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Title: TREATMENT OF INSULIN RESISTANCE AND OBESITY BY STIMULATING GLP-I RELEASE

Abstract:
The present invention relates to 1 or 2 C16-C18 acyl glycerol based compounds which are capable of activating G-protein coupled receptor 119 and thereby stimulate GLP-I release. Compounds of the present invention are useful in the prophylaxis and/or treatment of metabolic disorders and complications thereof, such as, type 2 diabetes mellitus (T2DM), obesity, insulin resistance, and cardiovascular disease.


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Treatment of insulin resistance and obesity by stimulating GLP-1 release

Field of invention
The present invention relates to compounds which are capable of activating G-protein coupled receptor 119 and thereby stimulate GLP-1 release. Compounds of the present invention are useful in the prophylaxis and/or treatment of metabolic disorders and complications thereof, such as, type 2 diabetes mellitus (T2DM), obesity, insulin resistance, and cardiovascular disease.

Background of invention
T2DM is a multifaceted metabolic disease characterized by a combination of resistance to insulin action and an inadequate compensatory insulin secretory response resulting in increased concentration of glucose in the blood [1]. The prolonged hyperglycemia of T2DM is associated with increased risk of developing severe vascular injury in eyes, kidney, heart and the nervous system. The risk of developing T2DM increases with age, obesity and lack of physical activity [1].

In the USA, the number of people with diagnosed diabetes (2007) now reaching 17.5 million is growing by approximately 1 million per year. More than 90% of those diagnosed diabetic have T2DM. This increase in the prevalence of T2DM is seen all over the world, and it is partly associated with the increased prevalence of obesity. The total estimated cost of diabetes in USA in 2007 is $174 billion, including $116 billion in excess medical expenditures and $58 billion in reduced national productivity [2]. A very large group of non-diabetic people have impaired fasting glucose levels (5.6 - 6.9 mmol/l) and they are now referred to as having pre-diabetes indicating their relatively high risk for developing T2DM [1].

The medical treatment of T2DM focuses on obtaining a tight control of blood glucose levels in order to minimize the risk of developing macrovascular and microvascular complications. A number of different antidiabetica exists. One relatively new group of antidiabetic medicines - the incretin-based medicines - enhances the glucose-stimulated insulin release by increasing the presence of the incretin hormone glucagon-like peptide-1 (GLP-1). GLP-1 is secreted from the hormone-producing L-cells of the intestinal tract in response to unknown components in the meal and GLP-1 increases the glucose-stimulated insulin release from the pancreas [3]. Furthermore, GLP-1 also
reduces gastric emptying, increases satiety and can result in weight loss [4;5]. The incretin effect is strongly reduced in patients with T2DM [6]. The incretin-based medicines increase the plasma concentration of GLP-1 either by inhibiting the GLP-1 catabolic enzyme DPP4 (called incretin enhancers), or by providing stable GLP-1 receptor agonists (called incretin mimetics) [7]. A third way of increasing GLP-1 secretion is to activate the GPR119, an orphan receptor that is found in the small intestine and in the pancreas [8;9]. It is known that stimulation of this receptor will increase GLP-1 secretion from intestinal cells in vitro [10] and in rodents in vivo [8]. Oleylethanolamide and lysophosphatidylcholine have been reported to be endogenous activators of GPR119 [11;12]. It is known that exogenous oleylethanolamide can inhibit food intake [13-15]. However, this anorexic effect of exogenous oleylethanolamide is not mediated via activation of intestinal GPR119 but probably via activation of intestinal PPARalpha [16;17].

Summary of invention

It has surprisingly been found that certain compounds can activate GPR119 in the intestine and thereby stimulate the intestinal GLP-1 release. Through stimulation of GLP-1 release the compounds can be used for treatment and/or prophylaxis of metabolic syndrome, diabetes-2, obesity, insulin resistance and cardiovascular disease.

Therefore, in a first aspect the invention relates to a compound selected from the group consisting of

![Formula XII](image1)

and

![Formula XIII](image2)

wherein R₁ and R₂ are individually selected from the group consisting of H, and C2-C8 acyl groups,

and wherein R₄ is selected from the group consisting of a C16 or C18 acyl group,

for use in the treatment and/or prophylaxis of metabolic syndrome, cardiovascular disease, diabetes-2, obesity or insulin resistance.

The invention also relates to use of a compound according to the invention for
activating G-protein coupled receptor 119.

In one aspect the invention relates to a pharmaceutical composition comprising a compound according to the invention and a pharmaceutically acceptable carrier, excipient and/or diluent.

Furthermore, there is provided a capsule comprising a compound according to the invention or a solvate of said compound, wherein the capsule is made of one or several from the group consisting of gelatine, a plant based gelling substance such as carrageenans, starch, cellulose, modified starch, and modified cellulose, such as hydroxypropyl methylcellulose, and derivatives of any of these. These capsules protect the compounds from hydrolysis in the stomach so that they can reach the target in the intestine.

The compounds may be formulated as a combination product comprising: (A) a compound according to the invention or a pharmaceutical composition according to the invention, and (B) another therapeutic agent that is useful in the treatment of metabolic syndrome diabetes type 2, obesity, insulin resistance, and/or cardiovascular disease, for simultaneous, successive or separate administration.

Furthermore, the invention relates to a method of activating G-protein coupled receptor 119 by administering a compound according to the invention to a cell expressing said receptor.

Also provided is a method of stimulating the release of GLP-1 in the gastrointestinal tract, in the pancreas and in the brain of a subject in need thereof comprising administering to said subject a therapeutically effective amount of a compound according the invention.

In an important aspect, the invention relates to a food or animal feed product comprising a compound selected from the group consisting of

\[
\begin{align*}
\text{R}_1 & \text{O} \\
\text{R}_2 & \text{O} \\
\text{R}_4 & \text{O}
\end{align*}
\]

[Formula XII]
wherein \( R_1 \) and \( R_2 \) are individually selected from the group consisting of \( H \), and C2-C8 acyl groups,
and wherein \( R_4 \) is selected from the group consisting of a C16 or C18 acyl group.

Most of the compounds of the invention are edible and are in the shape of oils that can be readily mixed into food and feed products and can replace a fraction of the oil or fat normally present in the food or feed.

Furthermore, the invention relates to use of a compound according to the invention as a low calorie fat substitute.

An aspect of the invention relates to use of a compound according to the invention for preparation of a dietary supplement, a food or feed product, or a beverage product for helping to sustain energy, helping control appetite, helping control blood sugar levels, reducing the risks associated with metabolic syndrome, reducing the risk associated with obesity and diabetes, reducing the risk associated with diabetes, helping to maintain healthy glucose and fat metabolism, or for helping to normalise production and release of GLP1 necessary for healthy glucose and fat metabolism, in a subject during and/or between meals or feedings comprising said dietary supplement, a food or feed product, or a beverage product.

A related aspect relates to a method for helping to sustain energy, helping control appetite, helping control blood sugar levels, reducing the risks associated with metabolic syndrome, reducing the risk associated with obesity and diabetes, reducing the risk associated with diabetes, helping to maintain healthy glucose and fat metabolism, or for helping to normalise production and release of GLP1 necessary for healthy glucose and fat metabolism, said method comprising administering to a subject a dietary supplement, a food or feed product, or a beverage product comprising a compound of the invention during and/or between meals or feedings.

The compounds of the invention, which are supplemented with the compounds of the invention, may provide a general improvement to human health. First and foremost, the compounds of the invention can protect against the harmful health effects associated with metabolic syndrome. It is also contemplated that the compounds of the invention can protect against the harmful health effects associated with type 2 diabetes, and
against the harmful health effects associated with obesity.

