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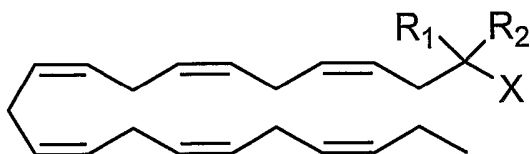
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(54) Title: FATTY ACID ANALOGUES, I.E. DHA DERIVATIVES FOR USES AS A MEDICAMENT



(I)

(57) Abstract: A compound of formula
(I), wherein one of R₁ and R₂ is a methyl
group and the other of R₁ and R₂ is a
hydrogen atom; wherein X represents
a carboxylic acid group, a carboxylate
group, or a carboxamide group; or
any pharmaceutically acceptable salt,
solvate, complex or pro-drug thereof,
for use as a medicament, is disclosed. A

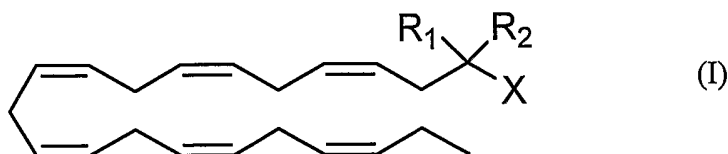
pharmaceutical composition and a fatty acid composition comprising a compound of formula (I) is also disclosed. The use of a
compound of formula (I) for the manufacture of a medicament, in particular for the treatment of diabetes type 2, is also disclosed.

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Fatty acid analogues, i.e. DHA derivatives for use as a medicament

Technical field

The present invention relates to compounds of the general formula (I):



5 for use as a medicament, in particular for the treatment of diabetes mellitus, type 2, and pre-stages thereof. It also relates to a pharmaceutical composition comprising a compound of formula (I), as well as to a fatty acid composition comprising a compounds of formula (I).

10 Background of the invention

The increasing incidence of type 2 diabetes mellitus worldwide poses an immense public health and medical challenge for the implementation of successful preventive and treatment strategies. The concurrent rise in overweight and obesity, which is tightly correlated to type 2 diabetes, interferes with diabetes treatment and
15 increases the likelihood of hypertension, dyslipidemia, and atherosclerosis related diseases.

The pathophysiologic condition precluding the development of type 2 diabetes is related to reduced effects of insulin on peripheral tissues, called insulin resistance. These tissues are mainly muscle, fat and liver. Muscle tissue is the main tissue
20 concerned by insulin resistance in type 2 diabetes. The syndrome characterised by insulin resistance, hypertension, dyslipidemia and a systemic proinflammatory state, is referred to as metabolic syndrome. The prevalence of metabolic syndrome in the adult population in developed countries is 22-39% (Meighs 2003)

Currently the most promising approach to mitigate and deter the metabolic
25 syndrome is lifestyle intervention with weight reduction, decreased consumption of saturated fat, increased physical activity in combination with appropriate pharmacotherapy. Healthy diets that avoid excess energy intake encompass substitution of mono and polyunsaturated fatty acids in exchange for saturated fat. In particular the long-chain omega-3 fatty acids from fatty fish, namely

eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have proven beneficial in prevention of type 2 diabetes.

EPA and DHA have effects on diverse physiological processes impacting normal health and chronic disease, such as the regulation of plasma lipid levels, cardiovascular and immune function, insulin action and neural development and visual function. Firm evidence exist for their beneficial role in the prevention and management of coronary heart disease, dyslipidemias, type 2 diabetes, insulin resistance, and hypertension (Simonopoulos 1999; Geleijnse 2002; Storlien 1998).

Recent studies suggest that omega-3 fatty acids serve as important mediators of gene expression, working via nuclear receptors like the peroxisome proliferator-activated receptors (PPARs) controlling the expression of the genes involved in the lipid and glucose metabolism and adipogenesis (Jump 2002). PPARs are nuclear fatty acid receptors that have been implicated to play an important role in obesity-related metabolic diseases such as hyperlipidemia, insulin resistance, and coronary heart disease.

The three subtypes, α , γ , and δ , have distinct expression pattern and evolved to sense components of different lipoproteins and regulate lipid homeostasis based on the need of a specific tissue. PPAR α potentiates fatty acid catabolism in the liver and is the molecular target of the lipid-lowering fibrates. PPAR γ on the other hand is essential for adipocyte differentiation and mediates the activity of the insulin-sensitizing thiazolidinediones (the glitazones) through mechanisms not fully understood. (Chih-Hao 2003; Yki-Järvinen 2004)

Recently, pharmaceuticals acting as ligands to the PPAR γ receptor have been introduced as treatment of type 2 diabetes (Yki-Järvinen 2004). These compounds called thiazolidinediones or glitazones are drugs that reverse insulin resistance which is the pathophysiologic basis for development of the metabolic syndrome and type 2 diabetes. These compounds, of which rosiglitazone and pioglitazone have been launched as pharmaceuticals, lower fasting and postprandial glucose concentrations (which is being manifest as a pathologic glucose tolerance test), plasma insulin as well as free fatty acid concentrations. In this respect the glitazones act as insulin sensitizers.

However, these improvements are generally accompanied by weight gain and an increase in the subcutaneous adipose-tissue mass (Adams 1997). The use of thiazolidinediones is not only associated with weight gain but a subgroup of patients also have fluid retention and plasma volume expansion, leading to peripheral oedema. The increase in body weight and oedema has been associated with an increase in the incidence of heart failure, which is the reason why the Food and Drug Administration

has included a warning in the prescription information for rosiglitazone (provided by Avandia) and pioglitazone (provided by Takeda). These adverse effects restrict the use of the glitazones especially in patients with coronary heart conditions. Clearly there is a potential for new drugs with positive effects on insulin resistance but with weight reduction activity and no fluid retention tendency.

The effect of the poly-unsaturated fatty acids (PUFAs) on PPARs are not only a result of fatty acid structure and affinity to the receptor. Factors contributing to the composition of the intracellular non-esterified fatty acids (NEFA) levels are also important. This NEFA pool is affected by the concentration of exogenous fatty acids entering the cell and the amount of endogenous synthesised fatty acids, their removal via incorporation into lipids as well as their oxidation pathways. (Pawar 2003)

Although omega-3 fatty acids are weak agonists of PPARs, when compared with pharmacological agonists like the thio-glitazones, these fatty acids have demonstrated improvement in glucose uptake and insulin sensitivity (Storlien 1987). It has been reported that adipocytes were more insulin sensitive and transported more glucose when the polyunsaturated to saturated fatty acid ratio in the diet was increased (Field 1990). Collectively, these data indicate that the 20- and 22-carbon fatty acids, namely EPA and DHA could play a preventive role in the development of insulin resistance.

Due to their limited stability in vivo and their lack of biological specificity, PUFAs have not achieved widespread use as therapeutic agents. Chemical modifications of the n-3 polyunsaturated fatty acids have been performed by several research groups in order to change or increase their metabolic effects.

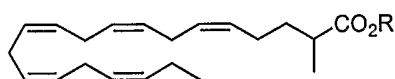
For example, the hypolipidemic effects of EPA was potentiated by introducing methyl or ethyl in α - or β -position of EPA. (Vaagenes 1999). The compounds also reduced plasma free fatty acid while EPA EE had no effect.

In a recent work published by L. Larsen (Larsen 2005) the authors show that the α -methyl derivatives of EPA and DHA increased the activation of the nuclear receptor PPAR α and thereby the expression of L-FABP compared to EPA/DHA. EPA with an ethyl group in the α -position activated PPAR α with equal strength as α -methyl EPA. The authors suggest that delayed catabolism of these α -methyl FA may contribute to their increased effects due to decreased β -oxidation in mitochondria leading to peroxisomal oxidation.

Alpha-methyl EPA has been shown to be a stronger inhibitor of platelet aggregation than EPA, both in vitro (Larsen 1998) and in vivo (Willumsen 1998).

Patent Abstract of Japan, publication number 05-00974 discloses DHA substituted in alpha-position with an OH-group, however only as an intermediate. No examination as to possible pharmaceutical effects of this compound is disclosed.

Laxdale Limited has also described the use of alpha substituted derivatives of EPA in the treatment of psychiatric or central nervous disorders (US6689812).



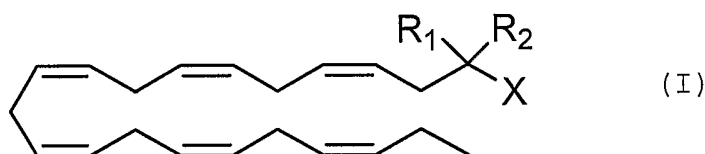
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R= H, CH₃, CH₂CH₃(A) α -methyl EPA

The vast research in the field of substituted fatty acids demonstrates the great interest in finding appropriate medical and pharmaceutical applications. However, so far the practical applications have been very limited, and there is thus a continuing need for finding useful application areas for fatty acid derivatives.

Summary of the invention

One aim of the present invention is to provide a useful medical application of DHA-derivatives. Accordingly, the present invention provides a compound of formula (I);



(I)

- 20 - wherein one of R₁ and R₂ is a methyl group and the other of R₁ and R₂ is a hydrogen atom;
- wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;
- or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof, for use as a medicament. The alpha-substituted DHA-derivative according to the invention has very surprisingly shown excellent results with regard to pharmaceutical activity. In particular, the fatty acid derivative according to the present invention possess a

huge potential to be used in the treatment and/or prevention of diabetes and pre-stages thereof.

The carboxylate group may be selected from the group consisting of ethyl carboxylate, methyl carboxylate, n-propyl carboxylate, isopropyl carboxylate, n-butyl carboxylate, sec.-butyl carboxylate, and n-hexyl carboxylate. Preferably, the carboxylate group is ethyl carboxylate.

