, CAS-ONLINE, EPODOC, MEDLINE, TXTE & WPI**

(54) Title of the Invention: **Novel combinations**
Abstract Title: **Pharmaceutical combination of a GPR119 agonist and a GPR40 agonist**

(57) A pharmaceutical combination comprising at least one GPR119 agonist and at least one GPR40 agonist. Provided is a pharmaceutical composition comprising at least one GPR119 agonist and at least one GPR40 agonist. The combination may alternatively comprise a single agent that has both GPR119 agonist and GPR40 agonist activity. Further provided are methodsof treating metabolic disorders, including type 2 diabetes and diseases associated with type 2 diabetes in a mammal, said methods comprising providing the mammal with a therapeutically effective combination of at least one GPR119agonist and at least one GPR40 agonist. The said combination has been found to be additive, more than additive and synergistic in nature in the treatment of the aforementioned disease states.

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FIGURES

Figure 1

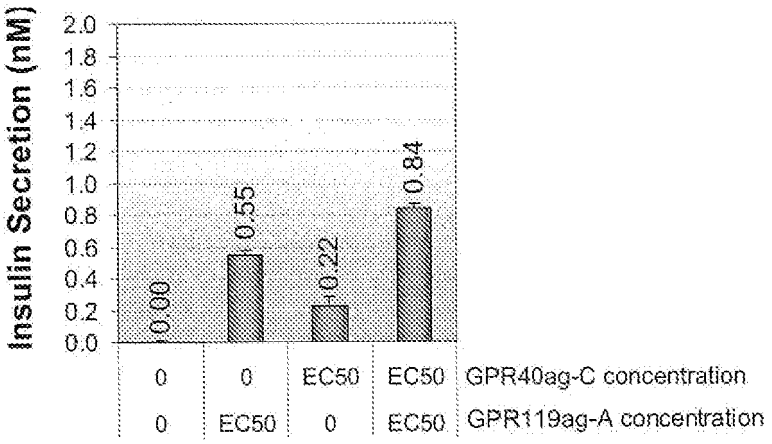


Figure 2

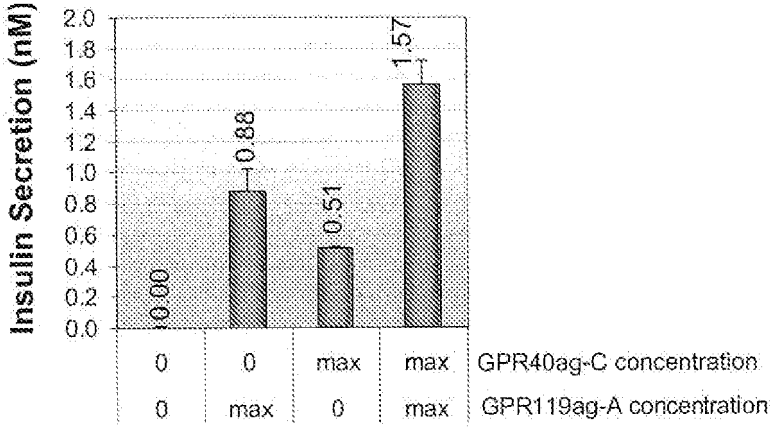


Figure 3

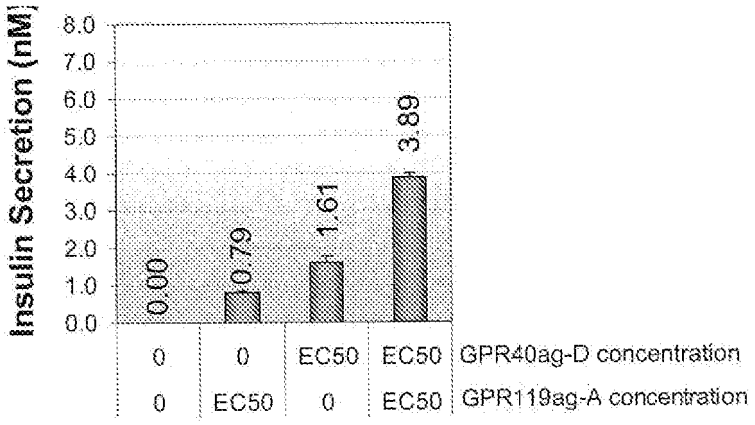


Figure 4

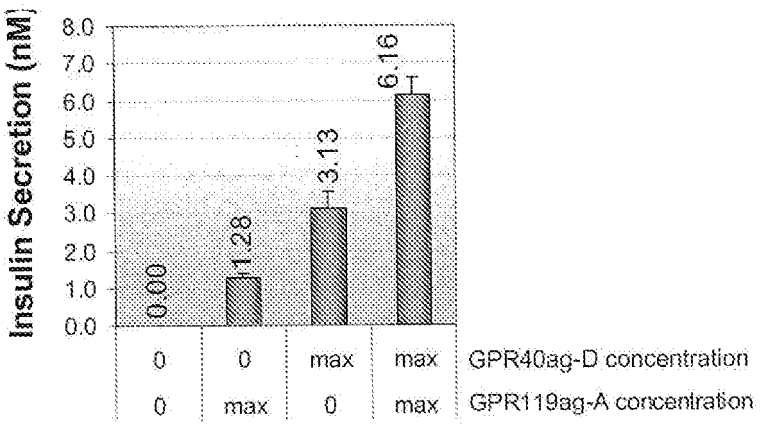


Figure 5

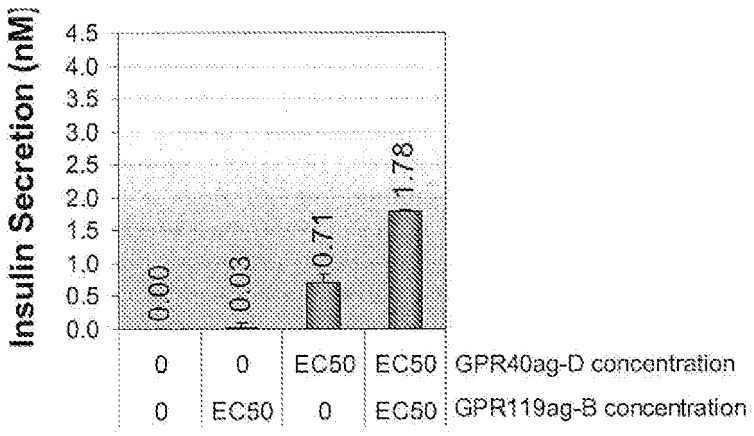


Figure 6

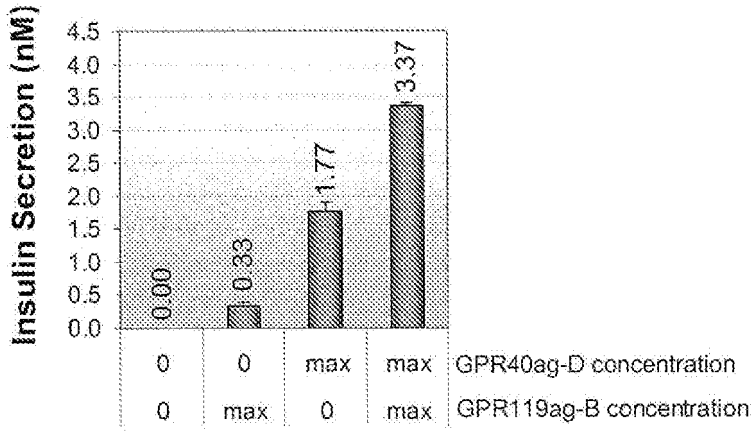
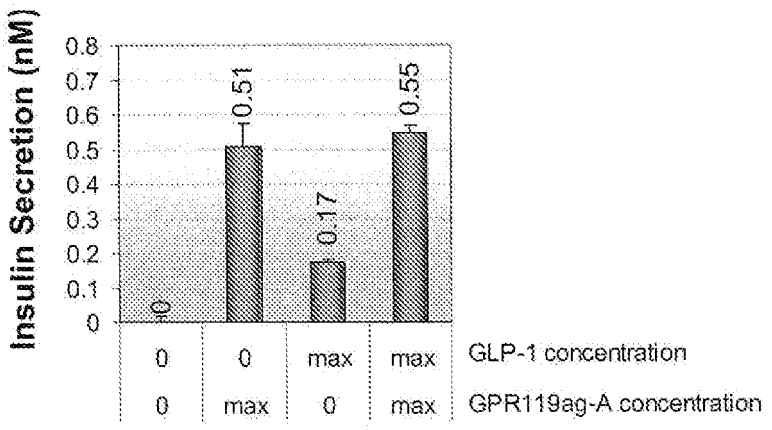


Figure 7



NOVEL COMBINATIONS

BACKGROUND OF THE INVENTION

The present invention is directed to a novel combination of biological mechanisms useful for the treatment of metabolic disorders including type II diabetes. The invention provides novel compositions comprising a GPR119 agonist and a GPR40 agonist and uses thereof. These compositions are useful for the treatment of metabolic disorders including type II diabetes.

Type 2 diabetes mellitus (T2DM) is one of the most common chronic diseases affecting over 200 million people wordwide. The disease is characterized by abnormally high blood glucose levels (hyperglycemia) which is believed to cause long-term microvascular and macrovascular complications, such as heart disease, stroke, high blood pressure, hyperlipidemia, kidney failure, blindness, limb amputation, nerve pain and other neurologic problems. (Xu J. et al, IDrugs vol7 no3 p249-256, 2004). Insulin plays a central role in controlling blood glucose levels, and secretion of this hormone is abnormal in patients with T2DM, for example being at levels that are insufficient to normalise blood glucose levels.

Obesity is characterized by an excessive adipose tissue mass relative to body size. Clinically, body fat mass is estimated by the body mass index (BMI; weight(kg)/height(m)^2), or waist circumference. Individuals are considered obese when the BMI is greater than 30 and there are established medical consequences of being overweight. It has been an accepted medical view for some time that an increased body weight, especially as a result of abdominal body fat, is associated with an increased risk for diabetes, hypertension, heart disease, and numerous other health complications, such as arthritis, stroke, gallbladder disease, muscular and respiratory problems, back pain and even certain cancers

A range of drug therapies have been tested or are in current use for the treatment of T2DM and related metabolic conditions. These include enhancement of insulin secretion by pancreatic islet beta cells using insulin secretagogues such as sulphonylurea drugs or glucagon-like peptide-1 (GLP-1)-based therapies, or direct administration of insulin.

There is a continuing need for novel antidiabetic agents, particularly ones that are more efficacious than current standards of care or are better tolerated with fewer adverse effects. There is a particular need for agents which could potentially ameliorate multiple risk factors.