In certain embodiments, the compounds of the invention can help reverse the harmful health effects associated with metabolic syndrome. It is also believed that the compounds products of the invention can help reverse the harmful health effects associated with obesity.

As shown by the examples the compounds of the invention can help normalise production and release of incretins involved in glucose homeostasis and fat metabolism. Normalisation of production and release of incretins is important to human health. The compounds of the invention can help stimulate production and release of incretins involved in glucose homeostasis and fat metabolism. More specifically the compounds of the invention can help normalise production and release of GLP1 necessary for efficient glucose homeostasis and fat metabolism. The compounds of the invention can help normalise production and release of GLP1 necessary for human health.

These beneficial health effects can also be brought about by using the food and feed products of the invention.

Description of Drawings

Figure 1. Ligand-induced GPR19 activation in CHO-K1 cells.
Five different ligands were tested. Oleylethanolamide, H-1 : 2-oleoylglycerol, H-2: 1-oleoylglycerol, H-4: oleic acid, and H-5: 2-palmitoylglycerol: cAMP was measured by a HTRF kit from CisBio International. Three experiments (each in duplicate) were performed using oleylethanolamide as positive control in each experiment. All dose-response curves were normalized to the efficacy of OEA in each experiment, and the data are shown as ligand-induced activation above basal receptor activity.

Figure 2. Ligand-induced GPR19 activation in COS-7 cells.
Three different ligands were tested: (C) oleylethanolamide (OEA), (A) 2-oleoylglycerol (2OG), and (D) oleic acid, by measurement of cAMP accumulation. Briefly, COS-7 cells were transiently transfected with the human GPR19 receptor (squares) or empty vector (negative control, triangle). The cells were seeded in plates one day after transfection, incubated for 24 hours with 2 µCi/ml of ³H-adenine. At the day of the
cAMP measurement, the cells incubated with the ligands for 25 minutes, lysed, and the cAMP purified using Dowex and alumina columns. The data represent sum-curves of three independent experiments (n=3), performed in duplicates. All dose-response curves were normalized to the efficacy of OEA in each experiment, and the data are shown as ligand-induced activation above basal receptor activity.

Figure 3. 2-OG-induced activation of GPR1 19-mediated cAMP formation.
Briefly, COS-7 cells were transiently transfected with human GPR1 19 receptor (squares) or empty vector (negative control, triangle). The cells were seeded in plates one day after transfection, incubated for 24 hours with 2 µCi/ml of 3H-adenine. At the day of the cAMP measurement, the cells were incubated with the ligand for 25 minutes, lysed, and the cAMP purified using Dowex and alumina columns. The data represent sum-curves of the 7 independent experiments, performed in duplicates. All dose-response curves were normalized to the efficacy of OEA in each experiment, and the data are showed as ligand-induced activation above basal receptor activity.

Figure 4. Stimulation of tGLP-1 release by 2OG in the intestinal lumen.
Eight healthy male volunteers were given enteral feeding by duodenal tube. Each volunteer was given three different liquid meals (two grams of 2OG(A), 1.54 grams of oleic (o) acid or vehicle (O)) on three different days. The vehicle consisted of 50 ml glycerol plus 5 ml ethanol. Data are mean values ± SEM and were analysed by repeated measurements ANOVA. The incremental area under the curve (iAUC) for the first 25 min was significantly increased (p=0.010) with the following values 0.07 ± 0.02 nMx25 min for 2OG,-0 0.04 ± 0.02 nMx25 min for oleic acid, and 0.03 ± 0.02 nMx25 min for vehicle.

Definitions
Treatment: "Treatment" can be performed in several different ways, including curative, ameliorating and as prophylaxis. Curative treatment generally aims at curing a clinical condition, such as a disease, which is already present in the treated individual. Ameliorating treatment generally means treating in order to improve in an individual an existing clinical condition. Prophylactic treatment generally aims at preventing a clinical condition from occurring or from developing further.
Detailed description of the invention

It has surprisingly been found that compounds selected from the group consisting of

\[
\begin{align*}
&\text{Y-X} - \text{O-R}_1, \\
&\text{X-Y} - \text{O-R}_2, \\
&\text{Y-X} - \text{OH}
\end{align*}
\]

and Y-OH, activate GPR19 in the intestine and thereby stimulate the intestinal GLP-1 release. Said compounds can be used for the prophylaxis and/or treatment of diabetes-2, obesity, insulin resistance, and cardiovascular disease.

Accordingly, in a first main aspect the invention relates to a compound selected from the above defined group for use as a medicament.

In another main aspect the invention relates to a method of treating metabolic syndrome, diabetes-2, obesity, insulin resistance, and/or cardiovascular disease comprising administering an effective amount of a compound as defined above to a subject in need of such treatment.

In a further aspect the invention relates to compounds as defined above capable of activating G-protein coupled receptor 119.

The present invention in its broadest aspect is directed to a compound selected from the group consisting of

\[
\begin{align*}
&\text{Y-X} - \text{O-R}_1, \\
&\text{X-Y} - \text{O-R}_2, \\
&\text{Y-X} - \text{OH}
\end{align*}
\]

and Y-OH [Formula IV],
wherein $R_1$ and $R_2$ are individually selected from the group consisting of $H$, and $O$

$$C - R_3$$ [Formula V] wherein $R_3$ is selected from the group consisting of alkyl groups comprising 1-12 carbon atoms,

$Y - X$ is selected from the group consisting of

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$Y$ is selected from the group consisting of an alkyl group comprising a carbon chain comprising at least 12 carbon atoms and comprising 0, 1, 2, 3 or 4 double bonds,

for use as a medicament.

In a preferred embodiment $R_1$ and/or $R_2$ are $C - R_3$. [Formula V]

In a more preferred embodiment $R_3$ is selected from an alkyl group consisting of 1-12 carbon atoms, such as 1-11, for example 1-10, such as 1-9, for example 1-8, such as 1-7, for example 1-6, such as 1-5, for example 1-4, such as 1-3 carbon atoms.

In an even more preferred embodiment $R_3$ is selected from an alkyl group consisting of 1-2 carbon atoms.

In a preferred embodiment $X$ is $O$. 

In a preferred embodiment $R_1$ and/or $R_2$ are $C - R_3$. [Formula V]
In one embodiment Y is selected from a group consisting of an alkyl group comprising a carbon chain consisting of 12, 14, 16, 18, or 20 carbon atoms. Said carbon chain further comprises 0, 1, 2, 3, or 4 double bonds.

In another embodiment Y is selected from a group consisting of an alkyl group comprising a carbon chain consisting of 13, 15, 17, or 19 carbon atoms. Said carbon chain further comprising 0, 1, 2, 3, or 4 double bonds.

In a preferred embodiment Y is selected from a group consisting of an alkyl group comprising a carbon chain consisting of 12 carbon atoms and 0 double bonds, an alkyl group comprising a carbon chain consisting of 14 carbon atoms and 0 double bonds, an alkyl group comprising a carbon chain consisting of 14 carbon atoms and 1 double bond, an alkyl group comprising a carbon chain consisting of 16 carbon atoms and 0 double bonds, an alkyl group comprising a carbon chain consisting of 16 carbon atoms and 1 double bond, an alkyl group comprising a carbon chain consisting of 18 carbon atoms and 0 double bonds, an alkyl group comprising a carbon chain consisting of 18 carbon atoms and 1 double bond, an alkyl group comprising a carbon chain consisting of 18 carbon atoms and 2 double bonds, an alkyl group comprising a carbon chain consisting of 18 carbon atoms and 3 double bonds, or an alkyl group comprising a carbon chain consisting of 20 carbon atoms and 0 double bonds.