The carboxamide group may be selected from the group consisting of primary carboxamide, N-methyl carboxamide, N,N-dimethyl carboxamide, N-ethyl carboxamide, and N,N-diethyl carboxamide.

The compounds of formula (I) are capable of existing in stereoisomeric forms. It will be understood that the invention encompasses all optical isomers of the compounds of formula (I) and mixtures thereof including racemates for use as a medicament.

The compound of formula (I) may also exist in the form of a phospholipid, a tri-, di- or monoglyceride, or in the form of a free acid.

Another aspect of the present invention relates to a pharmaceutical composition comprising a compound of formula (I) as an active ingredient. The pharmaceutical composition may further comprise a pharmaceutically acceptable carrier. Suitably, a pharmaceutical composition according to the invention is formulated for oral administration, e.g. in the form of a capsule or a sachet. A suitable daily dosage of a compound of formula (I) according to the present invention is 10 mg to 10 g, in particular 100 mg to 1 g of said compound per 24 hours.

In addition, the present invention relates to a fatty acid composition comprising a compound of formula (I). At least 60%, or at least 90% by weight of the fatty acid composition may be comprised of said compound. The fatty acid composition may further comprise (all-Z)-5,8,11,14,17-eicosapentaenoic acid (EPA), (all-Z)-4,7,10,13,16,19-docosahexaenoic acid (DHA), (all-Z)-6,9,12,15,18-heneicosapentaenoic acid (HPA), and/or (all-Z)-7,10,13,16,19-docosapentaenoic acid (DPA). The fatty acids may be present in the form of derivatives. A fatty acid composition according to the present invention may further comprise a pharmaceutically acceptable antioxidant, e.g. tocopherol. Within the scope of the present invention is also a fatty acid composition described above, for use as a medicament.

In a further aspect, the present invention relates to the use of a compound according to formula (I) for the manufacture of a medicament for controlling body weight reduction and/or for preventing body weight gain; for the manufacture of a medicament for the treatment and/or the prevention of obesity or an overweight

condition; for the manufacture of a medicament for the prevention and/or treatment of diabetes in an animal, in particular type 2 diabetes; for the manufacture of a medicament for the treatment and/or prevention of amyloidosis-related diseases; for the manufacture of a medicament for the treatment or prophylaxis of multiple risk factors for cardiovascular diseases, preferably for the treatment of elevated blood lipids for the manufacture of a medicament for prevention of stroke, cerebral or transient ischaemic attacks related to atherosclerosis of several arteries.

In addition, the present invention relates to a method for controlling body weight reduction and/or for preventing body weight gain; a method for the treatment and/or the prevention of obesity or an overweight condition; a method for the prevention and/or treatment of diabetes, in particular type 2 diabetes; a method for the treatment and/or prevention of amyloidosis-related diseases; a method for the treatment or prophylaxis of multiple risk factors for cardiovascular diseases; a method for the prevention of stroke, cerebral or transient ischaemic attacks related to atherosclerosis of several arteries, wherein a pharmaceutically effective amount of a compound of formula (I) is administered to a human or an animal. Suitably, the compound of formula (I) is administered orally to a human or an animal.

Brief description of the drawings

Fig. 1 shows the structural formula of alpha-methyl DHA ethyl ester.

Fig. 2 is a schematic overview of the free fatty acid pool theory.

Fig. 3 depicts the release of luciferase from transfected cells treated with the compound according to the invention.

Detailed description of the invention

In the research work leading to the present invention, it was found that the alpha-methyl-DHA shows excellent pharmaceutical activity.

Fatty acids enter cells passively or through G-protein coupled transporter systems, such as fatty acid transport proteins. Well inside the cells they are temporarily bound by binding proteins (Fatty acid binding proteins, FABP), which play an important role in directing fatty acids to various intracellular compartments for metabolism and gene expression (Pawar & Jump 2003). (Fig. 2 liver cell).

Esterification of fatty acids into triglycerides, polar lipids, and cholesterol esters and their beta-oxidation (mitochondrial and peroxisomal) requires conversion of fatty acids to acyl CoA thioesters. Other pathways, like microsomal NADPH-dependent mono-oxidation and eicosanoids synthesis, utilise non-esterified fatty acids as substrates. All these reactions are likely to influence cellular levels of free fatty

acids (non-esterified) and thereby the amount and type of fatty acids which could be used as ligands to nuclear receptors. Because PPARs are known to bind non-esterified fatty acids it is reasonable to expect that the composition of the free fatty acid pool is an important determinant in the control of PPAR activity.

5 The composition of the free fatty acid pool is affected by the concentration of exogenous fatty acids entering the cells, and their rate of removal via pathways listed above. Since short and medium chain fatty acids are effectively recruited to these pathways, in practice only the long-chain polyunsaturated fatty acids will be available for liganding to nuclear receptors. In addition, fatty acid structure may also be an
10 important determinant. Even if a series of mono and polyunsaturated fatty acids demonstrated affinity to the PPAR α receptor, EPA and DHA demonstrated the highest binding capacity in experiments with rat liver cells (Pawar & Jump 2003).

 Searching for fatty acid candidates available for genetic modification of proteins by interaction with nuclear receptors like the PPARs, it is important to verify
15 that the respective fatty acids will be enriched in the free fatty acid pool.

 DHA which enter cells are rapidly converted to fatty acyl-CoA thioesters and incorporated into phospholipids and due to this, the intracellular DHA level is relatively low. These DHA-CoA are also substrate for β -oxidation primarily in the peroxisomes that lead to retroconversion of DHA to EPA, see Fig. 2. Because of the
20 rapid incorporation into neutral lipids and the oxidation pathway DHA will not stay long in the free fatty acid pool. Due to this the effect of DHA on gene expression is probably limited.

 The present invention aims at achieving an accumulation of fatty acid derivatives in the free fatty acid pool, rather than incorporation into phospholipids. The
25 present inventors have surprisingly found that the introduction of a methyl substituent in the α -position of DHA will lead to a slower oxidation rate in addition to less incorporation into neutral lipids. This will lead to an increased effect on gene expression, since the DHA derivative will accumulate in the tissue particular within liver, muscle, and adipose cells and trigger local nuclear receptor activity to a greater
30 extent than DHA.

 EPA (all-Z)-5,8,11,14,17-eicosapentaenoic acid) has earlier been alkylated in α - and β -position to inhibit mitochondrial β -oxidation. DHA is not oxidised in the mitochondria, but rather incorporated into phospholipids. In the peroxisomes though some DHA is retroconverted to EPA. A substituent in the α -position of EPA and
35 DHA will due to this affect different metabolic pathways. It has earlier been shown that α -methyl EPA and β -methyl EPA is incorporated into phospholipids and triglycerids while α -ethyl EPA is not (Larsen 1998). In this study the derivatives were

tested as substrates and/or inhibitors of enzymes involved in the eicosanoid cascade. Since most of the substrates for these enzymes are fatty acids liberated from phospholipids it was desired that the derivatives were incorporated into phospholipids. In contrast to this, as mentioned before, this invention aims at
5 providing a derivative that will not incorporate into lipids, but rather accumulate in the NEFA pool.

It is to be understood that the present invention encompasses any possible pharmaceutically acceptable salts, solvates, complexes or prodrugs of the compounds of formula (I).

10 "Prodrugs" are entities which may or may not possess pharmacological activity as such, but may be administered (such as orally or parenterally) and thereafter subjected to bioactivation (for example metabolized) in the body to form the agent of the present invention which is pharmacologically active.

Where X is a carboxylic acid, the present invention also includes salts of the
15 carboxylic acids. Suitable pharmaceutically acceptable salts of carboxy groups includes metal salts, such as for example aluminium, alkali metal salts such as lithium, sodium or potassium, alkaline metal salts such as calcium or magnesium and ammonium or substituted ammonium salts.

A "therapeutically effective amount" refers to the amount of the therapeutic
20 agent which is effective to achieve its intended purpose. While individual patient needs may vary, determination of optimal ranges for effective amounts of each nitric oxide adduct is within the skill of the art. Generally the dosage regimen for treating a condition with the compounds and/or compositions of this invention is selected in accordance with a variety of factors, including the type, age, weight, sex, diet and
25 medical condition of the patient.

By "a medicament" is meant a compound according to formula (I), in any form suitable to be used for a medical purpose, e.g. in the form of a medicinal product, a pharmaceutical preparation or product, a dietary product, a food stuff or a
30 food supplement.

In the context of the present specification, the term "therapy" also includes "prophylaxis" unless there are specific indications to the contrary. The terms "therapeutic" and "therapeutically" should be constructed accordingly.

Treatment includes any therapeutic application that can benefit a human or non-human animal. The treatment of mammals is particularly preferred. Both human
35 and veterinary treatments are within the scope of the present invention. Treatment may be in respect of an existing condition or it may be prophylactic. It may be of an adult, a juvenile, an infant, a foetus, or a part of any of the aforesaid (e.g. an organ,

tissue, cell, or nucleic acid molecule). By "chronic treatment" is meant treatment that continues for some weeks or years.

"A therapeutically or a pharmaceutically active amount" relates to an amount that will lead to the desired pharmacological and/or therapeutic effects.

5 A compound according to the present invention may for example be included in a food stuff, a food supplement, a nutritional supplement, or a dietary product

Alpha-substituted DHA derivatives and EPA (or DHA for that matter) can be bound together and combined on triglyceride form by an esterification process between a mixture of alpha-derivatives, EPA and glycerol catalysed by Novozym 435 (a commercially available lipase from *Candida antarctica* on immobilised form).