However treatment with drugs modulating a single molecular target is frequently unable to produce the desired level of clinical efficacy, such as defined by reducing blood glucose levels or HbA1c, a marker of long term glucose control.

As a solution to inadequate efficacy achieved by agents modulating single targets, combination therapy is a strategy, delivered either by co-administration of single agents or by creation of fixed-dose combination products. It has been hypothesized that modulation of complex biological responses to achieve therapeutics with improved efficacy and/or safety may be achievable by targeting multiple biological targets or pathways.

Thus we have sought to identify novel combinations of biological mechanisms which, when modulated simultaneously, could give desirable anti-diabetic biological responses which are additive or synergistic when compared to modulation of either mechanism alone. In addition, we have sought to combine these dual or multiple biological activities into single small molecule drug candidates, called designed multiple ligands or polypharmacology compounds. Single compounds with multiple biological actions which display additive, better than additive or synergistic biological responses over single agents alone could have certain advantages over fixed-dose combination therapies, and over co-administration of existing single agents. For example, a fixed-dose combination pill delivers two or more separate entities which have individual drug disposition and pharmacokinetic properties, and the safety of the combination has to be ascertained independently from the safety of the two separate entities alone. In the case of co-administration of multiple drugs, there is a greater risk of drug-drug interactions and different dosing regimens for the individual drugs can cause poor patient compliance, and thus compromise patient benefit.

Therefore, we have sought to identify novel combinations of biological mechanisms which, when modulated simultaneously, could give desirable additive or synergistic biological responses and which are tractable to single small molecule modulation in designed multiple ligand suitable for oral administration.

Pancreatic islet cells, which include beta cells, express at least 20 types of G protein-coupled receptors (GPCR), which through ligand interactions may modulate secretion of key hormones of glucose control such as insulin (Ahren B, Nature Reviews Drug Discovery vol8 p369-385, 2009]. Since enhancement of insulin secretion is a known strategy for the treatment of T2DM, the identification of certain novel combinations of GPCR agonists that lead to additive or synergistic levels of insulin secretion relative to the modulation of either GPCR alone, are a promising avenue to achieve superior efficacy.

GPR119 (previously referred to as GPR116) is a GPCR identified as SNORF25 in WO00/50562 which discloses both the human and rat receptors, US 6,468,756 also discloses the mouse receptor (accession numbers: AAN95194 (human), AAN95195 (rat) and ANN95196 (mouse)). In humans, GPR119 is expressed in the pancreas, small intestine, large intestine and stomach. The expression profile of the human GPR119 receptor indicates its potential utility as a target for the treatment of diabetes. GPR119 agonists have been shown to stimulate the release of GLP-1 from the GI tract. In doing so, GPR119 agonists (1) enhance glucose-dependent insulin release from the pancreas leading to improvements in oral glucose tolerance; (2) attenuate disease progression by increasing β-cell cAMP concentrations; and (3) induce weight loss possibly through GLP-1's ability to reduce food intake.