In a more preferred embodiment Y is selected from a group consisting an alkyl group comprising a carbon chain consisting of 18 carbon atoms and 0 double bonds, an alkyl group comprising a carbon chain consisting of 18 carbon atoms and 1 double bond, an alkyl group comprising a carbon chain consisting of 18 carbon atoms and 2 double bonds, or an alkyl group comprising a carbon chain consisting of 18 carbon atoms and 3 double bonds, or an alkyl group comprising a carbon chain consisting of 18 carbon atoms and 4 double bonds.

In an even more preferred embodiment Y is an alkyl group comprising a carbon chain consisting of 18 carbon atoms and 1 double bond.

\[
\text{O} \\
\text{II}
\]

In a preferred embodiment \( R_1 \) and/or \( R_2 \) are \( \text{C} - R_s \) wherein \( R_s \) is an alkyl group consisting of 1-2 carbon atoms, X is O, and Y is an alkyl group comprising a carbon chain consisting of 18 carbon atoms and 1 double bond.
In another preferred embodiment the compound is Y-OH

Preferred compounds of the invention

A preferred aspect of the invention relates to compounds selected from the group consisting of

\[ R_1 \text{O} - \text{O-R}_2 \quad \text{and} \quad \text{H-O} - \text{O-R}_4 \]

[Formula XII] [Formula XIII]

wherein \( R_1 \) and \( R_2 \) are individually selected from the group consisting of H, and C2-C8 acyl groups,

and wherein \( R_4 \) is selected from the group consisting of a C16 or C18 acyl group,

for use in the treatment and/or prophylaxis of metabolic syndrome, cardiovascular disease, diabetes-2, obesity or insulin resistance.

Formula XII is a combination of above identified Formula I and Formula X. Likewise Formula XIII is a combination of above identified Formula II and Formula X. These compounds are glycerols with a long chain fatty acid in the 2 (Formula XII) or 1 (Formula XIII) position and optionally with short chain fatty acids in the 1 and 3 positions (Formula XII) and the 2 and 3 positions (Formula XIII) respectively.

Preferably \( R_1 \) and \( R_2 \) are linear and saturated as most edible short chain fatty acids are.

In one embodiment one of \( R_1 \) and \( R_2 \) is CH2-CH3 and the other is -H. Preferably, both \( R_1 \) and \( R_2 \) are CH2-CH3. Such acetylated glycerols will be hydrolysed in the stomach to the non-acetylated forms. \( R_1 \) and/or \( R_2 \) may also be -H.

Preferably, \( R_4 \) is linear. In a preferred embodiment, \( R_4 \) is unsaturated. In certain embodiments \( R_4 \) is C16 acyl group comprising 0, 1 or 2 double bonds. Examples of such \( R_4 \) groups include palmitic and palmitoleic. In other, preferred embodiments, \( R_4 \) is C18 acyl group comprising 0, 1, 2, 3, or 4 double bonds. Examples of such \( R_4 \) groups include oleic, linoleic, alpha-linoleic, elaidic, gamma linoleic, or stearidonic, preferably
oleic. 2-palmitoyl-glycerol has been shown to be an activator of GPR1 19 by the present inventors.

In a more preferred embodiment the compound is 1-oleoylglycerol. 1-oleoylglycerol is chemically stable in aqueous solution. In aqueous solution 1-OG isomerises spontaneously into 2-OG. Therefore, 1-OG may be regarded as a prodrug of 2-OG. When taken orally, the isomerisation starts already in the mouth and may continue in the stomach and intestine. This isomerisation process starts already in the mouth and continues into the stomach and the intestinal lumen. As 2-monoacylglycerols are taken up very rapidly, the equilibrium (1-OG/2-OG) may be shifted even more in favour of 2-OG in the intestine as 2-OG is taken up.

Similarly, it is expected that 1-palmitolyl-glycerol can act as a prodrug for 2-palmitoyl-glycerol.

Different 1-monoacylglycerols are widely commercially used as emulsifiers in the food, cosmetic and pharmaceutical industry [20] and 1-oleoylglycerol is cheap and easily available. 1-OG is generally recognised as safe by the FDA.

In yet a preferred embodiment the compound is 1-acetyl-2-oleoylglycerol, 1-acetyl-2-alpha-linoleylglycerol, 1-acetyl-2-elaidoylglycerol, and 1-acetyl-2-gamma-linoleylglycerol. Among these, 1-acetyl-2-oleoylglycerol is most preferred.

Even more preferred is 1,3 diacetyl-2-oleylglycerol, 1,3 diacetyl-2-linoleylglycerol, 1,3 diacetyl-2-alpha-linoleylglycerol, 1,3 diacetyl-2-elaidoylglycerol, and 1,3 diacetyl-2-gamma-linoleylglycerol. Among these, 1,3 diacetyl-2-oleylglycerol is most preferred.

Also preferred are glycerols with a C18 unsaturated acid in the 2 position. Examples of these are 2-oleylglycerol, linoleylglycerol, 2-elaidoylglycerol, and 2-gamma-linoleylglycerol.

In a most preferred embodiment the compound is 2-oleylglycerol. 2-oleylglycerol was found to activate GPR1 19 nearly as potently as oleylethanolamide (Example 1). 2-oleylglycerol can relatively easy be synthesized from olive oil or canola oil by the use of 1,3-specific lipases [19].
In a further embodiment, the compound is a triacylglycerol-like type of prodrug used to circumvent the chemical instability of 2-oleylglycerol. Such a prodrug is chemically stable and may be hydrolyzed to 2-oleylglycerol in the intestinal lumen. Preferred prodrugs are those with short-chain acyl (C2-C8) groups in the 1 and 3 positions. Short chain acyl groups in the 1 and 3 positions have the advantage that they provide less calories than triglycerides with two or three long fatty acids.

In a further embodiment the compound is the prodrug 1,3-di-acetyl-2-oleylglycerol.

The compounds of the present invention are capable of activating GPR19 and stimulating GLP-1 release in the gastrointestinal tract.

By activation/activating GPR 119 is meant binding to GPR1 19 and causing formation of cAMP and a subsequent increase in the level of intracellular cAMP.

GPR1 19 is found both in the intestine where it is involved in release of GLP-1 and in the pancreatic islet where it is involved in release of insulin [21]. GPR1 19 is also found in the brain (Chu et al, (2008) Endocrinology, 149; 2038-2047).


The compounds of the invention in one preferred embodiment are defined as having an EC50 of 50 μM or lower, such as 40 μM or lower, for example 30 μM or lower, such as 20 μM or lower, where EC50 is defined as the concentration of said compound needed for half-maximal activation of GPR1 19 measured by cAMP-formation. The EC50 as defined above is preferably determined in an assay as described in Example 1. The EC50 may be 18 μM or lower, such as 15 μM or lower, for example 12 μM or lower, such as 10 μM and lower, for example 8 μM or lower such as 5 μM or lower.

The compounds of the invention are useful as medicaments for prophylaxis, and/or treatment of metabolic syndrome, diabetes-2, obesity, insulin resistance, and
cardiovascular disease. The effect on GLP-1 release may be confirmed in an in vivo experiment such as described in Example 2.

In one embodiment the compounds of the invention are used for the treatment and/or prophylaxis of cardiovascular disease alone.