10 The compound of formula (I) has activity as pharmaceuticals, in particular as triggers of nuclear receptor activity. Thus, the present invention also relates to the compound of formula (I), pharmaceutically acceptable salts, solvates, complexes or pro-drugs thereof, as hereinbefore defined, for use as a medicament and/or for use in therapy. Preferably, the compound of formula (I), or pharmaceutically acceptable salts, solvates, complexes or pro-drugs thereof, of the invention may be used:

- for the prevention and/or treatment of diabetes mellitus in humans or animals;
- for controlling body weight reduction and/or for preventing body weight gain;
- for the prevention and/or treatment of obesity or an overweight condition in humans or in an animal;
- for the treatment and/or prevention of amyloidosis-related diseases;
- for the treatment or prophylaxis of multiple risk factors for cardiovascular diseases;
- for the prevention of stroke, cerebral or transient ischaemic attacks related to atherosclerosis of several arteries.
- for the treatment of TBC or HIV.

20 There are two major forms of diabetes mellitus. One is type 1 diabetes, which is known as insulin-dependent diabetes mellitus (IDDM), and the other one is type 2 diabetes, which is also known as non-insulin-dependent diabetes mellitus (NIDDM).
30 Type 2 diabetes is related to obesity/overweight and lack of exercise, often of gradual onset, usually in adults, and caused by reduced insulin sensitivity, so called peripheral insulin resistance. This leads to a compensatory increase in insulin production. This stage before developing full fetched type 2 diabetes is called the metabolic syndrome and characterized by hyperinsulinemia, insulin resistance, obesity, glucose
35 intolerance, hypertension, abnormal blood lipids, hypercoagulopathy, dyslipidemia and inflammation, often leading to atherosclerosis of the arteries. Later when insulin production ceases, type 2 diabetes mellitus develops.

In a preferred embodiment, the compound according to formula (I) may be used for the treatment of type 2 diabetes. The compound according to formula (I) may also be used for the treatment of other types of diabetes selected from the group consisting of metabolic syndrome, secondary diabetes, such as pancreatic, 5
extrapancreatic/endocrine or drug-induced diabetes, or exceptional forms of diabetes, such as lipotrophic, myotonic or a disease caused by disturbance of the insulin receptors. The invention also includes treatment of type 2 diabetes. Suitably, the compound of formula (I), as hereinbefore defined, may activate nuclear receptors, preferably PPAR (peroxisome proliferator-activated receptor) α and/or γ .

10 The compound of formula (I) may also be used for the treatment and/or prevention of obesity. Obesity is usually linked to an increased insulin resistance and obese people run a high risk of developing type 2 diabetes which is a major risk factor for development of cardiovascular diseases. Obesity is a chronic disease that afflict an increasing proportion of the population in Western societies and is associated, not 15
only with a social stigma, but also with decreasing life span and numerous problems, for instance diabetes mellitus, insulin resistance and hypertension. The present invention thus fulfils a long-felt need for a drug that will reduce total body weight, or the amount of adipose tissue, of preferably obese humans, towards their ideal body weight without significant adverse side effects.

20 The compound according to formula (I) may also be used for the prevention and/or treatment of amyloidosis-related diseases. Amyloidosis-related conditions or diseases associated with deposition of amyloid, preferably as a consequence of fibril or plaque formation, includes Alzheimer's disease or dementia, Parkinson's disease, amyotrophic lateral sclerosis, the spongiform encephalopathies, such as Creutzfeld- 25
jacob disease, cystic fibrosis, primary or secondary renal amyloidoses, IgA nephropathy, and amyloid deposition in arteries, myocardium and neural tissue. These diseases can be sporadic, inherited or even related to infections such as TBC or HIV, and are often manifested only late in life even if inherited forms may appear much earlier. Each disease is associated with a particular protein or aggregates of 30
these proteins are thought to be the direct origin of the pathological conditions associated with the disease. The treatment of a amyloidosis-related disease can be made either acutely or chronically.

The compound of formula (I) may also be used for the treatment due to reduction of amyloid aggregates, prevention of misfolding of proteins that may lead 35
to formation of so called fibrils or plaque, treatment due to decreasing of the production of precursor protein such as A β -protein (amyloid beta protein), and prevention and/or treatment due to inhibiting or slow down the formation of protein

fibrils, aggregates, or plaque. Prevention of fibril accumulation, or formation, by administering a compound of formula (I), as hereinbefore defined, is also included herein. In one embodiment, the compound of formula (I), pharmaceutically acceptable salts, solvates, complexes or pro-drugs thereof, as hereinbefore defined, are used for the treatment of TBC (tuberculosis) or HIV (human immunodeficiency virus).

Further, the compound of formula (I) may be administered to patients with symptoms of atherosclerosis of arteries supplying the brain, for instance a stroke or transient ischaemic attack, in order to reduce the risk of a further, possible fatal, attack.

The compound of formula (I) may also be used for the treatment of elevated blood lipids in humans.

Additionally, the compound of formula (I), as hereinbefore defined, are valuable for the treatment and prophylaxis of multiple risk factors known for cardiovascular diseases, such as hypertension, hypertriglyceridemia and high coagulation factor VII phospholipid complex activity. Preferably, the compound of formula (I) is used for the treatment of elevated blood lipids in humans.

The compound of formula (I) and pharmaceutically acceptable salts, solvates, pro-drugs or complexes thereof may be used on their own but will generally be administered in the form of a pharmaceutical composition in which the compound of formula (I) (the active ingredient) are in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

The present invention thus also provides a pharmaceutical composition comprising a therapeutically effective amount of the compound of formula (I) of the present invention and a pharmaceutically acceptable carrier, diluent or excipients (including combinations thereof).

This is a composition that comprises or consists of a therapeutically effective amount of a pharmaceutically active agent. It preferably includes a pharmaceutically acceptable carrier, diluent or excipients (including combinations thereof). Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as - or in addition to - the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s).

Pharmaceutical compositions within the scope of the present invention may include one or more of the following: preserving agents, solubilising agents, stabilising agents, s wetting agents, emulsifiers, sweeteners, colourants, flavouring agents, odourants, salts compounds of the present invention may themselves be provided in the form of a pharmaceutically acceptable salt), buffers, coating agents, 5 antioxidants, suspending agents, adjuvants, excipients and diluents.

A pharmaceutical composition according to the invention is preferably formulated for oral administration to a human or an animal. The pharmaceutical composition may also be formulated for administration through any other route where 10 the active ingredients may be efficiently absorbed and utilized, e.g. intravenously, subcutaneously, intramuscularly, intranasally, rectally, vaginally or topically.

In a specific embodiment of the invention, the pharmaceutical composition is shaped in form of a capsule, which could also be microcapsules generating a powder or a sachet. The capsule may be flavoured. This embodiment also includes a capsule 15 wherein both the capsule and the encapsulated fatty acid composition according to the invention is flavoured. By flavouring the capsule it becomes more attractive to the user. For the above-mentioned therapeutic uses the dosage administered will, of course, vary with the compound employed, the mode of administration, the treatment desired and the disorder indicated.

20 The pharmaceutical composition may be formulated to provide a daily dosage of 10 mg to 10 g. Preferably, the pharmaceutical composition is formulated to provide a daily dosage between 50 mg and 5 g of said composition. Most preferably, the pharmaceutical composition is formulated to provide a daily dosage between 100 mg and 1 g of said composition. By a daily dosage is meant the dosage per 24 hours.

25 The dosage administered will, of course, vary with the compound employed, the mode of administration, the treatment desired and the disorder indicated. Typically, a physician will determine the actual dosage which will be most suitable for an individual subject. The specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including 30 the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the individual undergoing therapy. The agent and/or the pharmaceutical composition of the present invention may be administered in 35 accordance with a regimen of from 1 to 10 times per day, such as once or twice per day. For oral and parenteral administration to human patients, the daily dosage level of the agent may be in single or divided doses.

A further aspect of the present invention relates to a fatty acid composition comprising a compound of formula (I). A fatty acid composition comprising a compound of formula (I) increases the natural biological effects of DHA that are a result of regulation of gene expression, and the derivatives according to the present invention will accumulate in the free fatty acid pool.

The fatty acid composition may comprise in the range of 60 to 100 % by weight of the compound of formula (I), all percentages by weight being based on the total weight of the fatty acid composition. In a preferred embodiment of the invention, at least 80% by weight of the fatty acid composition is comprised of a compound of formula (I). More preferably, the compound of formula (1) constitute at least 90% by weight of the fatty acid composition. Most preferably, the compound of formula (I) constitutes more than 95% by weight of the fatty acid composition. The fatty acid composition may further comprise at least one of the fatty acids (all-Z)-5,8,11,14,17-eicosapentaenoic acid (EPA), (all-Z)-4,7,10,13,16,19-docosahexaenoic acid (DHA), (all-Z)-6,9,12,15,18-heneicosapentaenoic acid (HPA), and (all-Z)-7,10,13,16,19-docosapentaenoic acid (DPAn-3), (all-Z)-8,11,14,17-eicosatetraenoic acid (ETAn-3), or combinations thereof. Further, the fatty acid composition may comprise (all-Z)-4,7,10,13,16-Docosapentaenoic acid (DPAn-6) and/or (all-Z)-5,8,11,14-eicosatetraenoic acid (ARA), or derivatives thereof. The fatty acid composition may also comprise at least these fatty acids, or combinations thereof, in the form of derivatives. The derivatives are suitably substituted in the same way as the DHA derivative of formula (I), as hereinbefore defined.

The fatty acid composition according to the invention may comprise (all-Z)-omega-3)-6,9,12,15,18-heneicosapentaenoic acid (HPA), or derivatives thereof, in an amount of at least 1% by weight, or in an amount of 1 to 4% by weight.

Further, the fatty acid composition according to the invention may comprise omega-3 fatty acids other than EPA and DHA that have 20, 21, or 22 carbon atoms, or derivatives thereof, in an amount of at least 1.5% by weight, or in an amount of at least 3% by weight.

In specific embodiments of the invention, the fatty acid composition is a pharmaceutical composition, a nutritional composition or a dietary composition.