GPR40 is a GPCR and has been shown to be highly expressed in the pancreatic islets of humans (and rodents) and insulin secreting cell lines. Saturated and unsaturated medium and long chain fatty acids have been identified as ligands for GPR40 (Itoh Y et al, Nature 422: 173-176, Briscoe et al *J Biol Chem* **278**: 11303-11311). The use of selective GPR40 agonists have confirmed the role of GPR40 in insulin secretion (Briscoe *et al Br J Pharmacol* **148** 619-628 and Tan *et al Diabetes* **57** 2211-2219). A selective GPR40 agonist has been shown to improve both fasting and postprandial glycaemic control in type 2 diabetic patients. (Leifke et al ADA 2011 414PP).

The inventors have identified novel combinations of biological mechanisms which, when modulated simultaneously, give desirable anti-diabetic biological responses which are more than additive or synergistic when compared to modulation of either mechanism alone. The invention provides novel combinations of GPR119

agonism and GPR40 agonism which, when modulated simultaneously, displays greater than additive insulin release in HIT-T15 islet-derived cells, and thus may be a useful approach to the treatment of metabolic disorders and type 2 diabetes.

The inventors have identified novel compositions comprising a GPR119 agonist and a GPR40 agonist that are useful for the treatment of metabolic disorders including type II diabetes. The invention provides composition, dosage forms comprising the compositions and methods of treating or preventing metabolic disorders, including type 2 diabetes and diseases associated with type 2 diabetes in a mammal, said methods comprising providing the mammal with a therapeutically effective amount of the composition or dosage form.

Combining GPCR agonists may elicit a response level that is either less than (sub-additive combination), equal to (additive combination), or greater than (synergistic combination) the simple arithmetic addition of response levels obtained with the same agents when tested separately. Alternatively the combined response may be less than that of just the single agents alone (antagonistic combination). In the context of combinations of drugs ultimately used for treatment of disease, synergistic or additive combinations are desirable. In this sense it is not possible to predict how two agonists of different GPR targets will interact, and it is therefore not possible to determine suitable therapeutic combinations based solely on their individual activity at their target receptor or downstream effector.

The inventors have now surprisingly discovered that when a GPR119 agonist and a GPR40 agonist are combined in a composition the response elicited is either additive, more than additive or synergistic.

SUMMARY OF THE INVENTION

The invention provides compositions comprising a GPR119 agonist and a GPR40 agonist wherein the agonists are separate agents or a single agent that is both a GPR119 agonist and a GPR40 agonist.

DETAILED DECRIPTION OF THE INVENTION

The invention will now be described in detail referring to the following figures:

FIGURE 1 shows the more than additive effect on insulin secretion of co-administration of Compound A and Compound C at EC_{50} concentrations.

FIGURE 2 shows the more than additive effect on insulin secretion of co-administration of Compound A and Compound C at optimum concentrations.

FIGURE 3 shows the more than additive effect on insulin secretion of co-administration of Compound A and Compound D at EC_{50} concentrations.

FIGURE 4 shows the more than additive effect on insulin secretion of co-administration of Compound A and Compound D at optimum concentrations.

FIGURE 5 shows the more than additive effect on insulin secretion of co-administration of Compound B and Compound D at EC_{50} concentrations.

FIGURE 6 shows the more than additive effect on insulin secretion of co-administration of Compound B and Compound D at optimum concentrations.

FIGURE 7 shows the less than additive effect on insulin secretion of co-administration of GLP-1 and Compound A at optimum concentrations.

The invention provides compositions comprising a GPR119 agonist and a GPR40 agonist wherein the agonists are separate agents or a single agent that is both a GPR119 agonist and a GPR40 agonist. In certain embodiments the compositions comprise a single agent that is both a GPR119 agonist and a GPR40 agonist. In further embodiments the compositions comprise at least one agent that is a GPR119 agonist and at least one further agent that is a GPR40 agonist. Compositions comprising two or more agents may be suitable for simultaneous or sequential administration of the agonists.

When used herein the term GPR119 agonist refers to any agent that binds to a GPR119 receptor and triggers a functional response in the GPR119 receptor. GPR119 agonists suitable for use in the present invention may be identified by methods known in the art and disclosed in, for example US7083933. Preferably the

GPR119 agonist is a selective GPR119 agonist. For the purpose of the present invention, the following protocol may be followed to determine whether a compound is a GPR119 agonist:

A stable cell line expressing recombinant human GPR119 is established by any method known in the art. This cell line is used to investigate the effect of a candidate compound on intracellular levels of cyclic AMP (cAMP). The cell monolayers are washed with phosphate buffered saline and stimulated at 37°C for 30 min with various concentrations of compound in stimulation buffer plus 1% DMSO. Cells are then lysed and cAMP content determined using the Perkin Elmer AlphaScreen™ (Amplified Luminescent ProximityHomogeneous Assay) cAMP kit. Buffers and assay conditions are as described in the manufacturer's protocol. Compounds found to display a concentration-dependent increase in intracellular cAMP levels with an EC_{50} of <20 μM are considered to be GPR119 agonists.

International Patent Applications WO 2005/061489, WO 2006/070208, WO 2006/067532, WO 2006/067531, WO 2007/003960, WO 2007/003961, WO 2007/003962, WO 2007/003964, WO 2007/116229, WO 2007/116230, WO 2008/081204, WO 2008/081205, WO 2008/081206, WO 2008/081207, WO 2008/081208, WO 2009/050522, WO 2009/050523, WO 2010/001166, WO 2010/004343, WO 2010/004344, WO 2010/004345, WO 2010/004346, WO 2010/004347 and WO 2010/004348 disclose GPR119 receptor agonists suitable for use in the invention.

Preferred GPR119 agonists include:

5-[1-(3-isopropyl-[1,2,4]oxadiazol-5-yl)-piperidin-4-ylmethoxy]-2-(4-methanesulfonylphenyl)-pyridine,

5-ethyl-2-{4-[4-(4-pentazol-1-ylphenoxymethyl)-thiazol-2-yl]-piperidin-1-yl}-pyrimidine,

4-[1-(2-fluoro-4-methanesulfonylphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yloxy]-piperidine-1-carboxylic acid isopropyl ester,

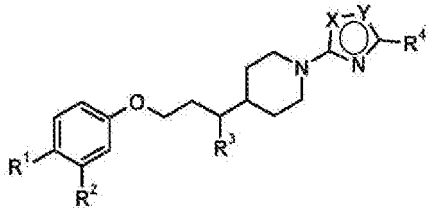
4-[5-methyl-6-(2-methylpyridin-3-yloxy)-pyrimidin-4-yloxy]-piperidine-1-carboxylic acid isopropyl ester,

4-[6-(6-methanesulfonyl-2-methylpyridin-3-ylamino)-5-methylpyrimidin-4-yloxy]-piperidine-1-carboxylic acid isopropyl ester,

4-[6-(6-methanesulfonyl-2-methylpyridin-3-ylamino)-5-methoxypyrimidin-4-yloxyl piperidine-1-carboxylic acid isopropyl ester

and 4-[5-methoxy-6-(2-methyl-6-[1,2,4]triazol-1-yl-pyridin-3-ylamino)-pyrimidin-4-yloxy]-piperidine-1-carboxylic acid isopropyl ester.