In a preferred embodiment, the compound according to the invention is for the treatment and/or prophylaxis of metabolic syndrome, diabetes-2, obesity or insulin resistance. Each of these disorders may be treated alone or in combination with one of the others. Likewise, the compounds of the invention may be used in the treatment of metabolic syndrome.

Accordingly the invention relates to a compound according to the invention for the treatment and/or prophylaxis of diabetes-2.

In another aspect it relates to a compound of the invention for the treatment and/or prophylaxis of obesity.

In another aspect it relates to a compound of the invention for the treatment and/or prophylaxis of metabolic syndrome.

Finally, the invention relates to a compound according to the invention for the treatment and/or prophylaxis of insulin resistance.

Accordingly, the invention relates to the use of one or more of the compounds as defined above as a medicament.

The invention also relates to the use of one or more of the compounds for the manufacture of a medicament.

**Pharmaceutical compositions**

Further, the invention relates to a pharmaceutical composition comprising a compound as defined above, or a solvate of said compound, and a pharmaceutically acceptable carrier, excipient and/or diluent. Suitable excipients and/or diluents can be, but are not
limited to lecithin, bile salts, Macrogol, sorbitan esters, polysorbates, ethanol, glycerol, medium-length triglycerides.


Formulations of the compounds of the invention can be prepared by techniques known to the person skilled in the art. The formulations may contain pharmaceutically acceptable carriers and excipients including microspheres, liposomes, microcapsules, nanoparticles or the like.

For formation of liposomes and nanoliposomes an emulsifier should be added. Examples of emulsifiers include lecithin, such as lecithin from egg yolk or soybean, honey, mustard powder, proteins and low molecular weight emulsifiers. Lipid vesicles are formed when e.g. phospholipids such as lecithin are placed in water and consequently form one bilayer or a series of bilayers, each separated by water molecules, once enough energy is supplied. Liposomes can e.g. be created by shaking or sonicating phospholipids in water.

The medicament of the invention comprises an effective amount of one or more of the compounds as defined above, or a composition comprising a compound as defined above, in combination with pharmaceutically acceptable additives. Such medicament may suitably be formulated for oral and intravenous administration routes. Oral administration is preferred.

Injectables are usually prepared either as liquid solutions or suspensions, solid forms suitable for solution in, or suspension in, liquid prior to injection. The preparation may also be emulsified. The active ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like, and
combinations thereof. In addition, if desired, the preparation may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or which enhance the effectiveness or transportation of the preparation.

The compounds according to the invention may preferably be administered by oral formulations.

The compounds according to the invention are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective. The quantity to be administered depends on the subject to be treated, including, e.g. the weight and age of the subject, the disease to be treated and the stage of disease.

Some of the compounds of the present invention are sufficiently active, but for some of the others, the effect will be enhanced if the preparation further comprises pharmaceutically acceptable additives and/or carriers. Such additives and carriers will be known in the art. In some cases, it will be advantageous to include a compound, which promotes delivery of the active substance to its target.

The preferred oral formulation comprising a compound according to the invention can be presented as units suitable for oral administration, such as capsules, tablets, or cachets.

In a preferred embodiment of the invention the oral formulation comprising a compound according to the invention is presented as capsules.

Suitable capsule materials can be, but are not limited to gelatine, a plant based gelling substance such as carrageenans, starch, cellulose, modified starch, and modified cellulose, such as hydroxypropyl methylcellulose.

Capsules can be formulated for sustained and/or controlled release.

Capsules can have an enteric coating. Different enteric coating polymers for enteric coated capsules can be, but are not limited to: Cellulose acetate phthalate (CAP), Methyl acrylate-methacrylic acid copolymers, Cellulose acetate succinate, Hydroxy propyl methyl cellulose phthalate, Hydroxy propyl methyl cellulose acetate succinate
(hypromellose acetate succinate), Polyvinyl acetate phthalate (PVAP), and Methyl methacrylate-methacrylic acid copolymers.

Suitable coatings for duodenum delivery can be, but are not limited to; EUDRAGIT® L 100-55 which contains an anionic copolymer based on methacrylic acid and ethyl acrylate, EUDRAGIT® L 30 D-55 which is the aqueous dispersion of an anionic copolymer based on methacrylic acid and ethyl acrylate.

Suitable coatings for jejunum/ileum delivery can be, but are not limited to; Eudragit® L100, Eudragit® S100, Eudragit® NE 30D

The capsule can be soluble or insoluble in gastric juice. Preferably, the capsule does not dissolve in gastric juice, but dissolves in the environment of the duodenum and upper ileum. The pH of gastric juice is strongly acidic, approximately pH 1-3. The pH of the duodenum is close to neutral, pH 6-6.5, and the pH of the ileum is neutral to slightly basic, pH 7-8. Thus a capsule, which is insoluble in gastric juice and soluble in the duodenum or ileum should be insoluble at low pH (1-3) and soluble at around neutral pH (6-8).

**Combination products**

The compounds of the invention can also be included in a combination product as herein described.

In one embodiment the combination product comprises; (A) a compound or a pharmaceutical composition according to the invention, and (B) a monoacylglycerol-lipase-inhibitor for simultaneous, successive or separate administration.

Monoacylglycerol-lipase inhibitors can be selected from, but are not limited to, the group consisting of JZL184, CAY10499, URB754, OMDM169, URB602, Disulfiram, Tetrahydrolipstatin, N-arachidonylemaleimide, Isopropyl dodecylfluorophosphonate (IDFP), Oxiran-2-ylmethyl (5Z,8Z,11Z,14Z)-icoso-5,8,11,14-tetraenoate, Tetrahydro-2H-pyran-2-ylmethyl (5Z,8Z,11Z,14Z)-icoso-5,8,11,14-tetraenoate, Cetilistat (ALT-962), and GT-398-255.
In a preferred embodiment the monoacylglycerol-lipase inhibitor is selected from the group consisting of JZL184, CAY10499, URB602, and OMDM169.

In a further embodiment the combination product comprises; (A) a compound or a pharmaceutical composition according to the invention, and (B) a monoacylglycerol-acyltransferase-inhibitor, for simultaneous, successive or separate administration.

In a preferred embodiment the monoacylglycerol-acetyltransferase inhibitor is sphingosine.

In another embodiment the combination product comprises; (A) a compound or a pharmaceutical composition according to the invention, and (B) another therapeutic agent that is useful in the treatment of metabolic syndrome, diabetes type 2, obesity and/or insulin resistance, for simultaneous, successive or separate administration. The therapeutic agent of (B) can be selected from, but are not limited to, the group consisting of sulphonylurea, biguanides, meglitinides, α-glucosidase inhibitors, and DPP-4 inhibitors.

In a further embodiment the combination product comprises; (A) a compound or a pharmaceutical composition according to the invention, and (B) another therapeutic agent that is useful in the treatment of cardiovascular disease.

The invention also provides methods for the prophylaxis and/or treatment of diabetes-2, obesity, insulin resistance, and cardiovascular disease comprising administering an effective amount of a compound according to the invention to a subject in need thereof. Preferably the subject is a human.

Generally, suitable dosage levels of a compound according to the invention are in the order of about 100 - 5000 mg, such as 500-5000 mg, for example 500-4000 mg, such as 500-3000 mg, for example 500-2000 mg. One or more dosages may be administered per day. A daily dosage can thus be up to 5 g/person, such as up to 10 g/person, for example up to 15 g/person, such as up to 20 g/person, for example 25 g/person for a subject of 70 kg. These values can be converted into dosages per kg/body weight per day.
Most preferred is a dose of 1000 mg of a compound according to the invention. For 2-oleylglycerol a preferred dosage is 2 g/dosage. For other compounds of the invention, a dosage giving the same dosage in moles is preferred. For example for 1,3-dioctyl-2-oleoylglycerol, an equivalent dosage is 3.5 g/dosage.