The fatty acid composition may further comprise an effective amount of a pharmaceutically acceptable antioxidant. Preferably, the antioxidant is tocopherol or a mixture of tocopherols. In a preferred embodiment the fatty acid composition further comprises tocopherol, or a mixture of tocopherols, in an amount of up to 4 mg per g of the total weight of the fatty acid composition. Preferably, the fatty acid

composition comprises an amount of 0.2 to 0.4 mg per g of tocopherols, based on the total weight of the composition.

Another aspect of the invention provides a fatty acid composition, or any pharmaceutically acceptable salt, solvate, pro-drug or complex thereof, comprising a compound of formula (I), as hereinbefore defined, for use as a medicament and/or in therapy. Such a fatty acid composition may be used to prevent and/or treat the same conditions as outlined for the compound of formula (I) above.

When the fatty acid composition is used as a medicament, it will be administered in a therapeutically or a pharmaceutically active amount.

In a preferred embodiment, the fatty acid composition is administered orally to a human or an animal.

The present invention also provides the use of a compound of formula (I), or a pharmaceutically acceptable salt, solvate, pro-drug or complex thereof, as hereinbefore defined, for the manufacture of a medicament for controlling body weight reduction and/or for preventing body weight gain; for the manufacture of a medicament for the treatment and/or the prevention of obesity or an overweight condition; for the manufacture of a medicament for the prevention and/or treatment of diabetes in a human or animal; for the manufacture of a medicament for the treatment and/or prevention of amyloidosis-related diseases; for the manufacture of a medicament for the treatment and prophylaxis of multiple risk factors known for cardiovascular diseases, such as hypertension, hypertriglyceridemia and high coagulation factor VII phospholipid complex activity; for the manufacture of a medicament for the treatment of TBC or HIV; for the manufacture of a medicament for prevention of stroke, cerebral or transient ischaemic attacks related to atherosclerosis of several arteries; for the manufacturing of a medicament for lowering triglycerides in the blood of mammals and/or elevating the HDL cholesterol levels in the serum of a human patients; or for the manufacturing of a medicament for the treatment and/or prevention of the multi metabolic syndrome termed "metabolic syndrome". All these embodiments also include the use of a fatty acid composition, as hereinbefore defined, comprising a compound of formula (I) for the manufacture of medicaments as outlined above.

The present invention also relates to a method for controlling body weight reduction and for preventing body weight gain, wherein a fatty acid composition comprising at least a compound of formula (I), as hereinbefore defined, is administered to a human or an animal.

Further, the invention relates to a method for the treatment and/or the prevention of obesity or an overweight condition, wherein a fatty acid composition

comprising at least a compound of formula (I), as hereinbefore defined, is administered to a human or an animal.

In a preferred embodiment of the invention, the present invention relates to a method for the prevention and/or treatment of diabetes mellitus, wherein a fatty acid composition comprising at least a compound of formula (I), as hereinbefore defined, is administered to a human or an animal. Preferably, diabetes mellitus is a type 2 diabetes.

Other aspects of the present invention relate to;

- a method for the treatment and/or prevention of amyloidosis-related diseases;
- 10 - a method for the treatment or prophylaxis of multiple risk factors for cardiovascular diseases;
- a method for prevention of stroke, cerebral or transient ischaemic attacks related to atherosclerosis of several arteries;

wherein a fatty acid composition comprising at least a compound of formula (I), as hereinbefore defined, is administered to a human or an animal.

The fatty acid derivative of formula (I) may be prepared most effectively from DHA. If the start material is not pure DHA (i.e. not 100% DHA) the final fatty acid composition will contain a mixture of DHA derivatives, as hereinbefore defined, and an amount of other fatty acids than DHA, wherein these fatty acids are substituted in the same way as the novel fatty acid analogue of formula (I). Such embodiments are also included herein.

In another embodiment of the invention, the compound of formula (I) is prepared from (all-Z)-4,7,10,13,16,19-docosahexaenoic acid (DHA), wherein said DHA is obtained from a vegetable, a microbial and/or an animal source, or combinations thereof. Preferably, said DHA is obtained from a marine oil, such as a fish oil.

The fatty acids in the composition may also be obtained from a vegetable, a microbial or an animal source, or combinations thereof. Thus, the invention also includes a fatty acid composition prepared from a microbial oil.

DHA is produced from biological sources like marine, microbial or vegetable fats. All possible raw materials are mixtures of fatty acids on triglyceride form where DHA constitutes only a fraction of the fatty acids. Typical DHA concentrations are 40% in microbial fats and 10-25% in marine fats. DHA-containing vegetable fats are during development and fats with high DHA concentrations are expected in the future.

The first process step will always be conversion of the triglycerides to free fatty acids or monoesters. Preferable esters are methyl or ethyl esters, but other esters

are possible. In this way the fatty acids bound together three by three on triglycerides are separated from each other and thereby making separation possible. Several methods of separating DHA from other fatty acids are available, the most common ones being short path distillation separating the fatty acids by volatility, and urea precipitation separating the fatty acids by degree of unsaturation. Other methods reported are silver nitrate complexation also separating the fatty acids on degree on combination with short path distillation and countercurrent extraction with supercritical carbon dioxide.

10 The most important challenges connected to production of pure DHA is to separate it from the other C20-22 highly unsaturated fatty acids present in all available sources. These fatty acids have properties so similar to DHA that none of the methods mentioned above provide sufficient degree of separation. For some microbial high DHA fats, which have very low levels of C20-22 highly unsaturated fatty acids, short path distillation alone or in combination of other methods mentioned may provide more than 90% purity.

15 Most DHA containing fats also contain considerable amounts of C20-22 highly unsaturated fatty acids, e.g. EPA (20:5n-3), n-3DPA (22:5n-3), HPA (21:5n-3) and others. The only available method for separating DHA from such fatty acids is preparative High Performance Liquid Chromatography, the stationary phase being silica gel or silver nitrate impregnated silica gel, the mobile phase being selected organic solvents or supercritical carbon dioxide. With this method DHA with more than 97% purity is available. However, it has to be noted that the production costs increases strongly with concentration, as an example is production cost for 97% DHA more 5 times higher than for 90% DHA.

20 DHA having a purity of 90, 95 eller 97% contains small amounts of other fatty acids. As an example, DHA having a purity of 97% contains n-3DPA (22:5n-3), but also long chain fatty acids, e.g. EPA (20:5n-3), HPA (21:5n-3), and others. However, the other fatty acids will react in a way similar to DHA and provide alpha-substituted derivatives.

25 Organic synthesis may provide a purification method since DHA and n-6DPA (and 22:5n-6 which normally is present in very low concentrations) are the only known fatty acids that can provide gamma-lactones by cyclisation with the first double bond. Lactonisation followed by purification and hydrolysis back to DHA may be a possibility, but it is expected that this pathway is even more expensive than HPLC.

35

Examples

The invention will now be described in more detail by the following example, which is not to be constructed as limiting the invention.

5 Synthesis protocol

Preparation of α -methyl DHA EE (PRB-1)

Butyllithium (228 ml, 0.37 mol, 1.6 M in hexane) was added dropwise to a stirred solution of diisopropylamine (59.5 ml, 0.42 mol) in dry THF (800 ml) under
10 N₂ at 0 °C. The resulting solution was stirred at 0 °C for 30 min., cooled to -78 °C and stirred an additional 30 min. before dropwise addition of DHA EE (100 g, 0.28 mol) in dry THF (500 ml) during 2 h. The dark-green solution was stirred at -78 °C for 30 min. before MeI (28 ml, 0.45 mol) was added. The solution was allowed to reach -20 °C during 1.5 h, then poured into water (1.5 l) and extracted with heptane (2 x 800
15 ml). The combined organic phases were washed with 1 M HCl (1 l), dried (Na₂SO₄), filtered and evaporated *in vacuo*. The product was purified by dry flash chromatography on silica gel eluting with heptane/EtOAc (99:1) to give 50 g (48%) of the titled compound as a slightly yellow oil;

¹H-NMR (200MHz, CDCl₃) δ 1.02 (t, *J* 7.5 Hz, 3H), 1.20 (d, *J* 6.8 Hz, 3H),
20 1.29 (t, *J* 7.1 Hz, 3H), 2.0-2.6 (m, 5H), 2.8-3.0 (m, 10H), 4.17 (t, *J* 7.1 Hz, 2H), 5.3-5.5 (m, 12H);

MS (electrospray); 393 [M+Na].

The enantiomeric pure compounds can be prepared by resolving a racemic
25 compound of formula (I), as hereinbefore defined. The resolution of a compound of formula (I) may be carried out using known resolution procedures, for example by reacting the compound of formula (I) with an enantiomerically pure auxiliary to provide a mixture of diastereomers that can be separated by chromatography. Thereafter the two enantiomers of compound (I) may be regenerated from the
30 separated diastereomers by conventional means, such as hydrolysis.

There is also a possibility to use stoichiometric chiral auxiliaries to effect an asymmetric introduction of the substituent, as hereinbefore defined, in the α -position of DHA. The use of chiral oxazolidin-2-ones has proved to be a particularly effective methodology. The enolates derived from chiral N-acyloxazolidines can be quenched
35 with a variety of electrophiles in a highly stereoregulated manner (Ager, Prakash, Schaad, Chem. Rev. 1996, 96, 835).

Experiments

A number of experiments has been performed in order to demonstrate that the compound according to the invention is effective in particular for the treatment and/or prevention of diabetes mellitus.