Further GPR119 agonists that may be used in the invention include a compound of formula (I), or a pharmaceutically acceptable salt thereof:



(I)

wherein one of X and Y is O and the other is N;

R1 is -CONHR5;

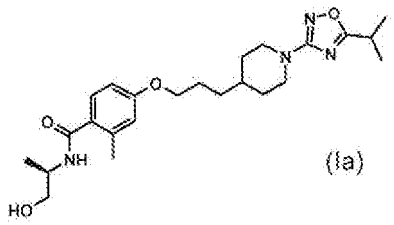
R2 is hydrogen, halo or methyl;

R3 is hydrogen or methyl;

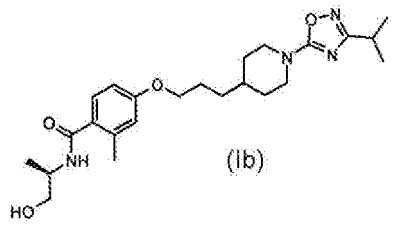
R4 is C2-5 alkyl; and

R5 is hydrogen, C1–3alkyl, or C2–3alkyl substituted by hydroxy.

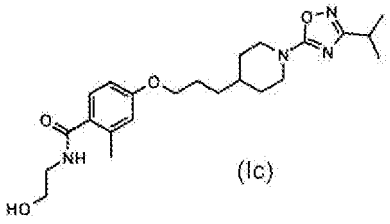
Examples of such agonists include the following:



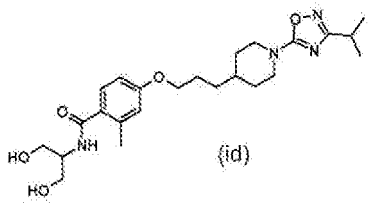
(Ia)



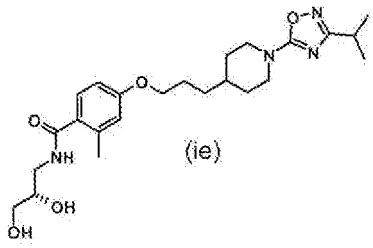
(Ib)



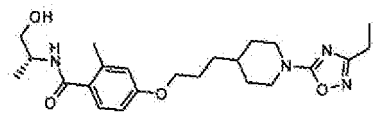
(Ic)



(id)



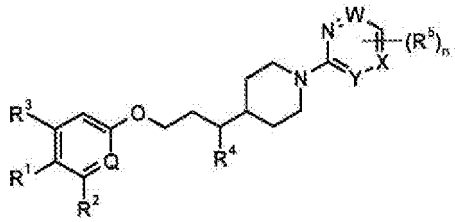
(ie)



(If)

and pharmaceutically acceptable salts thereof.

Still further GPR119 agonists that may be used in the invention include a compound of formula (I), or a pharmaceutically acceptable salt thereof:



(I)

wherein Q is CH or N;

one of W, X and Y is N or CH and the others are CH where the H may be replaced by R5 when present;

R1 is -SO2Me or -CONHR6;

R2, R3 and R4 are independently selected from hydrogen and methyl;

n is 0, 1 or 2;

R5 is independently C1-4 alkyl, C1-4 alkoxy, fluoro, chloro, C1–3 fluoroalkyl or benzyl;

R6 is hydrogen, 3-azetidinyl, 3-pyrrolidinyl, 3-piperidinyl or 4-piperidinyl, wherein the azetidinyl, pyrrolidinyl and piperidinyl rings may be optionally substituted with OH, CH2OH or CH3; C1–3 alkyl, C2–4 alkyl substituted by -N(R7)2 and/or one or two hydroxy groups, or C1–4 alkyl substituted by a 4- to 6-membered nitrogen-containing heterocyclic ring; and

R7 is independently hydrogen or methyl.

When used herein the term GPR40 agonist refers to any molecule that binds to a GPR40 receptor and triggers a functional response in the GPR40 receptor. GPR40 agonists suitable for use in the present invention may be identified by methods known in the art. Preferably, the GPR40 agonist is a selective GPR40 agonist. For example, the following protocol may be followed to determine whether a compound is a GPR40 agonist:

A stable HEK293 cell line expressing recombinant human GPR40 receptors is established using methods known in the art. This cell line is used to investigate the effect of candidate compounds on mobilisation of intracellular calcium through the GPR40 receptor. Cells of the HEK293 cell line are cultured in growth medium overnight in black, clear bottom, poly-D-lysine-coated, 96-well plates at 37°C with 5% CO_2. Growth medium is then removed from each well and replaced immediately with Fluo-4 Direct™ reagent (Invitrogen). Cell plates are subsequently incubated for 60 min at 37°C + 5% CO_2. Cell plates are then transferred to a FLIPR tetra® plate reader where test compounds are added and levels of intracellular fluorescence measured (Ex 470-495nm, Em 515-575nm). Compounds that stimulate a calcium response in a concentration-dependent manner with an EC_{50} value <20μM may be considered to be GPR40 agonists.

GPR40 agonists are well known in the art. For example, GPR40 agonists are disclosed in the following references, the contents of which are incorporated by reference: Shawn D. Walker, *et al*, Development of a Scalable Synthesis of a GPR40 Receptor Agonist, Organic Process Research and Development, especially AMG837.