Such a dosage could preferably be administered per meal. The dosage can be administered before, after or together with a meal.

### Food and feed products

In certain aspects, the invention relates to a food or animal feed product comprising a compound selected from the group consisting of

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\begin{align*}
\text{R}_1 & \text{O} - \text{R}_2 \\
\text{R}_4 & \text{O} - \text{R}_3 \\
\end{align*}
\]

[Formula XII]

wherein \( R_1 \) and \( R_2 \) are individually selected from the group consisting of H, and C2-C8 acyl groups, and wherein \( R_4 \) is selected from the group consisting of a C16 or C18 acyl group.

The term food or animal feed product includes dietary supplements and beverages.

Preferably \( R_1 \) and \( R_2 \) are linear and saturated, as most edible short chain carboxylic acids are linear and saturated. More preferably one of \( R_1 \) and \( R_2 \) is \( \text{CH}_2 \cdot \text{CH}_3 \) and the other is -H. Still more preferably \( R_1 \) and \( R_2 \) are \( \text{CH}_2 \cdot \text{CH}_3 \). Preferred compounds that are 1, and or 3 substituted include 1-acetyl-2-oleoylglycerol and even more preferably 1,3-diacyl-2-oleoylglycerol.

Compounds that are acylated or acetylated in the 1 and or 3 position are degraded in the stomach to glycerols that are only substituted in the 2-position. This protects the compounds as they pass through the stomach so that more of the active 2-substituted glycerols reach the intestine and activate GPR19. Furthermore, compounds of the invention that are substituted in both the 1- and the 3-positions are oils that can be readily mixed with other oils and are oils that can be used for cooking. Thus it is expected that essentially pure 1,3-acetyl-2-oleoylglycerol can be used as a major
fraction of salad oil and can be used in an essentially pure form as a cooking or frying oil.

In other embodiments, \( R_i \) and/or \( R_j \) is - H.

Preferably, \( R_1 \) is linear. In a preferred embodiment, \( R_4 \) is unsaturated. In certain embodiments \( R_4 \) is C16 acyl group comprising 0, 1 or 2 double bonds. Examples of such \( R_4 \) groups include palmitic and palmitoleic. In other, preferred embodiments, \( R_4 \) is C18 acyl group comprising 0, 1, 2, 3, or 4 double bonds. Examples of such \( R_4 \) groups include oleic, linoleic, alpha-linoleic, elaidic, gamma linoleic, or stearidonic, preferably oleic. 2-palmitoyl-glycerol has been shown to be an activator of GPR1 19 by the present inventors.

A compound according to the invention can be incorporated into a food product. The food product can be any food product. Examples of food products are; processed food items, such as bread, diary products, such as yoghurt, smoothies, cheese and ice cream, non dairy products, dietary products, spreads, products for diabetics, and salad oil. Also included are high fat products such as mayonnaise, butter, margarine, oils, such as salad oil, cooking oil, and frying oil. Further examples of food products include cakes, cookies, and snacks.

The compound of the invention may amounts to a minimum of 10 weight% of other fats in the product, such as at least 20 weight%, for example at least 30%, such as at least 40%, for example at least 50%, such as at least 60%, for example at least 70%, such as at least 80%, for example at least 90%, such as essentially 100%.

In such high-fat products, the compounds of the invention can serve as low calorie fat substitutes, as the compounds of the invention, in particular glycerols with C18 (such as oleic) in the 2 position and short chain acyl groups in the 1 and 3 positions contain less calories than fatty triglycerides. Such compounds are oils and can be readily mixed with other oils and fats. Such products of course also have an effect on the release of gastric hormones.

A compound according to the invention can be incorporated into an animal feed product, for example a feed product for dogs or cats.
The food and feed products of the invention may reduce the risk or likelihood or extent of dyslepidemia, obesity, type 2 diabetes, metabolic syndrome, and/or may served to release gastric hormones and/or regulate glucose in the blood.

In certain embodiments the food and/or food product may provide one or more of the following effects on an individual eating the food or feed product:

1) Help sustain energy
2) Help control appetite
3) Help control blood sugar levels
4) Reduce the risks associated with metabolic syndrome
5) Reduce the risk associated with obesity and diabetes
6) Reduce the risk associated with diabetes
7) Help to maintain healthy glucose and fat metabolism
8) Help to normalise production and release of GLP1 necessary for healthy glucose and fat metabolism.

The invention thus relates to a method of achieving one or more of the cited effects in an individual in need thereof by providing the individual with an effective amount of the food or feed product of the invention.

In certain embodiments and if the compound incorporated into a food or animal feed product is 1-oleoylglycerol, 1-linoleoylglycerol, 2-oleoylglycerol with short chain esters (R3 = short chain alkyls), or 2-linoleoylglycerol with short chain esters (R3 = short chain alkyls), the food product is preferably a low fat product wherein said compound amount to a minimum of 10 weight% of other fats.

In one embodiment the compound in the food product or animal feed product is encapsulated in a capsule material which is soluble in gastric juice.

In another embodiment the compound in the food product or animal feed product is encapsulated in a capsule material which is insoluble in gastric juice. This ensures that the compounds of the invention are released in the duodenum or ileum.

Examples

Example 1. In vitro experiments with GPR1 19-expressing cells.
GPR1 19 receptor signalling experiments were carried out using COS-7 cells transiently transfected with the human GPR1 19 receptor. The COS-7 cells were grown at 10% CO2 and 37°C in Dulbecco’s modified Eagle’s medium with glutamax (Gibco, Cat. No 21885-025) adjusted with 10% fetal bovine serum (FBS), 180 u/ml penicillin and 45 ug/mL streptomycin (PenStrep). Transfection of the COS-7 cells was performed by the calcium phosphate precipitation method. The cells were seeded in 24 well plates (1.5 x 10^5 cells/well) one day after transfection, and were subsequently incubated for 24 hours with 2 µCi/ml of 3H-adenine in 0.5 ml growth medium per well. At the day of the cAMP measurement, the cells were washed twice in HBS buffer (25 mM Hepes, pH 7.2, supplemented with 0.75 mM NaH2PO4, 140 mM NaCl and 0.05% (w/v) bovine serum albumin), and 0.5 ml HBS buffer supplemented with 1mM of the phosphodiesterase inhibitor IBMX (3-isobutyl-1-methylxanthine, Sigma chemicals Co., St. Louis, Missouri, USA) was added together with increasing concentrations of the different compounds (2OG, 1OG, OEA, oleic acid or 2-PG). After 25 min incubation at 37°C, the cells were placed on ice, the medium was removed, and the cells were lysed in 1 ml of 5% (w/v) trichloroacetic acid, supplemented with 0.1 mM cAMP and 0.1 mM ATP for 30 min. The lysate mixtures were loaded onto Dowex columns (Bio-Rad, Hercules, California, USA), which were washed with 2 ml of water and placed onto the top of alumina columns (Sigma) and washed again with 10 ml of water. The alumina columns were eluted with 6 ml of 0.1 M imidazole into 15 ml scintillation fluid (Highsafe III). Columns were re-used up to 15 times. Dowex columns were regenerated by adding 10 ml of 2 N HCl followed by 10 ml of water; the alumina columns were regenerated by adding 2 ml of 1 M imidazole, 10 ml of 0.1 M imidazole, and finally 5 ml of water. Determinations were made in duplicate.