5 In the following experiments, alpha-methyl-DHA EE is denoted "PRB-1".

Example 1. Analysis of intracellular free fatty acids (non-esterified fatty acids) in liver cells

10 **Background**

Liver tissue from animals fed PRB-1 was analysed with respect to free unesterified fatty acids. The animals were recruited from Experiment 4 (pharmacodynamic effects of DHA derivatives in an animal model of metabolic syndrome). The animals had been given DHA (15% of fat content of the diet) or the
15 DHA-derivative (1,5% of the fat content in their diet) for 8 weeks and were supposed to be in a steady-state situation with stable levels of DHA and the DHA-derivative intracellularly. Liver tissue was chosen due to the fact that the metabolisation rate is very high in liver.

20 **Method**

The liver samples were homogenized in cold PBS buffer, and extracted immediately with chloroform: methanol (2:1) containing 0.2mM butylated hydroxytoluene (BHT) using cis-10-heptadecenoic acid as internal standard. The organic phases were dried under nitrogen, re-dissolved in acetonitrile with 0.1%
25 acetic acid and 10µM BHT for RP-HPLC MS/MS analysis. Total protein content was measured using Bio-Rad method after homogenization.

Agilent 1100 system was used for reverse phase column (Supelco Ascentis C₁₈ column, 25cm x 4.6mm, i.d. 5µm) separation within 22min. The flow phase was iso-gradient acetonitrile-H₂O (87+13, v/v) containing 0.1% acetic acid. The column
30 oven temperature was set at 35°C. The column elute was identified and quantified in the negative electrospray ionisation applying multiple reaction monitoring mode by triple tandem quadrupole mass/mass (ABI Qtrap-4000). The parent-daughter ion pairs were 341.3/341.3 (PRB-1), under unit resolution. The signal collection dwell time was all 100 msec except for FA 17:1 which was set at 200msec. Accurate verification
35 of isomeric PRB compounds was done by combination of the retention time and characteristic mass/charge ratio. The quadratic regression standard curve was used for quantification after internal standard calibration.

Results

The concentration of the DHA-derivative according to the invention was about 10 μg per g of total amount of protein in the liver cells. This means that PRB-1 will be available as a ligand to nuclear receptors, a pattern which could be translated into therapeutic effects in handling of blood glucose and blood lipids.

Example 2

Computer based affinity testing

10

Background

Nuclear receptors have been sequenced and the amino acid sequence is known for the PPARs and other relevant receptors engaged in the genetic control of glucose and fat. X-ray crystallography and NMR spectroscopy of the PPAR receptors are available and computerised affinity testing of fatty acids liganding to the receptors can be used to estimate binding kinetics. The binding geometrics, often called binding modes or poses, include both positioning of the ligand relative to the receptor and the conformational state of the ligand and the receptor. Effective ligand docking can therefore be analysed.

Affinity of the ligand to the receptor is defined by two different parameters: docking of the ligand (DHA derivative) into the binding site of the receptor and electrostatic bonding between certain amino acids of the receptor and the carboxyl group or side chains in the head of the fatty acid. (Krumrine).

As previously known, the PPAR α receptor is more promiscuous compared to PPAR γ , meaning that PPAR α will accept more fatty acids as ligands compared to PPAR γ . However, since patients with metabolic syndrome or type 2 diabetes are usually obese or overweight and have pathologic blood lipids, mainly elevated triglycerides and low High-Density Cholesterol (HDL-cho) activation of the PPAR α receptor is important. An ideal drug for treatment of metabolic syndrome or type 2 diabetes should act as ligand to both these receptors, preferably with the highest affinity to the PPAR γ receptor.

Method

Ranking of PRB-1 according to its binding affinity was calculated and given as lowest binding affinity (LBA) and average binding affinity (ABE).

PRB-1 was tested with the computerized docking method (both r and s enantiomers). The PPAR γ ligands rosiglitazone and pioglitazone, both in the r and s

form, were also tested for comparison. These compounds are registered pharmaceuticals for treatment of diabetes.

Results

The results are shown in Table 1, presenting the parameters Lowest binding energy of single confirmation (LBE), average binding energy (ABE) of the correctly posed confirmation and fraction of correctly posed confirmation of the ICM-saved 20 lowest energy confirmation (f_{bound}) of the compounds tested. Affinity to the RXR α was tested in the same setting. The RXR α receptor interacts with the PPAR receptor forming a heterodimer by liganding of a fatty acid.

10

Table 1

	PPAR α			PPAR γ			RXR α		
	LBE	ABE	f_{bound}	LBE	ABE	f_{bound}	LBE	ABE	f_{bound}
DHA	-16.14	-13.29 (0.47)	0.60	-11.34	-10.51 (0.21)	0.35	-12.15	-10.72 (0.29)	0.40
cr-PRB1	-16.29	-14.25 (0.53)	0.50	-12.96	-11.82 (0.38)	0.30	-15.68	-14.25 (0.35)	0.30
cs-PRB1	-15.97	-14.01 (0.30)	0.80	-12.74	-10.24 (0.48)	0.65	-17.17	-14.48 (0.44)	0.50
sROSI				-9.47	-9.01 (0.17)	0.20			
sROSI				-10.05	-7.89 (0.91)	0.20			
rPIO				ND					
sPIO				-7.59	-7.59	0.05			

15 ND= Not docked, c = the double bonds in all-cis form. r= R enantiomer, s= S enantiomer. ROSI= Rosiglitazone, PIO= Pioglitazone

PRB-1 has a high LBE and ABE score for the PPAR α and PPAR γ receptors compared to the mother compound DHA but also to the PPAR γ ligands rosiglitazone and pioglitazone, both in the r and s form. This is an interesting observation indicating that PRB-1 could be promising competitors to the established anti-diabetics rosiglitazone and pioglitazone.

In conclusion, the DHA-derivative according to the invention demonstrated interesting affinities to the PPAR α and PPAR γ receptors with binding affinities better than rosiglitazone and pioglitazone.

25

Example 3

Affinity testing in transfected cells

Background

30 Release of luciferase is correlated to transcription of genes. Binding of a ligand to a nuclear receptor such as PPAR γ induces transcription of the respective

gene thereby releasing luciferase. This technique therefore provides a measure of ligand affinity to the receptor as well as activation of the responsible gene.

Method

5 Transient transfection of COS-1 cells was performed in 6-well plates as described by Graham and van der Eb (Graham). For full length PPAR transfection studies, each well received 5 µg reporter construct, 2.5 µg pSV-β-galactosidase as an internal control, 0.4 µg pSG5-PPARγ2. The cells were harvested after 72 h, and the luciferase activity was measured according to the protocol (Promega). The luciferase activity was normalised against β-galactosidase activity. The adipocytes were 10 transfected at D11 of differentiation using 16 µl LipofectaminPlus reagent, 4 µl Lipofectamine (Life Technologies Inc.), 0.2 µg pSG5-PPARγ, and 100 ng pTK Renilla luciferase as control of transfection efficiency. Three hours after transfection, cells were cultured in serum containing medium and incubated for 48 hours in the 15 same medium containing appropriate agents. The luciferase activities were measured as recommended by the manufacturer (Dual Luciferase assay, Promega). All transfections were performed in triplicate.

Fatty acids (BRL or DHA) and PRB-1 (stock solutions) were solubilized to 0.1 M final concentration in DMSO. Then, Fatty solubilized to 10mM in DMSO and 20 stored in 1.5 ml tubes (homopolymer, plastic tubes) flushed with argon and stored at -20°C. 10 µM of PRB-1 or fatty acids and DMSO (control) was added to the media 5h after transfection. Transfected cells were maintained for 24h before lysis by reporter lysis buffer. Binding of PRB-1 or fatty acids to the LBD of PPAR activates GAL4 binding to UAS, which in turn stimulates the tk promoter to drive luciferase 25 expression. Luciferase activity was measured using a luminometer (TD-20/20 luminometer; Turner Designs, Sunnyvale, CA) and normalized against protein content.

Results

30 Fig. 3 depicts the release of luciferase from transfected cells treated with PRB-1. The results indicate that PRB-1 has a high release of luciferase.

Example 4

35 **Pharmacodynamic effects of DHA derivatives in an animal model of metabolic syndrome**

Background

An animal model of the metabolic syndrome using the adipose prone mice of the C57BL/6J strain was used to document effects on typical laboratory and pathological anatomical features common for the metabolic syndrome. When given a high fat diet containing about 60% of fat, the animals are getting obese developing high insulin plasma levels, pathological glucose tolerance test, elevated serum triglycerides and non-esterified fatty acids, and fat liver.

Example 4a

10 **Effect of the DHA derivative according to the invention in adipose prone mice during 4 months of dietary interventions**

Method

All experiments were performed on male C57BL/6 mice, either a substrain C57BL/N (supplier: Charles River, Germany, n = 160, experiments A-C, see below), or a substrain C57BL/6J (supplier: the Jackson laboratory, Bar Harbor, ME, USA, n = 32, experiment D). Total numbers of animals used were higher (n = 170 and 36, respectively), because of culling. In the latter case, animals were bred for several generations (< 20) at the Institute of Physiology. At the beginning of the treatment, animals were 14-week-old and their body weight range was 23.6 – 27.1g. One week before the study start, animals were sorted according to their body weight and assigned to subgroups (n = 8) of similar mean body weight. This method allowed for culling of about 5 – 10 % of animals showing the lowest and highest body weight, respectively. The animals eliminated from the study at this stage were sacrificed by cervical dislocation. Complete health check of mice was performed by the supplier Charles River and at the start of study serological tests were performed by ANLAB (Prague, Czech Republic). In addition, regular health checks were performed in the animal house in 3-mo-intervals using sentinel mice and serological examinations (ANLAB). In all the tests, the animals were free of specific pathogens.