Stephen C. McKeown, *et al*, Solid phase synthesis and SAR of small molecule agonists for the GPR40 receptor, Bioorganic & Medicinal Chemistry Letters 17 (2007) 1584–1589, especially compounds 1- 45. Elisabeth Christiansen *et al*, Structure-Activity Study of Dihydrocinnamic Acids and Discovery of the Potent FFA1 (GPR40) Agonist TUG-469, ACS Med. Chem. Lett., 2010, 1 (7), pp 345–349, especially any of the compounds in Figure 1, and any of compounds 1-31. Shawn P. Walsh *et al*, 3-Substituted 3-(4-Aryloxyaryl)-Propanoic Acids as GPR40 Agonists; Bioorg Med Chem Lett. 2011 Jun 1;21(11):3390-4, especially compounds 1-25. Celia P. Briscoe *et al*, Pharmacological regulation of insulin secretion in MIN6 cells through the fatty acid receptor GPR40: identification of agonist and antagonist small molecules, British Journal of Pharmacology (2006) 148, 619–628, especially GW9508 of figure 1. Dulce M. Garrido *et al*, Synthesis and activity of small molecule GPR40 agonists; Bioorganic & Medicinal Chemistry Letters 16 (2006) 1840–1845, especially compounds 2A-14A, 2B-7B, 10B-12B and 14B-15B. Changyou Zhou *et al*; Discovery of 5-aryloxy-2,4-thiazolidinediones as potent GPR40 agonists; Bioorganic & Medicinal Chemistry Letters 20 (2010) 1298–1301, especially the compounds in Tables 1-5. Fengbin Song, *et al*, Synthesis and Biological Evaluation of 3-Aryl-3-(4-phenoxy)-propionic Acid as a Novel Series of G Protein-Coupled Receptor 40 Agonists; J Med Chem. 2007 Jun 14;50(12):2807-17, especially compounds 1-61. Nobuyuki Negoro *et al*, Discovery of TAK-875: A Potent, Selective, and Orally Bioavailable GPR40 Agonist; ACS Med. Chem. Lett., 2010, 1 (6), pp 290–294, especially compounds 1-9.

Preferred GPR40 agonist include:

{(S)-6-[4'-(3-methanesulfonylpropoxy)-2',6'-dimethylbiphenyl-3-ylmethoxy]-2,3-dihydrobenzofuran-3-yl}-acetic acid,

(1S,1aS,6aR)-4-(2-difluoromethoxy-4-trifluoromethylphenoxy)-1,1a,6,6a-tetrahydro cyclopropa[a]indene-1-carboxylic acid,

(S)-3-[4-(4'-trifluoromethylbiphenyl-3-ylmethoxy)-phenyl]-hex-4-ynoic acid,

3-[4-(3-phenoxybenzylamino)-phenyl]-propionic acid,

and 5-[4-(3-Chloro-5-methyl-pyridin-2-yloxy)-2-methyl-benzyl]-thiazolidine-2,4-dione.

When used herein the term agent refers to the active compound or to a substance that provides or delivers the active compound, e.g. a carrier or matrix comprising the active compound.

The compositions may suitably comprise further therapeutic ingredients or adjuvants.

The compositions may be conveniently presented in unit dosage form. The compositions may be prepared by any of the methods well known in the art of pharmacy. The agonists may be combined either together or separately as the active ingredient(s) 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. In general, such methods include a step of bringing into association the active ingredient with the carrier that constitutes one or more necessary ingredients. In general, the compositions may be prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both. The product can then be conveniently shaped into the desired presentation.

The invention also provides dosage forms comprising a composition as described herein and a pharmaceutically acceptable carrier. In certain embodiments the dosage forms comprise a single agent that is both a GPR119 agonist and a GPR40 agonist. In further embodiments the dosage forms comprise at least one agent that is a GPR119 agonist and at least one further agent that is a GPR40 agonist. In certain embodiments the dosage forms are in a fixed-dose combination. The dosage form may be solid liquid or semi-solid depending on the intended route of administration. Suitable dosage forms include oral dosage forms and , parenteral injectionssuch as pills, tablets, capsules, liquids, suspensions, powders, granules, solid crystals and pastes. Further dosage forms include controlled release means and delivery devices.

The dosage forms are provided for use in the treatment of a disorder selected from the group comprising diabetes, obesity, metabolic syndrome (syndrome X), impaired glucose tolerance, insulin resistance, pancreatic beta-cell insufficiency, hyperglycemia, enteroendocrine cell insufficiency, glucosuria, metabolic acidosis, cateracts, diabetic nephropathy, diabetic retinopathy, diabetic coronary artery disease, diabetic cerebrovascular disease, diabetic peripheral vascular disease, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia, low HDL levels,

hypertension, myocardial infarction, atherosclerosis and stroke, preferably for use in the treatment or prophylaxis of the disorder is diabetes.

The pharmaceutical carrier employed can be, for example, a solid, liquid, or gas. Examples of solid carriers include lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, and stearic acid. Examples of liquid carriers are sugar syrup, peanut oil, olive oil, and water. Examples of gaseous carriers include carbon dioxide and nitrogen.

In preparing compositions for oral dosage form any convenient pharmaceutical media may be employed. For example water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like may be used to form oral liquid preparations such as suspensions, elixirs and solutions; while carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like may be used to form oral solid preparations such as powders, capsules and tablets. Because of their ease of administration, tablets and capsules are the preferred oral dosage units whereby solid pharmaceutical carriers are employed. Optionally, tablets may be coated by standard aqueous or nonaqueous techniques.

A tablet containing the composition may be prepared by compression or moulding, optionally with one or more accessory ingredients or adjuvants. Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. Each tablet may suitably contain from about 100 mg to about 2 g of one or more active ingredient and each cachet or capsule may suitably contain from about 100 mg to about 2 g of one or more active ingredient.

For example, a formulation intended for the oral administration to humans may contain from about 100 mg to about 2 g of one or more of the active agent(s), compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95 percent of the total composition. Unit dosage forms will generally contain between from about 250 mg to about 2 g of the active

ingredient, typically 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 800 mg or 1000 mg.

Pharmaceutical compositions for use by parenteral administration may be prepared as solutions or suspensions of the active compounds in water. A suitable surfactant can be included such as, for example, hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof in oils. Further, a preservative can be included to prevent the detrimental growth of microorganisms.

The pharmaceutical compositions must be stable under the conditions of manufacture and storage; thus, preferably should 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), vegetable oils, and suitable mixtures thereof.

In addition to the aforementioned carrier ingredients, the pharmaceutical formulations described above may include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, flavoring agents, binders, surface-active agents, thickeners, lubricants, preservatives (including anti-oxidants) and the like. Furthermore, other adjuvants can be included to render the formulation isotonic with the blood of the intended recipient. Compositions containing a compound or pharmaceutically acceptable salts thereof for use in accordance with the present invention may also be prepared in powder or liquid concentrate form.

The present invention also provides the use of a GPR119 agonist or a GPR40 agonist in the preparation of a composition or dosage form as described herein.

The present invention also provides methods of preparing a pharmaceutical composition comprising admixing a GPR119 agonist and a GPR40 agonist with at least one pharmaceutically acceptable carrier. Included are methods that comprise admixing a single agent that is both a GPR119 agonist and a GPR40 agonist with at least one pharmaceutically acceptable carrier. Also included are methods that comprise admixing at least one agent that is a GPR119 agonist and at least one further agent that is a GPR40 agonist with at least one pharmaceutically acceptable carrier.