GPR1 19 receptor signalling experiments were carried out using CHO-K1 cells transiently transfected with the human GPR1 19 receptor. CHO-K1 cells expressing GPR1 19 grown to mid-log phase prior to the test in culture media without antibiotics and supplemented with doxycycine (final concentration 200 ng/ml) were detached by gentle flushing with PBS-EDTA (5 mM EDTA), recovered by centrifugation and resuspended in Ham’s F12 culture medium containing 10% FCS and no antibiotic. Cells were counted, centrifuged in a 50 ml Falcon tube and resuspended in KRH-IBMX (5 mM KCL, 1.25 mM MgSO4, 124 mM NaCl, 25 mM HEPES, 13.3 mM glucose, 1.25 mM KH2PO4, 1.45 mM CaCl2, 0.6 mg/ml BSA and 10 mg/ml Phenol red, pH 7.4) at a concentration of 6.25 x 10^5 cells/ml. Cells were then filled in 96 well plates (total vol 24
µl/well) and stimulated with agonists in serial dilution for 30 min. cAMP was then measured using a HTRF kit from CisBio International (cat no. 62AM2PEB). Determinations were made in duplicate.

Results:
2OG and some of its structural analogues were found to increase cAMP formation in CHO-K1 cells transiently expressing the GPR19 (Fig 1). 2OG was found to have and EC50 nearly as low as that seen for the recognised GPR19 agonist, oleoylthanolamide (Table 1). 2OG was also tested in another laboratory using COS-7 cells transiently expressing GPR19 (Fig 2). Also in these cells was 2OG nearly as potent as oleoylthanolamide (Fig 2). Thus 2OG can readily activate GPR19 and appear to be a full agonist.

Table 1 EC-values (µM) for activation of GPR19 in two different expression systems, CHO-K1-cells and COS-7-cells. In both cell types the cAMP formation was quantified.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CHO-exp-1</th>
<th>CHO-exp-2</th>
<th>CHO-exp-3</th>
<th>COS-cells (n=3)</th>
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<tr>
<td>Oleoylthanolamide</td>
<td>1.43</td>
<td>4.65</td>
<td>1.67</td>
<td>1.4</td>
</tr>
<tr>
<td>2-Oleoylglycerol</td>
<td>1.55</td>
<td>1.12</td>
<td></td>
<td>2.8</td>
</tr>
<tr>
<td>oleic acid</td>
<td></td>
<td></td>
<td></td>
<td>&gt;30</td>
</tr>
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</table>

Table 2. shows EC50 values (microM) for activation of GPR19 in transiently transfected COS-7-cells (as described previously) by four different compounds using the assay described above. Data for 2-OG are also shown in Figure 3.

Table 2

<table>
<thead>
<tr>
<th>Compound</th>
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<tbody>
<tr>
<td>2OG</td>
<td>4.2 (mean, n = 7)</td>
</tr>
<tr>
<td>oleic acid</td>
<td>&gt;30 (n = 2)</td>
</tr>
<tr>
<td>20G-ether</td>
<td>7.9 (mean, n = 3)</td>
</tr>
<tr>
<td>2-palmitolylglycerol (2PG)</td>
<td>&gt;30 (n = 2)</td>
</tr>
</tbody>
</table>

Example 2A. In vivo experiment
Six healthy male volunteers that have fasted for 10 hours, are given enteral feeding by a duodenal tube. Each volunteer is given four different liquid meals (bolus, 55-65 ml) on four different days (A-D)

Day A: 2OG (2 g) in 50 ml glycerol + 5 ml ethanol

Day B: oleic acid (1.54 g) in 50 ml glycerol + 5 ml ethanol (=control Day A)

Day C: 2OG (2 g) + glucose (10-2Og in 10 ml water) in 50 ml glycerol + 5 ml ethanol

Day D: oleic acid (1.54 g) + glucose (10-2Og in 10 ml water) in 50 ml glycerol + 5 ml ethanol (=control Day C)

Blood samples are collected at the following time points (min): -15, -10, 0, 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 240.

From duodenal lumen is collected 1 ml sample (at 15 and 30 min)

In serum is measured: insulin and C-peptide

In plasma is measured: glucose, GLP-1, glucose-dependent insulinotropic polypeptide (GIP), glucagon, peptide-YY, cholecystokinin,

In duodenal sample is measured: bilirubin

The methods for these assays have been described previously [18].

Example 2B. Clinical experiment

8 healthy male volunteers (20-30 years, BMI 20-25) that had fasted 10 hours, were given enteral feeding by duodenal tube. The liquid meals were prepared 5 min before use. Each volunteer was given three different liquid meals (clear solution, bolus 55 ml) on three different days (A-C).

Day A: 2OG (2g) in 50 ml glycerol + 5 ml ethanol

Day B: oleic acid (1.54 g) in 50 ml glycerol + 5 ml ethanol

Day C: 50 ml glycerol + 5 ml ethanol

Blood Samples were collected at the following points (min): -15, -10, 0, 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 240.

In serum was measured: insulin and C-peptide

In plasma was measured: glucose, tGLP-1, Cholecystokinin (CCK).

The methods for these assays have been described previously (ref 18).
Results:

As can be seen from figure 4, during the first 25 minutes there was a significant increased levels of tGLP-1 in the plasma of the individuals as a response to the duodenal bolus of 2 g of 2OG, compared to both vehicle and to oleic acid. The 1.54 g of oleic acid is in mol amount equivalent to the 2 g 2OG, and this amount of oleic acid did not increase plasma levels of GLP-1. As expected, there were no effect of the increased level of tGLP-1 on insulin or c-peptide levels (data not shown), since these persons were fasted when the bolus meal were given. GLP-1 is an incremental hormone that will increase insulin release only when plasma glucose levels are elevated (ref. 3). The plasma levels of glucose started in all three groups to increase after 25 min (no difference between groups, data not shown) due to gluconeogenesis from the glycerol in the vehicle. Likewise no difference was seen in plasma cholecystokinin levels (data not shown).

Clearly, 2OG in the intestine can stimulate GLP-1 release in humans probably via activation of GPR19 in intestinal L-cells.

References


Claims

1. A compound selected from the group consisting of

   [Formula XII]  [Formula XIII]

   wherein \( R_1 \) and \( R_2 \) are individually selected from the group consisting of H, and C2-C8 acyl groups,
   and wherein \( R_4 \) is selected from the group consisting of a C16 or C18 acyl group,
   for use in the treatment and/or prophylaxis of metabolic syndrome, cardiovascular disease, diabetes-2, obesity or insulin resistance.

2. A compound according to claim 1, wherein \( R_1 \) and \( R_2 \) are linear and saturated.

3. A compound according to claim 1, wherein one of \( R_1 \) and \( R_2 \) is \( \text{CH}_2 - \text{CH}_3 \) and the other is \(-\text{H}\).

4. A compound according to claim 1 wherein \( R_1 \) and \( R_2 \) are \( \text{CH}_2 - \text{CH}_3 \).

5. A compound according to claim 1 wherein \( R_1 \) and/or \( R_2 \) is \(-\text{H}\).

6. A compound according to any of the preceding claims, wherein \( R_4 \) is linear.

7. A compound according to any of the preceding claims, wherein \( R_4 \) is unsaturated.

8. A compound according to any of the preceding claims, wherein \( R_4 \) is C16 acyl group comprising 0, 1 or 2 double bonds.

9. A compound according to any of the preceding claims 1-6, wherein \( R_4 \) is C18 acyl group comprising 0, 1, 2, 3, or 4 double bonds.

