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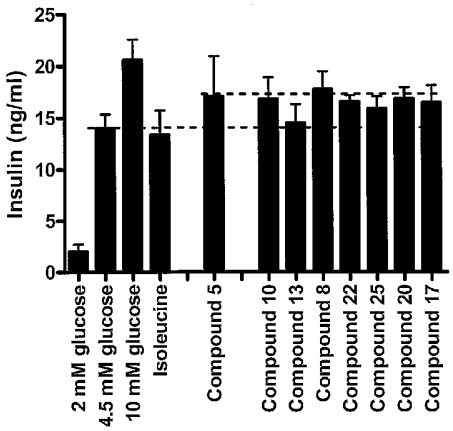
(54) Title: DIASTEREOISOMERS OF 4-HYDROXYISOLEUCINE AND USES THEREOF



(57) Abstract: The invention relates to configurational isomers 4-hydroxyisoleucine, and to lactones, pharmaceutically acceptable salts, and prodrugs thereof, to processes for their preparation, and to pharmaceutical compositions comprising the same. The isomers of the invention exhibit insulinotropic activity and thus may be useful for the prevention and treatment of disorders of carbohydrate or lipid metabolism, including diabetes mellitus (type 1 and type 2 diabetes), pre-diabetes, and Metabolic Syndrome.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

DIASTEREOISOMERS OF 4-HYDROXYISOLEUCINE AND USES THEREOF

BACKGROUND OF THE INVENTION

a) Field of the invention

The invention relates to isomers of 4-hydroxyisoleucine, and to lactones, pharmaceutically acceptable salts and prodrugs thereof, to processes for their preparation, to pharmaceutical compositions comprising the same and to their use for preventing and treating disorders of carbohydrate or lipid metabolism, including diabetes mellitus (type 1 and type 2 diabetes), pre-diabetes, and Metabolic Syndrome.

b) Brief description of the related art

Diabetes mellitus is a disorder of carbohydrate metabolism, and develops when the body cannot effectively control blood glucose levels. The disease is characterized by inadequate secretion or utilization of insulin, high glucose levels in the blood and urine, and excessive thirst, hunger, weight loss, and urine production. It can lead to a number of serious complications, including cardiovascular disease, kidney disease, blindness, nerve damage, and limb ischemia.

Diabetes is divided into two types, 1 and 2, with the latter accounting for about 90% of cases. In type 1 diabetes, the body destroys the insulin-producing β cells of the pancreas, resulting in the inability of the body to produce insulin. Type 1 diabetes typically occurs in children or young adults, and generally is managed by insulin administration, strict diet, and exercise. Type 1 diabetes is observed as well in older adults following therapeutic failure of type 2 diabetes. Type 2 diabetes is characterized by impaired insulin secretion due to altered β cell function, as well as decreased ability of normally insulin sensitive tissues (e.g., the liver and muscle) to respond to insulin. Type 2 diabetes generally develops in those over 45, but is recently also being detected in younger people. The disease is associated with risk factors such as age, family history, obesity, lack of regular exercise, high blood pressure, and hyperlipidemia. Treatment involves strict diet and exercise regimens, oral medications (e.g., medications that increase insulin secretion and/or insulin sensitivity), and, in some cases, insulin administration.

Type 2 diabetes is rapidly increasing in its importance as a major public health concern in the Western world. While one hundred years ago it was a relatively

rare disease, today there are more than 200 million type 2 diabetics worldwide, and this number is estimated to increase to greater than about 300 million by the year 2025. This dramatic increase in the incidence of type 2 diabetes parallels an increase in the prevalence of obesity in Western cultures. Further, as more cultures adopt Western dietary habits, it is likely that type 2 diabetes will reach epidemic proportions throughout the world. Given the seriousness of the complications associated with this disease, as well as its rapidly increasing incidence, the development of effective approaches to treatment is a primary concern in the field of medicine.

In 1973, Fowden et al., in *Phytochemistry* 12:1707-1711, 1973, reported the presence of (2*S*,3*R*,4*R*)-4-hydroxy-3-methylpentanoic acid (4-hydroxyisoleucine or 4-OH) in the seeds of fenugreek (*Trigonella foenum-graecum*), an annual herbaceous plant that is widespread in regions of Asia, Africa, and Europe. Its absolute configuration was subsequently restudied and corrected as being (2*S*,3*R*,4*S*) by Alcock et al. in *Phytochemistry* 28:1835-1841, 1989. This unusual substance represents about 0.6% of the seed weight and more that 85% of the free amino acids in the seeds, with two coexisting isomers: the (2*S*,3*R*,4*S*) isomer (~90%) and the (2*R*,3*R*,4*S*) isomer (~10%) (Sauvaire et al., *Herbs, Botanicals and Teas* (2000), Edited by G. Mazza and B.P. Oomah, p. 107-129). It has been demonstrated that the (2*S*,3*R*,4*S*) isomer possesses insulinotropic and insulin sensitizing activities (Broca et al., *Am. J. Physiol.* 277:E617-E623, 1999; Broca et al., *Eur. J. Pharmacol.* 390:339-345, 2000; Broca et al., *Am. J Physiol. Endocrinol. Metab.* 287:E463-E471, 2004) and that compound has since been developed for the treatment of diabetes (PCT publication Nos. WO 97/32577 and WO 01/15689). On the other hand, it has been reported that the (2*R*,3*R*,4*S*) isomer has no or much less biological activity than the (2*S*,3*R*,4*S*) isomer (Broca et al., *Eur. J. Pharmaco.* 390:339-345, 2000).