The present invention also provides a composition as described herein for use in a method of treatment of the human or animal body.

The present invention also provides the use of a composition as described herein in the manufacture of a medicament for the treatment or prevention of diabetes or a condition related thereto.

EXAMPLES

As a model system to investigate the effects of combining islet GPCR modulators, insulin secretion is measured using the HIT-T15 islet-derived cell line (Santerre RF et al, *PNAS* vol78 no7 p4339-4343 1981). HIT-T15 is derived from hamster pancreatic beta cells and allows the precise measurement of insulin levels in response to test agents or combinations thereof. Functional expression of several islet GPCR genes is known to be retained in this cell line compared to primary islet cells, as well as the effects of specific agonist compounds on the rate of insulin secretion

METHODOLOGY

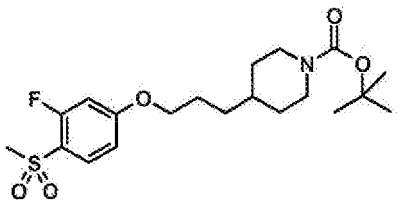
HIT–T15 cells are seeded in 96 well cell culture microtitre plates at a density of 80,000 cells/ well in 0.2ml / well RPMI-1640 medium supplemented with 10% fetal calf serum. Enhanced reproducibility is obtained by avoiding edge wells and by allowing cells to settle at room temperature on the bench for 45 min immediately following seeding. Culture plates are incubated at 37°C in a humidified 5% CO2 atmosphere for 24 hr. All assay buffers and diluted compounds are pre-warmed to 37°C prior to addition. Plates are maintained on a 37°C hotplate during manipulations. Medium is discarded from assay plate wells. Cells are washed twice with 0.2ml and pre-incubated for 30 min in 0.1 ml Krebs-Ringer-HEPES assay buffer (KRHB; Poitout V et al, Diabetes vol44 p306-313, 1995) supplemented with 0.1% BSA which is then discarded. Test compounds in single or combination doses are prepared in KRHB-BSA buffer supplemented with 5.6 mM glucose, at a final DMSO concentration of 0.5% and added to wells of the 96-well plate, which is incubated at 37°C for 30 min. This glucose concentration in the HIT-T15 cell line represents a stimulatory concentration, in effect mimicking hyperglycemia. Samples containing secreted insulin are collected, clarified by centrifugation at 2500 rpm for 5 minutes, and measured for insulin concentration using a commercial ELISA kit which includes standards of known insulin concentration (Rat insulin ELISA kit; Mercodia). Absorbance data from test samples is interpolated against the ELISA standard curve

to generate nM insulin concentrations. Experiments are designed with duplicate wells for each test combination. Data is subtracted from adjacent vehicle control wells containing 0.5% DMSO alone, generating an increment above control insulin secretion value.

Data is provided showing effects of co-dosing agonist compounds of GPR119 and GPR40, both of which are individually functionally active in HIT-T15 cells in causing an increase in insulin secretion levels. It is shown that the combinations of GPR40 and GPR119 agonists elicit a greater than additive interaction. This occurs irrespective of the selection of alternative agonist compounds for each GPCR, and irrespective of whether maximal or EC50 concentrations are used. In contrast, combining agonists to GPR119 with GLP-1 receptor or GIP receptor, generates a total response that is sub-additive. This occurs irrespective of whether maximal or EC50 concentrations are used. Although agonists of all GPCRs tested are active on their own, the outcome of combining them reveals an unexpected beneficial response for the combination of GPR119 and GPR40 agonism.

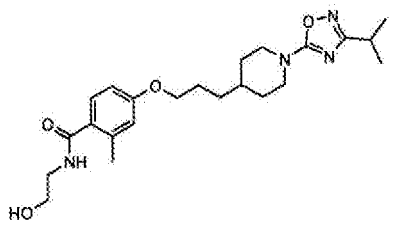
The following compounds are used as tool GPCR agonists:

Compound A- GPR119 Agonist (GPR119agA)



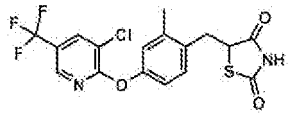
4-{3-(3-Fluoro-4-methanesulfonylphenoxy)propyl] piperidine-1-carboxylic acid tert-butyl ester. The synthesis of this compound is disclosed in PCT/GB2006/050178.

Compound B- GPR119 Agonist (GPR119agB)



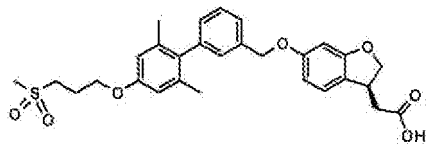
N-(2-Hydroxyethyl)-4-{3-[1-(3-isopropyl-[1,2,4]oxadiazol-5-yl)piperidin-4-yl]propoxy}-2 methylbenzamide. The synthesis of this compound is disclosed in PCT/GB2008/050011.

Compound C- GPR40 Agonist (GPR40agC)



identified as Cpd B in Tan CP et al, *Diabetes* vol57 p2211-2219, 2008.

Compound D- GPR40 Agonist (GPR40agD)



identified as TAK-875 in Negoro N et al, *ACS Med. Chem. Lett.* Vol1 No6 p290-294.

Example 1

Compound A and Compound C were added to HIT-T15 cells at concentrations equal to their individual EC_{50} concentrations for enhancement of insulin secretion as determined from a full dose-response test in the same experiment (Compound A: 100nM; Compound C: 4000 nM). Responses for the two compounds at this concentration either alone or in combination delivered simultaneously are shown graphically in Figure 1. Increase in insulin secretion in response to combined agents (0.84 nM insulin) exceeds that of simple addition of the levels achieved with respective single agents (0.55+0.22 nM insulin).

Example 2

Compound A and Compound C were added to HIT-T15 cells at concentrations equal to their most effective concentrations for enhancement of insulin secretion as determined from a full dose-response test in the same experiment (Compound A: 1000nM, Compound C: 15000nM). Responses for the two compounds at this concentration either alone or in combination delivered simultaneously are shown graphically in Figure 2. Increase in insulin secretion in response to combined agents (1.57 nM insulin) exceeds that of simple addition of the levels achieved with respective single agents (0.88+0.51 nM insulin).

Example 3

Compound A and Compound D were added to HIT-T15 cells at concentrations approximately equal to their individual EC_{50} concentrations for enhancement of insulin secretion as determined from a full dose-response test in the same experiment (Compound A: 30nM, Compound D: 15000nM). Responses for the two compounds at this concentration either alone or in combination delivered simultaneously are shown graphically in Figure 3.

Increase in insulin secretion in response to combined agents (3.89 nM insulin) exceeds that of simple addition of the levels achieved with respective single agents (0.79+1.61 nM insulin).