30

Diets

Animals were fed 3 types of experimental diets:

- (i) Chow diet (ssniff R/M-H from SSNIFF Spezialdieten GmbH, Soest, Germany; see also <http://ssniff.de>) with protein, fat and carbohydrate forming 33, 9, and 58 energy %, respectively
- (ii) High-fat diet prepared in the laboratory (cHF diet) with protein, fat and carbohydrate forming 15, 59, and 26 energy %, respectively, and well

characterized fatty acid composition (with most of the lipids coming from corn oil; see Ruzickova 2004)

- (iii) cHF diets in which 0.15, 0.5, and 1.5 % of fat (specifically the corn oil constituent) was replaced by various PRB-compounds, namely PRB1, PRB2, PRB5, PRB7, and PRB8, or by DHA. All these compounds were in the form of ethyl esters, provided by Pronova Biocare a.s. in sealed containers. Chemical composition of the PRB-compounds was unknown to the laboratory performing the experiments (Institute of Physiology, Academy of Sciences Prague, Czech Republic).

After arrival, the PRB-compound were stored in a refrigerator in original containers. The containers were opened just before preparation of the experimental diets. Diets were kept in plastic bags flushed by nitrogen and stored at -70°C in small aliquots sufficient for feeding animals for one week. Fresh ratios were given in 2-day intervals or daily.

Outline of the study

The study was based on 4 individual experiments. In each of the experiments, PRB-1 (or DHA, respectively) admixed to cHF diet in three different concentrations (0.15, 0.5, and 1.5 % of the fat content) were tested. In each experiment, a subgroup of plain cHF diet-fed mice was included and served as a control. Mice were caged in groups of 4 and fed standard chow diet until 3 mo of age, when animals (n = 8-13) were randomly assigned to the different test diets. After 2 mo on this new diet (at 5 mo of age), animals were fasted overnight and in the morning, intraperitoneal Glucose Tolerance Test (GTT) was performed. Animals were sacrificed after 4 months on the experimental diets, at 7 mo of age, and the end-point analysis were performed.

Study parameters.

The parameters in the study were: Body weight gain (grams), area under the curve (AUC) from intraperitoneal glucose tolerance tests (mMol x 180 min), plasma insulin (ng/ml), serum triglycerides (TAGs, mmol/l), and non-esterified fatty acids (NEFA, mmol/l).

Results

The results are shown in the following tables 2, 3 and 4. (* = significant differences compared to cHF diets (P<0.05).)

Table 2 shows the effects in animals given 1.5% concentration of the PRB test compounds compared to animals given standard chow (STD), composite high fat diet (cHF) or 97% DHA. A pronounced reduction in AUC from glucose tolerance tests was seen in the animals given PRB-1. Plasma insulin was low in the PRB-1 treated animals.

Table 3 shows the effects in animals given a lower concentration, 0.5%, of the PRB test compounds compared to animals given standard chow (STD), composite high fat diet (cHF) or 97% DHA.

Table 4 shows the results from the lowest PRB concentration given, 0.15%. Here, the differences were small. Weight gain was somewhat lower in the PRB-1 group. Plasma insulin was lower in PRB-1.

Table 2 The effect of PRB-1 after 4 months of treatment with 1.5% concentration

Parameter	STD	cHF	PRB-1	DHA
Body weight (grams)	32.4 ± 0.7	49.6 ± 0.6	44.0 ± 1.5*	47.1 ± 0.7*
Body wt. gain (grams)	7.8 ± 0.4	25.2 ± 0.5	20.2 ± 1.3*	23.0 ± 0.8*
Food intake (grams/mouse/day)	3.64 ± 0.04	2.70 ± 0.02	2.64 ± 0.03	2.63 ± 0.02
AUCglucose (mM x 180min)	1124 ± 57	1625 ± 151	913 ± 68*	2132 ± 288*
Fasted glucose (mg/dL)	77 ± 3	145 ± 7	130 ± 14	138 ± 7
Insulin (ng/mL)	1.03 ± 0.09	5.35 ± 0.36	2.73 ± 0.33	6.55 ± 0.31
TAGs (mmol/L)	1.41 ± 0.09	1.45 ± 0.07	1.58 ± 0.08	1.91 ± 0.26*
NEFA (mmol/L)	0.57 ± 0.05	0.61 ± 0.04	0.63 ± 0.03*	0.98 ± 0.07

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Table 3 The effect of PRB-1 after 4 months of dietary interventions: 0.5% concentration.

Parameter	STD	CHF	PRB-1	DHA
Body weight (grams)	32.4 ± 0.7	49.6 ± 0.6	47.4 ± 0.6	46.9 ± 0.7*
Body wt. gain (grams)	7.8 ± 0.4	25.2 ± 0.5	23.8 ± 0.5	22.9 ± 0.7*
Food intake (grams/mouse/day)	3.64 ± 0.04	2.70 ± 0.02	2.67 ± 0.04	2.70 ± 0.03
AUCglucose (mM x 180min)	1124 ± 57	1625 ± 151	1596 ± 205	1816 ± 182
Fasted glucose (mg/dL)	77 ± 3	145 ± 7	131 ± 7	136 ± 8
Insulin (ng/mL)	1.03 ± 0.08	5.35 ± 0.36	3.93 ± 0.59	5.82 ± 0.47
TAGs (mmol/L)	1.41 ± 0.09	1.45 ± 0.07	2.03 ± 0.22	1.78 ± 0.08*
NEFA (mmol/L)	0.57 ± 0.05	0.61 ± 0.04	0.73 ± 0.04*	0.89 ± 0.03

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Table 4. The effect of PRB-1 after 4 months of dietary interventions: 0.15% concentration.

Parameter	STD	cHF	PRB-1	DHA
Body weight (grams)	32.4 ± 0.7	49.6 ± 0.6	47.2 ± 1.3	48.3 ± 0.6
Body wt. Gain (grams)	7.8 ± 0.4	25.2 ± 0.5	22.9 ± 1.1	24.3 ± 0.8
Food intake (grams/mouse/day)	3.64 ± 0.04	2.70 ± 0.02	2.63 ± 0.04	2.79 ± 0.03
AUCglucose (mM x 180min)	1124 ± 57	1625 ± 151	1291 ± 172	1477 ± 214
Fasted glucose (mg/dL)	77 ± 3	145 ± 7	126 ± 15	141 ± 10
Insulin (ng/mL)	1.03 ± 0.08	5.35 ± 0.36	3.50 ± 0.29	4.31 ± 0.39*
TAGs (mmol/L)	1.41 ± 0.09	1.45 ± 0.07	1.75 ± 0.08	1.50 ± 0.13
NEFA (mmol/L)	0.57 ± 0.05	0.61 ± 0.04	0.62 ± 0.04*	0.96 ± 0.07

5

In conclusion, testing of PBR-1 during 4 months in adipose prone animals with insulin resistance and metabolic syndrome demonstrated a clear and unsuspected effect on insulin resistance and symptoms of the metabolic syndrome such as weight reduction, reduced AUC in the intraperitoneal glucose tolerance test, lower insulin/plasma levels as well as reduced triglyceride and non-esterified free fatty acids. Effects were observed in the dose of 1.5% as well as in the 0.5% group. Some effects were even noticed in the lowest concentration group of 0.15%.

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Example 4b

Testing of DHA derivatives on liver fat

Method

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Tissue samples from animals in the experiments with DHA derivatives was histologically analysed. After paraffination, tissue samples from liver, adipose tissue, skeletal muscle, pancreas, and kidney were stained with eosin-hematoxylin.

Results

There were no pathological findings in the tissues examined with exception from liver. Control animals fed high fat diet had developed fat liver (liver steatosis).
5 Fat droplets in the liver can easily be distinguished from normal liver cells. Animals treated with PRB-1 had low degree of fat liver.

This is an extremely important finding and very relevant for treatment of patients with insulin resistance, obesity and type 2 diabetes. Liver steatosis is a common finding in these patients which is usually related to an overload of fatty acids
10 and triglycerides, biological markers present in the development of insulin resistance and the metabolic syndrome. DHA-derivatives reduce liver steatosis.

Discussion and conclusions

The present application clearly shows that alpha-methyl-DHA activates
15 nuclear receptors, especially PPAR γ and PPAR α , thereby offering a series of therapeutic effects in the treatment of insulin resistance, the metabolic syndrome, type 2 diabetes, cardiovascular disease and other atherosclerotic related diseases.

In testing of affinity to PPAR γ and PPAR α using computerized docking technology, the DHA-derivative according to the present invention showed affinities
20 to both receptors, not least PPAR γ which probably is the most important nuclear receptor engaged in the activation of genes responsible for metabolism of blood glucose. Alpha-methyl DHA has two stereoisomers, the r and the s form. Using the docking technology both stereoisomers possessed about the same affinity to PPAR γ and PPAR α meaning that neither the r or the s form should have advantages
25 compared to the racemic form. In fact the racemic form may have advantages over each one of the stereoisomers.

When affinity was tested in transfected cells carrying the nuclear receptor and the subsequent DNA response element, the compound according to the invention demonstrated good affinity measured as release of luciferase.
30

The DHA derivative according to the invention has been tested in the C57BL/6 mouse model developing insulin resistance and the metabolic syndrome when fed high fat diet. The derivative demonstrated significant biological effects.

Comparing with pure DHA, alfa-methyl DHA (PRB-1) seems to be more potent than DHA. These findings and the potency compared to the mother molecule
35 DHA are not predictable and highly unexpected.

Since alfa-methyl DHA (PRB-1) seems to work by simultaneous liganding to the nuclear receptors PPAR α and PPAR γ the compound would not only possess

therapeutic interesting effects on glucose and lipid metabolism, not least in patients with insulin resistance, metabolic syndrome and type 2 diabetes but also have weight reduction as well as a general anti-inflammatory effect. Directly or through positive intervention on risk factors alfa-methyl DHA (PRB-1) would have a preventive effect
5 on the development of cardiovascular disease such as myocardial infarction and cerebral stroke as well as having a preventive effect on cardio-vascular mortality.