Notwithstanding the growing body of evidence on the positive activities of 4-hydroxyisoleucine for the treatment of diabetes, no one has ever demonstrated that configurational isomers of 4-hydroxyisoleucine, other than the (*2S,3R,4S*) isomer, could be useful for the prevention and/or treatment of metabolic diseases such as diabetes.

In view of the above, there is a need for alternative and improved compounds for preventing and treating disorders of carbohydrate and lipid metabolism, particularly diabetes.

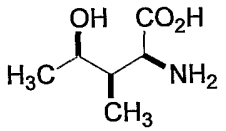
There is also a need for pharmaceutical compositions and therapeutic methods of stimulating glucose uptake and/or of stimulating insuling secretion.

The present invention provides such compounds along with methods for their use. Accordingly, the present invention fulfils the above-mentioned needs and also other needs as it will be apparent to those skilled in the art upon reading the following specification.

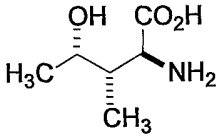
SUMMARY OF THE INVENTION

The present inventors have found that configurational isomers of (*2S,3R,4S*)-4-hydroxyisoleucine also exhibit insulinotropic activity in *in vitro* assays: one that monitors the glucose-dependent stimulation of insulin secretion in insulin secreting INS-1 cells and another that monitors glucose uptake in differentiated 3T3-L1 adipocytes.

Accordingly, the present invention features the use of isomers of 4-hydroxyisoleucine (4-OH) as defined herein, for therapeutic and/or prophylactic purposes. In preferred embodiments, the isomers according to the invention are isomers of *2S,3R,4S* 4-hydroxyisoleucine selected from the group consisting of:

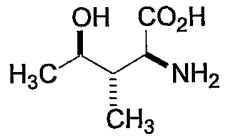


[8] (*2S,3R,4R*),

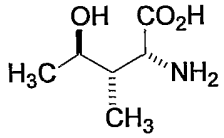


[10]

(*2S,3S,4S*),

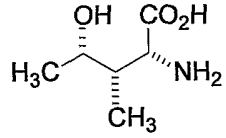


[13] (*2S,3S,4R*),

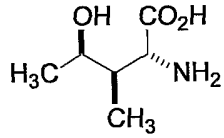


[17]

(*2R,3S,4R*),

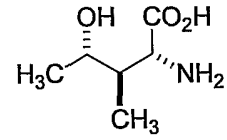


[20] (*2R,3S,4S*),



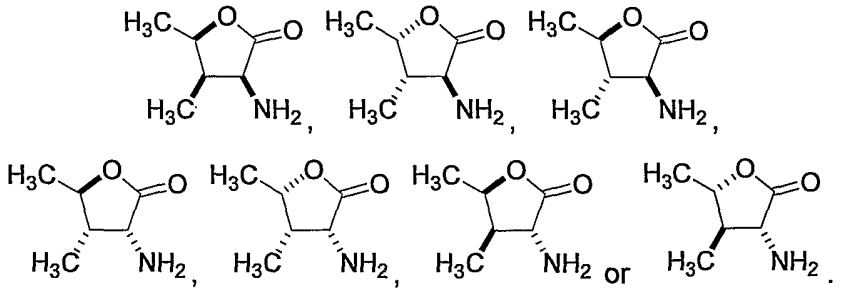
[22]

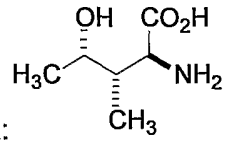
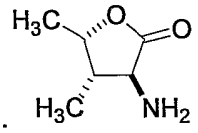
(*2R,3R,4R*), and



[25] (*2R,3R,4S*).

Exemplary prodrugs include those compounds in which the carboxylate group and the hydroxyl group are condensed to form one of the following lactones:



Another aspect of the invention features the following compound:  [10] (*2S,3S,4S*), or its lactone prodrug. 

In another aspect, the invention features pharmaceutical compositions comprising one or more of such isomers, and a pharmaceutically acceptable excipient.

In another aspect, the invention provides a method for treating a mammal having a disorder of carbohydrate or lipid metabolism that includes administering to the mammal one or more isomer of 4-OH as defined herein. Preferably, the disorder is non-insulin dependent diabetes mellitus, more preferably type 2 diabetes mellitus. In one embodiment, the method can further include administering a second agent to the mammal, where the agent can be, for example, an antidiabetic agent.