10. A compound according to claim 6, wherein \( R_4 \) is oleic, linoleic, alpha-linoleic,
elaidic, gamma linoleic, or stearidonic, preferably oleic.

11. A compound according to any of the preceding claims, wherein the compound is 2-oleoyl-glycerol.

12. A compound according to claim 1, wherein the compound is 1-oleoyl-glycerol.

13. A compound according to claim 1, wherein the compound is 1-acetyl-2-oleoylglycerol.

14. A compound according to claim 1, wherein the compound is 1,3-diacetyl-2-oleoylglycerol.

15. A compound according to any of the preceding claims, wherein the compound is an agonist of G-protein coupled receptor 119.

16. A compound according to any of the preceding claims for the treatment and/or prophylaxis of cardiovascular disease.

17. A compound according to any of the preceding claims for the treatment and/or prophylaxis of metabolic syndrome, diabetes-2, obesity or insulin resistance.

18. A compound according to claim 17, for the treatment and/or prophylaxis of metabolic syndrome

19. A compound according to claim 17, for the treatment and/or prophylaxis of diabetes-2.

20. A compound according to claim 17, for the treatment and/or prophylaxis of obesity.

21. A compound according to claim 17, for the treatment and/or prophylaxis of insulin resistance.

22. A compound as described in any of claims 1-21.
23. A method of treating diabetes-2 comprising administering an effective amount of a compound according to any of claims 1-21 to a subject in need of such treatment.

24. A method of treating obesity comprising administering an effective amount of a compound according to any of claims 1-21 to a subject in need of such treatment.

25. A method of treating insulin resistance comprising administering an effective amount of a compound according to any of claims 1-21 to a subject in need of such treatment.

26. A method of treating metabolic syndrome comprising administering an effective amount of a compound according to any of claims 1-21 to a subject in need of such treatment.

27. A method of treating cardiovascular disease comprising administering an effective amount of a compound according to any of claims 1-21 to a subject in need of such treatment.

28. A method according to any of claims 23 to 26, wherein the subject is a human.

29. Use of a compound according to any of claims 1-21 for activating G-protein coupled receptor 119.

30. Use of a compound according to any of claims 1-21 for the manufacturing of a medicament for the treatment and/or prophylaxis of diabetes-2, obesity, insulin resistance, and cardiovascular disease.

31. A pharmaceutical composition comprising a compound according to any of claims 1 to 21 and a pharmaceutically acceptable carrier, excipient, emulsifier, surfactant and/or diluent.

32. A pharmaceutical composition comprising a solvate of a compound according to
any of claims 1 to 21, and a pharmaceutically acceptable carrier, excipient, emulsifier, surfactant and/or diluent.

33. A capsule comprising a compound according to any of claims 1-21 or a solvate of said compound, wherein the capsule is made of one or several from the group consisting of gelatine, a plant based gelling substance such as carrageenans, starch, cellulose, modified starch, and modified cellulose, such as hydroxypropyl methylcellulose, and derivatives of any of these.

34. A capsule according to claim 33, wherein the capsule is soluble in gastric juice.

35. A capsule according to claim 33, wherein the capsule is insoluble in gastric juice, preferably wherein the capsule is soluble in the duodenum and upper ileum.

36. A capsule according to any of claims 33 to 35 coated for delayed release.

37. A combination product comprising; (A) a compound according to any of claims 1 to 21, or a pharmaceutical composition according to claims 31 or 32, and (B) a monoacylglycerol-lipase-inhibitor for simultaneous, successive or separate administration.

38. A combination product according to claim 37 wherein the monoacylglycerol-lipase-inhibitor is selected from the group consisting of JZL184, CAY10499, URB602, OMDM169.

39. A combination product comprising; (A) a compound according to any of claims 1 to 21, or a pharmaceutical composition according to claims 31 or 32, and (B) a monoacylglycerol-acyltransferase-inhibitor, for simultaneous, successive or separate administration.

40. A combination product according to claim 39 wherein the monoacylglycerol-acyltransferase-inhibitor is sphingosine.

41. A combination product comprising; (A) a compound according to any of claims
1 to 21 or a pharmaceutical composition according to claims 31 or 32, and (B) another therapeutic agent that is useful in the treatment of metabolic syndrome, diabetes type 2, obesity, insulin resistance, and/or cardiovascular disease, for simultaneous, successive or separate administration.

42. A combination product according to any of the preceding claims 37 to 41, for use in the treatment of metabolic syndrome, diabetes type 2, obesity, insulin resistance, and/or cardiovascular disease.

43. A method of activating G-protein coupled receptor 119 by administering a compound according to any of claims 1-21 to a cell expressing said receptor.

44. A method of stimulating the release of GLP-1 in the gastrointestinal tract of a subject in need thereof comprising administering to said subject a therapeutically effective amount of a compound according to any of claims 1-21.

45. A method according to claim 44, wherein the stimulation of the release of GLP-1 occurs in the duodenum.

46. A method according to claim 45, wherein the stimulation of the release of GLP-1 occurs in the ileum.

47. A method of stimulating the release of GLP-1 in the pancreas of a subject in need thereof comprising administering to said subject a therapeutically effective amount of a compound according to any of claims 1-21.

48. A method of stimulating the release of GLP-1 in the brain of a subject in need thereof comprising administering to said subject a therapeutically effective amount of a compound according to any of claims 1-21.

49. A method according to any of claims 41 to 48, wherein the subject is a human.

50. A food or animal feed product comprising a compound selected from the group consisting of
[Formula XII]

wherein \( R_1 \) and \( R_2 \) are individually selected from the group consisting of \( H \), and C2-C8 acyl groups, and wherein \( R_4 \) is selected from the group consisting of a C16 or C18 acyl group.

51. A food or animal feed product according to claim 50, wherein \( R_1 \) and \( R_2 \) are linear and saturated.

52. A food or animal feed product according to claim 50, wherein one of \( R_1 \) and \( R_2 \) is \( \text{CH}_2 \cdot \text{CH}_3 \) and the other is -H.

53. A food or animal feed product according to claim 50 wherein \( R_1 \) and \( R_2 \) are \( \text{CH}_2 \cdot \text{CH}_3 \).

54. A food or animal feed product according to claim 50 wherein \( R_1 \) and/or \( R_2 \) is -H.

55. A food or animal feed product according to any of the preceding claims 50 to 54, wherein \( R_4 \) is linear.

56. A food or animal feed product according to any of the preceding claims 50 to 55, wherein \( R_4 \) is unsaturated.

57. A food or animal feed product according to any of the preceding claims 50 to 56, wherein \( R_4 \) is C16 acyl group comprising 0, 1 or 2 double bonds.

58. A food or animal feed product according to any of the preceding claims 50-56, wherein \( R_4 \) is C18 acyl group comprising 0, 1, 2, 3, or 4 double bonds.

59. A food or animal feed product according to claim 58, wherein \( R_4 \) is oleic, linoleic, alpha-linoleic, elaidic, gamma linoleic, or stearidonic, preferably oleic.
60. A food or animal feed product according to any of the preceding claims 50, wherein the compound is 2-oleoyl-glycerol.

61. A food or animal feed product according to claim 50, wherein the compound is 1-acetyl-2-oleoylglycerol.

62. A food or animal feed product according to claim 50, wherein the compound is 1,3-diacetyl-2-oleoylglycerol.

63. The food product according to any of the claims 50 to 62, in the form of bread, snacks, cookies, energy bars, diary products, such as yoghurt, smoothies, cheese, and ice cream, non-dairy products, spread, mayonnaise, butter, margarine, oil, cooking oil, frying oil, and salad oil.