Pharmaceuticals acting as PPAR γ ligands are already on the market but even if these compounds are having positive effects on glucose metabolism, they are hampered by adverse effects such as elevated triglycerides, weight increase and
10 oedema. The alfa-substituted DHA derivative presented in this application has a combined PPAR γ and PPAR α effect which is probably both relevant and advantageous for patients with insulin resistance, metabolic syndrome and type 2 diabetes. Furthermore, these combinative actions should have important effects also on blood lipids, inflammatory events, atherosclerosis, and thereby cardiovascular
15 disease.

The invention shall not be limited to the shown embodiments and examples.

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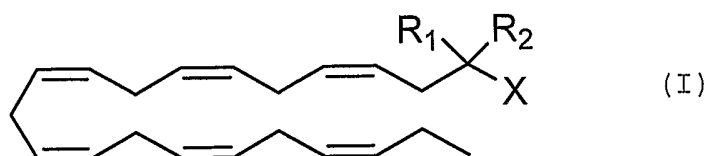
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Claims

1. A compound of formula (I);

5



- wherein one of R₁ and R₂ is a methyl group and the other of R₁ and R₂ is a hydrogen atom;
- 10 - wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;

or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof,

15 for use as a medicament.

2. A compound according to claim 1, wherein said carboxylate group is selected from the group consisting of ethyl carboxylate, methyl carboxylate, n-propyl carboxylate, isopropyl carboxylate, n-butyl carboxylate, sec.-butyl carboxylate, and
20 n-hexyl carboxylate.

3. A compound according to claim 2, wherein said carboxylate group is ethyl carboxylate.

25 4. A compound according to claim 1, wherein said carboxamide group is selected from the group consisting of primary carboxamide, N-methyl carboxamide, N,N-dimethyl carboxamide, N-ethyl carboxamide, and N,N-diethyl carboxamide.

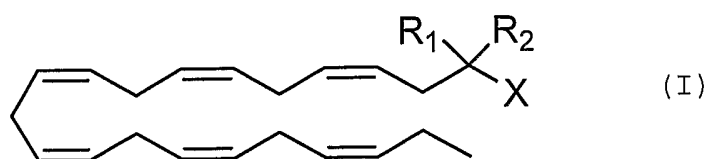
30 5. A compound according to any claim 1, in the form of a phospholipid, a tri-, di- or monoglyceride, or in the form of a free acid.

6. A compound according to claim 1 in racemic form.

7. A compound according to claim 1 in the form of its R stereoisomer.

8. A compound according to claim 1 in the form of its S stereoisomer.

5 9. A fatty acid composition comprising a compound of formula (I);



10 - wherein one of R_1 and R_2 is a methyl group and the other of R_1 and R_2 is a hydrogen atom;

- wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;

15

or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof.

10. A fatty acid composition according to claim 9, wherein at least 60% by weight of the fatty acid composition is comprised of said compound.

20

11. A fatty acid composition according to claim 10, wherein at least 90% by weight of the fatty acid composition is comprised of said compound.

25 12. A fatty acid composition according to any one of the claims 9 to 11, further comprising fatty acids selected from (all-Z)-5,8,11,14,17-eicosapentaenoic acid (EPA), (all-Z)-4,7,10,13,16,19-docosahexaenoic acid (DHA), (all-Z)-6,9,12,15,18-heneicosapentaenoic acid (HPA), and/or (all-Z)-7,10,13,16,19-docosapentaenoic acid (DPA).

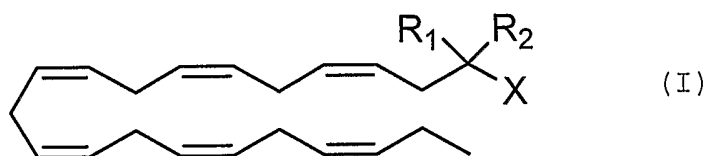
30 13. A fatty acid composition according to claim 12, wherein said fatty acids are present in the form of derivatives.

14. A fatty acid composition according to any one of claims 9 to 13, further comprising a pharmaceutically acceptable antioxidant.

15. A fatty acid composition according to claim 14, wherein said antioxidant is tocopherol.

16. A fatty acid composition according to any one of the claims 9 to 15, for use as a medicament.

17. A pharmaceutical composition comprising, as an active ingredient, a compound of formula (I);



- wherein one of R₁ and R₂ is a methyl group and the other of R₁ and R₂ is a hydrogen atom;
- wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;

or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof.

18. A pharmaceutical composition according to claim 17, further comprising a pharmaceutically acceptable carrier.

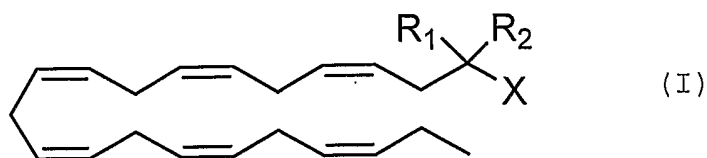
19. A pharmaceutical composition according to claim 18, formulated for oral administration.

20. A pharmaceutical composition according to claim 19, in the form of a capsule or a sachet.

21. A pharmaceutical composition according to any one of the claims 17 to 20, formulated to provide a daily dosage of 10 mg to 10 g of said compound.

22. A pharmaceutical composition according to claim 21, formulated to provide a daily dosage of 100 mg to 1 g of said compound.

23. Use of a compound of of formula (I);



5 - wherein one of R₁ and R₂ is a methyl group and the other of R₁ and R₂ is a hydrogen atom;

- wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;

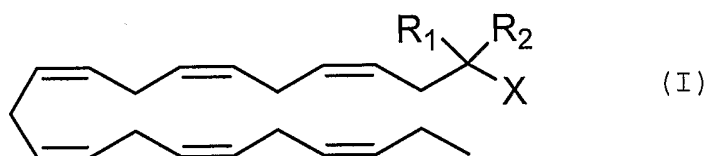
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or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof,

for controlling body weight reduction and/or for preventing body weight gain.

15

24. Use of a compound of of formula (I);



20 - wherein one of R₁ and R₂ is a methyl group and the other of R₁ and R₂ is a hydrogen atom;

- wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;

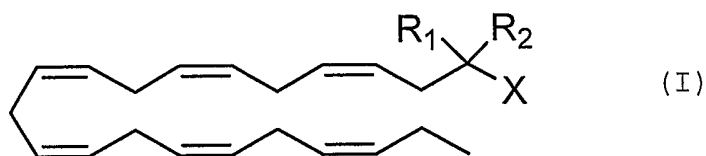
25

or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof,

for the treatment and/or the prevention of obesity or an overweight condition.

30

25. Use of a compound of formula (I);



- 5 - wherein one of R₁ and R₂ is a methyl group and the other of R₁ and R₂ is a hydrogen atom;
- wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;

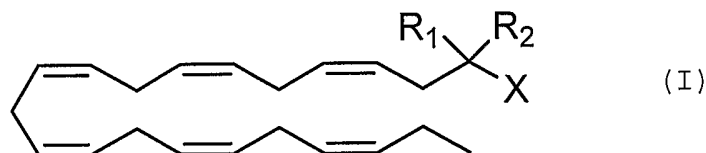
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or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof, for the prevention and/or treatment of diabetes.

15

26. Use according to claim 25, wherein the diabetes is type 2 diabetes.

27. Use of a compound of formula (I);



20

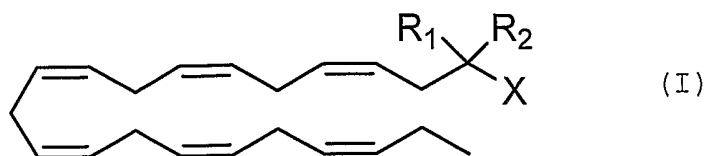
- wherein one of R₁ and R₂ is a methyl group and the other of R₁ and R₂ is a hydrogen atom;
- wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;

25

or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof, for the treatment and/or prevention of amyloidosis-related diseases.

30

28. Use of a compound of formula (I);



- 5 - wherein one of R₁ and R₂ is a methyl group and the other of R₁ and R₂ is a hydrogen atom;
- wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;

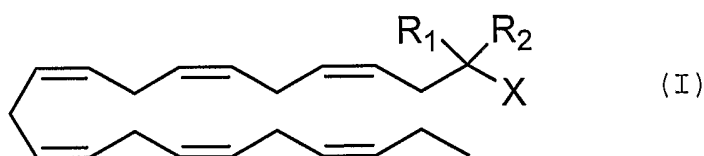
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or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof,

for the treatment or prophylaxis of multiple risk factors for cardiovascular diseases, preferably for the treatment of elevated blood lipids.

15

29. Use of a compound of formula (I);



- 20 - wherein one of R₁ and R₂ is a methyl group and the other of R₁ and R₂ is a hydrogen atom;
- wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;

25

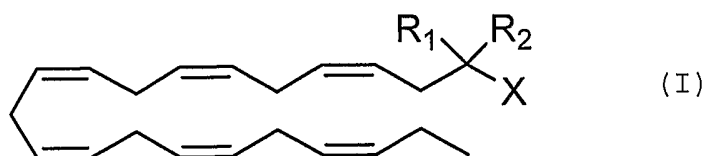
or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof,

for prevention of stroke, cerebral or transient ischaemic attacks related to atherosclerosis of several arteries.

30

30. A method for controlling body weight reduction and/or for preventing body weight gain, wherein a pharmaceutically effective amount of a compound of formula (I);

5



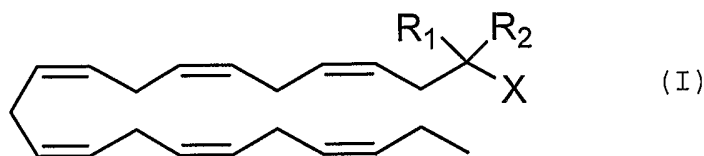
- wherein one of R₁ and R₂ is a methyl group and the other of R₁ and R₂ is a hydrogen atom;
- 10 - wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;

or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof, is administered to a human or an animal.