Example 4

Compound A and Compound D were added to HIT-T15 cells at concentrations equal to their most effective concentrations for enhancement of insulin secretion as determined from a full dose-response test in the same experiment (Compound A: 1000nM, Compound C: 30000nM). Responses for the two compounds at this concentration either alone or in combination delivered simultaneously are shown graphically in Figure 4. Increase in insulin secretion in response to combined agents (6.16 nM insulin) exceeds that of simple addition of the levels achieved with respective single agents (1.28+3.13 nM insulin).

Example 5

Compound B and Compound D were added to HIT-T15 cells at concentrations approximately equal to their individual EC50 concentrations for enhancement of insulin secretion as determined from a full dose-response test in the same experiment (Compound B: 100nM, Compound D: 15000nM). Responses for the two compounds at this concentration either alone or in combination delivered simultaneously are shown graphically in Figure 5. Increase in insulin secretion in response to combined agents (1.78 nM insulin) exceeds that of simple addition of the levels achieved with respective single agents (0.03+0.71 nM insulin).

Example 6

Compound B and Compound D were added to HIT-T15 cells at concentrations equal to their most effective concentrations for enhancement of insulin secretion as determined from a full dose-response test in the same experiment (Compound B: 1000nM, Compound D: 30000nM). Responses for the two compounds at this concentration either alone or in combination delivered simultaneously are shown graphically in Figure 6. Increase in insulin secretion in response to combined agents (3.37 nM insulin) exceeds that of simple addition of the levels achieved with respective single agents (0.33+1.77 nM insulin).

Comparative Example 7 GLP-1+ GPR119agA- Comparative Example

GLP-1 and Compound A were added to HIT-T15 cells at concentrations equal to their most effective concentrations for enhancement of insulin secretion as determined from a full dose-response test in the same experiment (GLP-1: 300nM, Compound A: 1000 nM). Responses for the two compounds at this concentration either alone or in combination delivered simultaneously are shown graphically in Figure 7. Increase in insulin secretion in

response to combined agents (0.55 nM insulin) is less than that of simple addition of the levels achieved with respective single agents (0.51+0.17 nM insulin).

CLAIMS:

1. A composition comprising a GPR119 agonist and a GPR40 agonist wherein the agonists are separate agents or a single agent that is both a GPR119 agonist and a GPR40 agonist.

2. A composition as claimed in Claim 1 comprising a single agent that is both a GPR119 agonist and a GPR40 agonist.

3. A composition as claimed in Claim 1 comprising at least one agent that is a GPR119 agonist and at least one further agent that is a GPR40 agonist.

4. A composition as claimed in any one of Claims 1 to 3 wherein the GPR119 agonist is a selective GPR119 agonist.

5. A composition as claimed in any one of claims 1 to 4 wherein the GPR119 agonist is selected from:

5-[1-(3-isopropyl-[1,2,4]oxadiazol-5-yl)-piperidin-4-ylmethoxy]-2-(4methanesulfonylphenyl)-pyridine,

5-ethyl-2-{4-[4-(4-pentazol-1-ylphenoxymethyl)-thiazol-2-yl]-piperidin-1-yl}-pyrimidine,

4-[1-(2-fluoro-4-methanesulfonylphenyl)-1H-pyrazolo[3,4d]pyrimidin4-yloxy]-piperidine-1-carboxylic acid isopropyl ester,

4-[5-methyl-6-(2-methylpyridin-3-yloxy)-pyrimidin-4-yloxy]-piperidine-1-carboxylic acid isopropyl ester,

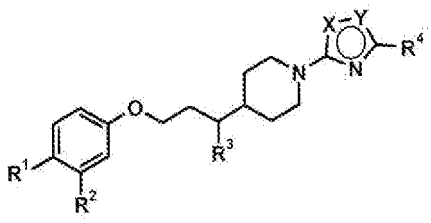
4-[6-(6-methanesulfonyl-2-methylpyridin-3-ylamino)-5-methylpyrimidin-4-yloxy]-piperidine-1-carboxylic acid isopropyl ester,

4-[6-(6-methanesulfonyl-2-methylpyridin-3-ylamino)-5-methoxypyrimidin-4-yloxyl piperidine-1-carboxylic acid isopropyl ester,

4-[5-methoxy-6-(2-methyl-6-[1,2,4]triazol-1-yl-pyridin-3-ylamino)-pyrimidin-4-yloxy]-piperidine-1-carboxylic acid isopropyl ester

and pharmaceutically acceptable salts thereof.

6. A composition as claimed in any one of claims 1 to 4 wherei.. ... ...
agonist is a compound of formula (I), or a pharmaceutically acceptable salt thereof:



(I)

wherein one of X and Y is O and the other is N;

R1 is -CONHR5;

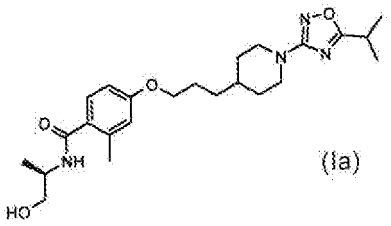
R2 is hydrogen, halo or methyl;

R3 is hydrogen or methyl;

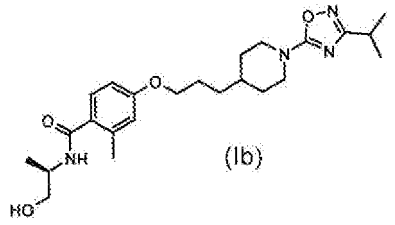
R4 is C2-5 alkyl; and

R5 is hydrogen, C1–3alkyl, or C2–3alkyl substituted by hydroxy.

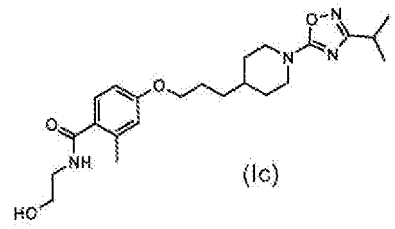
7. A composition as claimed in Claim 6, wherein the compound is selected from



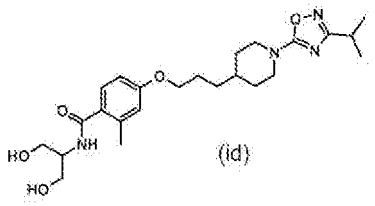
(Ia)



(Ib)



(Ic)



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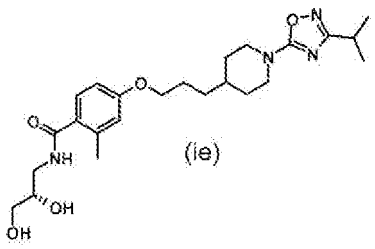

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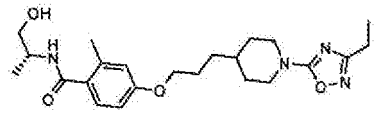
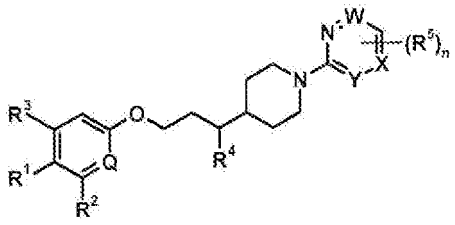


(If)

and pharmaceutically acceptable salts thereof.