64. The food or animal feed product according to any of the claims 50 to 63, wherein said compound amounts to a minimum of 10 weight% of other fats in the product, such as at least 20 weight%, for example at least 30%, such as at least 40%, for example at least 50%, such as at least 60%, for example at least 70%, such as at least 80%, for example at least 90%, such as essentially 100%.

65. A food or animal feed product according to any of the claims 50 to 64, wherein said compound is encapsulated in a capsule material which is soluble in gastric juice.

66. A food or animal feed product according to any of the claims 50 to 64, wherein said compound is encapsulated in a capsule material which is insoluble in gastric juice, preferably wherein the capsule is soluble in the duodenum and/or ileum.

67. Use of a compound according to any of claims 1-21 as a low calorie fat substitute.

68. Use of a compound according to any of claims 1-21 for preparation of a dietary supplement, a food or feed product, or a beverage product for helping to sustain
energy, helping control appetite, helping control blood sugar levels, reducing the risks associated with metabolic syndrome, reducing the risk associated with obesity and diabetes, reducing the risk associated with diabetes, helping to maintain healthy glucose and fat metabolism, or for helping to normalise production and release of GLP1 necessary for healthy glucose and fat metabolism, in a subject during and/or between meals or feedings comprising said dietary supplement, a food or feed product, or a beverage product.

69. A method for helping to sustain energy, helping control appetite, helping control blood sugar levels, reducing the risks associated with metabolic syndrome, reducing the risk associated with obesity and diabetes, reducing the risk associated with diabetes, helping to maintain healthy glucose and fat metabolism, or for helping to normalise production and release of GLP1 necessary for healthy glucose and fat metabolism, said method comprising administering to a subject a dietary supplement, a food or feed product, or a beverage product comprising a compound of any of the claims 1-21 during and/or between meals or feedings.
Exp-1

Fig. 1
Exp-2

Fig. 1, contd
Exp-3

![Graph of Oleoyl ethanolamide activation](image1)

![Graph of H-2 activation](image2)

![Graph of H-4 activation](image3)

Fig. 1, contd
Fig. 3
INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2010/050161

A CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet
According to International Patent Classification (IPC) or to both national classification and IPC

B FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC:A23D, A61K, A61P

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

SE, DK, FI, NO classes as above

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

EPO-Internal, PAJ, WPI data, BIOSIS, CHEM ABS Data, EMBASE, MEDLINE

C DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>WO 2008069004 A1 (KAO CORP ET AL), 12 June 2008 (2008-06-1 2); abstract; page 1, paragraph [0001] - page 1, paragraph [0002]; Examples 5-6, pages 21-23</td>
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</table>

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents
  "A" document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search 11-10-2010
Date of mailing of the international search report

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<td>GUILLION J., et al. &quot;First total synthesis of 1,3-diacyl- and dibutryroyl-2-oleoylglycerol, previously isolated from natural products&quot; 1999, Pharmacy and pharmacology communications, Vol. 5, No. 5, pp 311-313.; abstract; page 311, column 1, line 1 - page 311, column 1, line 7</td>
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<td>GHAFOURI, N. et al. &quot;Inhibition of monoacylglycerol lipase and fatty acid amide hydrolase by analogues of 2-arachidonoylglycerol&quot; 2004, Brit. J. Pharm., Vol. 143, pp. 774-784.; abstract; Table 1, compounds 2OG, 2LG</td>
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<td>WILSON, T. A. et al. &quot;Structured triglycerides containing caprylic (8:0) and oleic (18:1) fatty acids reduce blood cholesterol concentrations and aortic cholesterol accumulation in hamsters&quot; 2006, BBA, Vol. 1761, pp. 345-349.; abstract; page 346, column 2, line 46 - page 347, column 1, line 32</td>
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<td>X</td>
<td>US 6369252 B1 (AKOH CASIMIR C), 9 April 2002 (2002-04-09); column 8, line 51 - column 8, line 69; column 9, line 21 - column 9, line 29; claim 1</td>
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<td>SOFTLY B. J., &quot;Composition of representative SALATRIM fat preparations&quot; 1994, J. Agrc. Food Chem. Vol 42, No. 2, pp 461-467.; page 481, column 1, line 1 - page 481, column 1, line 9; Table 4.5</td>
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### International Search Report

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

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

1. **IXI** claims Nos. 23-28 and 43-49  
   because they relate to subject matter not required to be searched by this Authority, namely:

   Claims 23-28 and 43-49 relate to a method for treatment of the human or animal body by therapy, as well as diagnostic methods, see PCT rule 39.1 (iv). Nevertheless, a search has been made for these claims. The search has been directed to the technical content of the claims.

2. **☐** Claims Nos. 15, 29, 43-49  
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

   The expressions "agonist of G-protein coupled receptor 119", "activating G-protein...

3. **☐** Claims Nos.  
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III**  
**Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

The following separate inventions were identified:

1. Claims 1-36 directed to compounds for use in the treatment of metabolic syndrome, cardiovascular disease, diabetes-2, obesity or insulin resistance.

2. Claims 37-42 directed to a combination product comprising the compounds of the present application in combination with a monoacylglycerol-lipase-inhibitor or a monoacylglycerol-acyltransferase-inhibitor.

   .../...

1. **☐** As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. **☐** As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. **☐** As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. **☐** No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims, it is covered by claims Nos.

**Remark on Protest**

- **☐** The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.

- **☐** The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation.

- **☐** No protest accompanied the payment of additional search fees.
Continuation of: Box No. Ill

3: Claims 50-69 directed to a food or animal feed product comprising the compounds of the present application.

A search has been carried out, which relates to all the inventions mentioned above.

The present application has been considered to contain 3 inventions which are not linked such that they form a single general inventive concept, as required by Rule 13 PCT for the following reasons:

The single general concept of the present application is the teaching that mono-, di- and tri-acylglyceroles can be used in the treatment of metabolic syndrome, cardiovascular disease, diabetes-2, obesity or insulin resistance.

Document D1 discloses such a compound and a method for evaluating or screening an obesity controller, a blood insulin regulator, or a blood sugar regulator.

Thus, the single general concept is obvious and cannot be considered as a single general inventive concept in the sense of Rule 13.1 PCT.

No other features can be distinguished which can be considered as the same or corresponding special technical features in the sense of Rule 13.2 PCT.

Thus, the application lacks unity of invention.
Continuation of: Box No. II

coupled receptor 119" and "stimulating release of GLP-1 " in claims 15, 29, 43-49 relate to a large or undefined number of different disorders which cannot be clearly defined by these expressions. The application provides support for use of the compounds in the treatment of only a very limited number of such disorders. Claims 15, 29 and 43-49 do therefore not meet the requirements of Article 6 PCT that claims shall be clear, concise and supported by the description. Because of this, a meaningful search over the whole of the claimed scope cannot be performed. Consequently, the search has been carried out only for the diseases mentioned in claim 1.
Continuation of: second sheet

International Patent Classification (IPC)

A61K 31/23 (2006.01)
A23D 9/007 (2006.01)
A61K 31/231 (2006.01)
A61K 31/232 (2006.01)
A61P 3/04 (2006.01)
A61P 3/10 (2006.01)

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Use the application number as username. The password is IMXAXUKQZR.

Paper copies can be ordered at a cost of 50 SEK per copy from PRV InterPat (telephone number 08-782 28 85).

Cited literature, if any, will be enclosed in paper form.
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