15

31. A method for the treatment and/or the prevention of obesity or an overweight condition, wherein a pharmaceutically effective amount of a compound of formula (I);

20

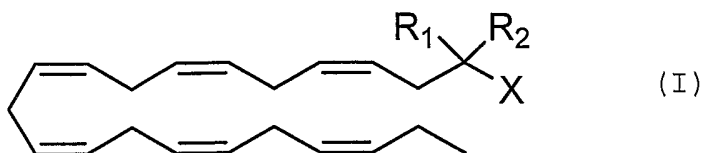


- wherein one of R₁ and R₂ is a methyl group and the other of R₁ and R₂ is a hydrogen atom;
- 25 - wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;

or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof, is administered to a human or an animal.

30

32. A method for the prevention and/or treatment of diabetes, wherein a pharmaceutically effective amount of a compound of formula (I);



5

- wherein one of R₁ and R₂ is a methyl group and the other of R₁ and R₂ is a hydrogen atom;
- wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;

10

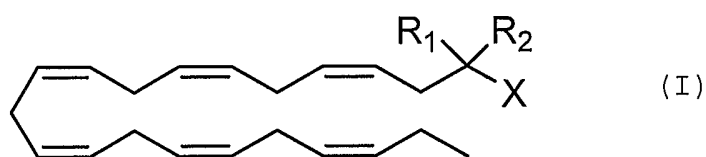
or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof, is administered to a human or an animal.

15

33. A method according to claim 32, wherein the diabetes is type 2 diabetes.

34. A method for the treatment and/or prevention of amyloidosis-related diseases, wherein a pharmaceutically effective amount of a compound of formula (I);

20



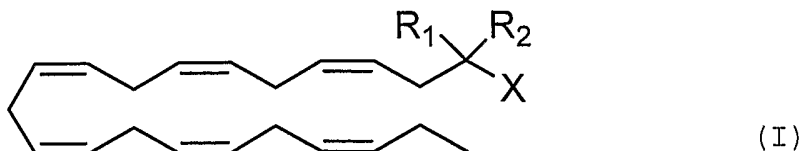
- wherein one of R₁ and R₂ is a methyl group and the other of R₁ and R₂ is a hydrogen atom;
- wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;

25

or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof, is administered to a human or an animal.

30

35. A method for the treatment or prophylaxis of multiple risk factors for cardiovascular diseases, wherein a pharmaceutically effective amount of a compound of formula (I);



5

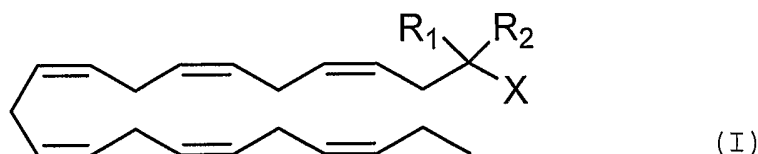
- wherein one of R₁ and R₂ is a methyl group and the other of R₁ and R₂ is a hydrogen atom;
- wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;

10

or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof, is administered to a human or an animal.

36. A method for the prevention of stroke, cerebral or transient ischaemic attacks related to atherosclerosis of several arteries, wherein a pharmaceutically effective amount of a compound of formula (I);

15



20

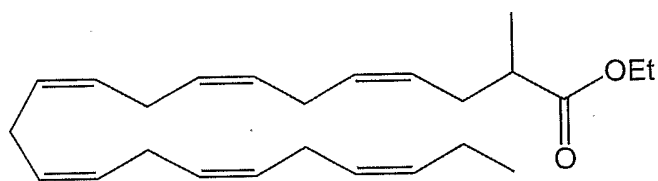
- wherein one of R₁ and R₂ is a methyl group and the other of R₁ and R₂ is a hydrogen atom;
- wherein X represents a carboxylic acid group, a carboxylate group, or a carboxamide group;

or any pharmaceutically acceptable salt, solvate, complex or pro-drug thereof, is administered to a human or an animal.

25

37. A method according to any one of the claims 30 to 36, wherein the compound of formula (I) is administered orally to a human or an animal.

1/3



PRB-1 α -methyl docosahexaenoic acid ethyl ester

Fig. 1

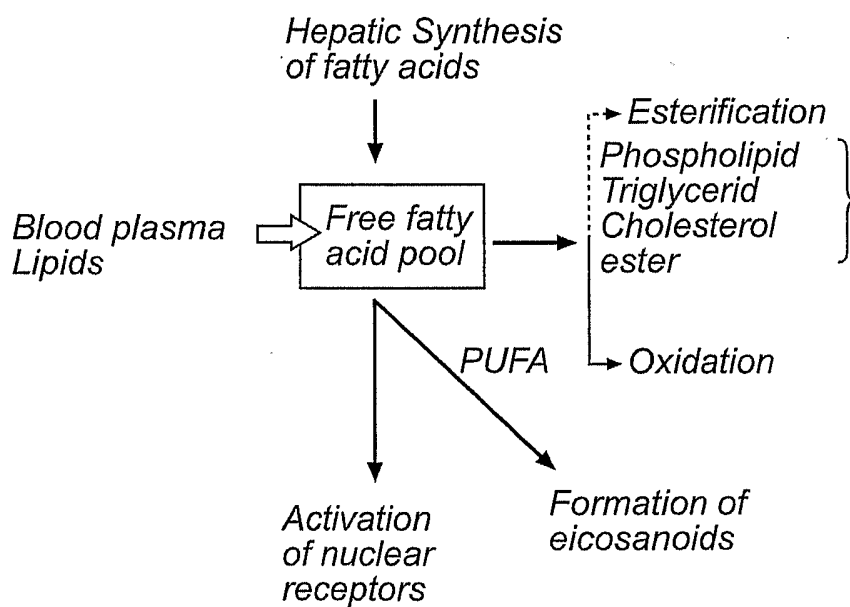
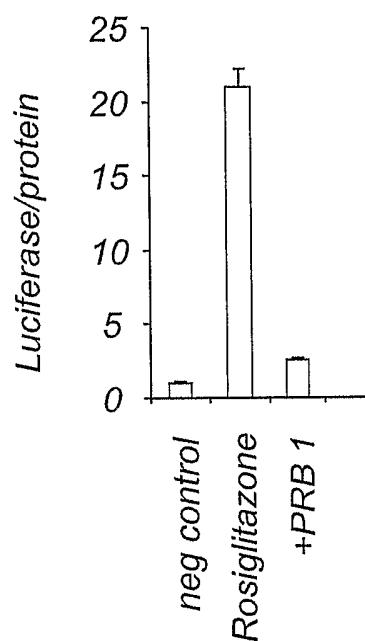


Fig. 2

Affinity testing in transfected cells containing PPARgamma receptors



All ligands 10µM

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2006/001164

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: A61K, C07D

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

EPO-INTERNAL, WPI DATA, PAJ, CHEM.ABS DATA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 1310249 A1 (QUATEX N.V.), 14 May 2003 (14.05.2003), claims 1,13-14,18-19, paragraph [0021], abstract --	1-22
Y	WO 2004078166 A2 (SOLVAY PHARMACEUTICALS GMBH), 16 Sept 2004 (16.09.2004), claims 8-17, abstract --	1-22
Y	US 5656667 A (BREIVIK ET AL), 12 August 1997 (12.08.1997), claims 14,24,49,52, abstract --	1-22

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

8 August 2006

Date of mailing of the international search report

10 -08- 2006

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Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2006/001164

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	EP 1544281 A1 (KAO CORPORATION), 22 June 2005 (22.06.2005), claims 1-3,6, paragraph [0065], abstract --	1-22
Y	LARSEN LAILA N. ET AL, "Sulfur-Substituted and alfa-Methylated Fatty Acids as Peroxisome Proliferator-Activated Receptor Activators", Lipids 2005, Vol. 40, No. 1, p. 49-57, Figure 1, Results, abstract --	1-22
A	HEGE VAAGENES et al, "Methylated Eicosapentaenoic Acid and Tetradecylthioacetic Acid: Effects on Fatty Acid Metabolism", Biochemical Pharmacology, 1999, vol. 58, pages 1133-1143, fig. 1 --	1-37
A	DATABAS WPI Week 199306 Derwent Publications Ltd., London, GB; Class C07C, AN 1993-233465, & JP 500974 A (SAGAMI CHEM RES CENTRE), 8 January 1993 (1993-01-08) abstract, STN-Caplus, RN 147544-49-8 --	1-37
A	US 20040162348 A1 (PEET ET AL), 19 August 2004 (19.08.2004), claims 1,13, abstract --	1-37
A	EP 1466597 A1 (CLINIGENETICS), 13 October 2004 (13.10.2004) -- -----	1-37

International patent classification (IPC)

C11C 3/00 (2006.01)
A61K 31/202 (2006.01)
A61K 31/232 (2006.01)
A61P 3/04 (2006.01)
A61P 3/10 (2006.01)
A61P 9/10 (2006.01)
C07C 57/03 (2006.01)
C07C 69/587 (2006.01)

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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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB06/001164

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

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

- 1. Claims Nos.: 23-37
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 23-37 relate to a method of treatment of the human or animal body by surgery or by therapy, as well as diagnostic
.../...
- 2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
- 3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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:

- 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 an additional fee, this Authority did not invite payment of any additional fee.
- 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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB06/001164

Box II.1

methods /Rule 39.1(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds.

INTERNATIONAL SEARCH REPORT
Information on patent family members

04/03/2006

International application No.

PCT/IB2006/001164

EP	1310249	A1	14/05/2003	IT	MI20012384 A	12/05/2003
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				IE	892317 L	11/02/1990
				IL	91275 A	19/01/1996
				IT	1235879 B	23/11/1992
				IT	8921521 D	00/00/0000
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