8. A composition as claimed in any one of claims 1 to 4 wherein the GPR119 agonist is a compound of formula (I), or a pharmaceutically acceptable salt thereof:



(I)

wherein Q is CH or N;

one of W, X and Y is N or CH and the others are CH where the H may be replaced by R5 when present;

R1 is -SO2Me or -CONHR6;

R2, R3 and R4 are independently selected from hydrogen and methyl;

n is 0, 1 or 2;

R5 is independently C1-4 alkyl, C1-4 alkoxy, fluoro, chloro, C1–3 fluoroalkyl or benzyl;

R6 is hydrogen, 3-azetidinyl, 3-pyrrolidinyl, 3-piperidinyl or 4-piperidinyl, wherein the azetidinyl, pyrrolidinyl and piperidinyl rings may be optionally substituted with OH, CH2OH or CH3; C1–3 alkyl, C2–4 alkyl substituted by -N(R7)2 and/or one or two hydroxy groups, or C1–4 alkyl substituted by a 4- to 6-membered nitrogen-containing heterocyclic ring; and
R7 is independently hydrogen or methyl.

9. A composition as claimed in any one of Claims 1 to 8 wherein the GPR40 agonist is a selective GPR40 agonist.

10. A composition as claimed in any one of Claims 1 to 9 wherein the GPR40 agonist is selected from:

{(S)-6-[4'-(3-methanesulfonylpropoxy)-2',6'-dimethylbiphenyl-3-ylmethoxy]-2,3-dihydrobenzofuran-3-yl}-acetic acid,

(1S,1aS,6aR)-4-(2-difluoromethoxy-4-trifluoromethylphenoxy)-1,1a,6,6a-tetrahydro cyclopropa[a]indene-1-carboxylic acid,

(S)-3-[4-(4'-trifluoromethylbiphenyl-3-ylmethoxy)-phenyl]-hex-4-ynoic acid,

3-[4-(3-phenoxybenzylamino)-phenyl]-propionic acid,

5-[4-(3-Chloro-5-methyl-pyridin-2-yloxy)-2-methyl-benzyl]thiazolidine-2,4-dione

and pharmaceutically acceptable salts thereof.

11. A dosage form comprising a composition as claimed in any one of Claims 1 to 10 and a pharmaceutically acceptable carrier.

12. A dosage form as claimed in Claim 11 comprising a single agent that is both a GPR119 agonist and a GPR40 agonist.

13. A dosage form as claimed in Claim 11 comprising at least one agent that is a GPR119 agonist and at least one further agent that is a GPR40 agonist.

14. A dosage form as claimed in Claim 13 wherein the agents are in a fixed-dose combination.

15. A dosage form as claimed in any one of Claims 11 to 14 wh... dosage form.

16. A dosage form as claimed in Claim 15 which is a tablet, capsule, granule or pellet.

17. A dosage form as claimed in any one of Claims 11 to 16 for use in the treatment or prophylaxis of a disorder selected from the group comprising diabetes, obesity, metabolic syndrome (syndrome X), impaired glucose tolerance, insulin resistance, pancreatic beta-cell insufficiency, hyperglycemia, enteroendocrine cell insufficiency, glucosuria, metabolic acidosis, cateracts, diabetic nephropathy, diabetic retinopathy, diabetic coronary artery disease, diabetic cerebrovascular disease, diabetic peripheral vascular disease, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dislypidaemia, low HDL levels, hypertension, myocardial infarction, atherosclerosis and stroke.

18. A dosage form as claimed in Claim 17 wherein the disorder is diabetes.

19. Use of a GPR119 agonist in the preparation of a composition as claimed in any one of Claims 1 to 10 or in the preparation of a dosage form as claimed in any one of Claims 11 to 16.

20. Use of a GPR40 agonist in the preparation of a composition as claimed in any one of Claims 1 to 10 or in the preparation of a dosage form as claimed in any one of Claims 11 to 16.

21. A method of preparing a pharmaceutical composition comprising admixing a GPR119 agonist and a GPR40 agonist with at least one pharmaceutically acceptable carrier.

22. A method as claimed in Claim 21 comprising admixing a single agent that is both a GPR119 agonist and a GPR40 agonist with at least one pharmaceutically acceptable carrier.

23. A method as claimed in Claim 21 comprising admixing at least one agent that is a GPR119 agonist and at least one further agent that is a GPR40 agonist with at least one pharmaceutically acceptable carrier.

24. A composition as claimed in any one of Claims 1 to 10 or a dosage form as claimed in any one of Claims 11 to 16 for use in a method of treatment of the human or animal body.

25. Use of a composition as Claimed in any one of Claims 1 to 10 in the manufacture of a medicament for the treatment or prevention of diabetes or a condition related thereto.

Application No:	GB1201720.8	**Examiner:**	Dr Bill Thomson
Claims searched:	1-25	**Date of search:**	3 May 2012

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1, 3, 4, 9, 11, 13-21 and 23-25 at least	WO 2008/054675 A2 (MERCK & CO., INC.) - See whole document, in particular page 16, line 16 - page 18, line 2
X	1, 3, 4, 9, 11, 13-21 & 23-25 at least	WO 2008/001931 A2 (TAKEDA PHARMACEUTICAL COMPANY LIMITED) - See whole document, in particular page 60, line 21 - page 62, line 10
X	1, 3, 4, 9, 11, 13-21 and 23-25 at least	EP 2399914 A1 (TAKEDA PHARMACEUTICAL COMPANY LIMITED) - See whole document, in particular paragraphs 320-323 and 338
X	1, 3, 4, 9, 11, 13-21 and 23-25 at least	US 2009/0258921 A1 (NOMURA ET AL) - See whole document, in particular paragraphs 53-54

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

The following online and other databases have been used in the preparation of this search report

BIOSIS, CAS-ONLINE, EPODOC, MEDLINE, TXTE & WPI

International Classification:

Subclass	Subgroup	Valid From
A61K	0031/4523	01/01/2006
A61K	0031/343	01/01/2006
A61K	0031/4427	01/01/2006
A61K	0031/445	01/01/2006
A61P	0003/00	01/01/2006
A61P	0003/10	01/01/2006
A61P	0009/00	01/01/2006