According to another aspect, the invention is directed to a method of treatment of disease in a mammal treatable by administration a compound stimulating insulin secretion, which method comprises administration of a therapeutically effective amount of a pharmaceutical composition comprising a therapeutically effective amount of at least one isomer of 4-OH according to the invention, and a pharmaceutically acceptable carrier or excipient, either alone or in combination with other pharmacologically active agents

In another aspect, this invention is directed to a method for stimulating glucose uptake by muscle cells and/or adipocytes, comprising contacting such cells with an effective amount of isomer(s) according to the invention.

In another aspect, this invention is directed to a method for stimulating insulin secretion by beta-cells in the pancreatic islets, comprising contacting said cells with an effective amount of isomer(s) according to the invention.

In yet another aspect, this invention is directed to pharmaceutical compositions and more particularly to the use of isomer(s) according to the invention in the preparation of a medicine for use in the treatment of a disorder of carbohydrate or lipid metabolism in which elevated circulating glucose levels are problematic, including but not limited to diabetes mellitus (type 1 and type 2 diabetes), pre-diabetes, Metabolic Syndrome, hyperglycemia, diabetic neuropathy and diabetic nephropathy.

In a further aspect of the present invention there are provided processes for the preparation of isomer(s) according to the invention.

An advantage of the invention is that it provides novel useful stimulators of glucose uptake and stimulators of insulin secretion. The invention also provides compounds, compositions and methods for the unmet medical need of carbohydrate or lipid metabolism, and more particularly type 2 diabetes.

Additional objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of preferred embodiments with reference to the accompanying drawings which are exemplary and should not be interpreted as limiting the scope of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a synthetic scheme showing the synthesis of each eight (8) configurational isomers of 4-hydroxyisoleucine.

Figure 2 is a graph showing the effect of configurational isomers of 4-hydroxyisoleucine on the stimulation of glucose uptake by differentiated 3T3-L1 adipocyte cells.

Figure 3 is a graph showing the glucose-dependent stimulation of insulin secretion in INS-1 cells by configurational isomers of 4-hydroxyisoleucine.

DETAILED DESCRIPTION OF THE INVENTION

The invention features compounds, pharmaceutical compositions, and methods that include isomers of (2*S*,3*R*,4*S*)-4-hydroxyisoleucine, a compound that has been shown to stimulate insulin secretion in a glucose dependent manner and to decrease insulin resistance (see, e.g., U.S. Patent No. 5,470,879; WO 01/15689;

Broca et al., *Am. J. Physiol.* 277:E617-E623, 1999; and Broca et al., *Am. J Physiol. Endocrinol. Metab.* 287:E463-E471, 2004). More particularly, the invention features (2*S*,3*S*,4*S*)-4-hydroxyisoleucine and pharmaceutical compositions and methods that include one or more of the (2*S*,3*S*,4*S*), (2*S*,3*R*,4*R*), (2*S*,3*S*,4*R*), (2*R*,3*S*,4*R*), (2*R*,3*S*,4*S*), (2*R*,3*R*,4*R*) or (2*R*,3*R*,4*S*) configurational isomers of 4-hydroxyisoleucine for the treatment of mammals having a disorder of carbohydrate or lipid metabolism.

In order to provide an even clearer and more consistent understanding of the specification and the claims, including the scope given herein to such terms, the following definitions are provided:

A) Definitions

The term "**administration**" or "**administering**" refers to a method of giving a dosage of a pharmaceutical composition to a mammal, such as a human, where the method is, e.g., oral, subcutaneous, topical, intravenous, intraperitoneal, or intramuscular. The preferred method of administration can vary depending on various factors, e.g., the components of the pharmaceutical composition, site of the potential or actual disease, and severity of disease.

By "**disorder of carbohydrate metabolism**" is meant a metabolic disorder in which the subject having the disorder cannot properly metabolize sugars. Examples of such disorders include, for example, diabetes mellitus (type 1 and type 2), pre-diabetes, hyperglycemia, impaired glucose tolerance, Metabolic Syndrome, glucosuria, diabetic neuropathy and nephropathy, obesity, and eating disorders.

By "**disorder of lipid metabolism**" is meant a metabolic disorder in which the subject having the disorder cannot properly metabolize, distribute and/or store fat. Examples of such disorders include, but are not limited to type 2 diabetes, pre-diabetes, and Metabolic Syndrome.

By "**effective amount**" is meant the amount of a compound required to treat or prevent a disorder of carbohydrate or lipid metabolism, such as, for example, diabetes and Metabolic Syndrome. The effective amount of active compound(s) used to practice the present invention for therapeutic or prophylactic treatment of conditions caused by or contributed to by a disorder of carbohydrate or lipid metabolism varies depending upon the manner of administration, and the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. An effective

amount can also be that which provides some amelioration of one or more symptoms of the disorder or decreases the likelihood of incidence of the disorder.

Compounds that have the same molecular formula but differ in the nature or sequence of bonding of their atoms or the arrangement of their atoms in space are termed "**isomers**". Isomers in which the connectivity between atoms is the same but which differ in the arrangement of their atoms in space are termed "**stereoisomers**". Stereoisomers that are not mirror images of one another are termed "**diastereomers**" and those that are non-superimposable mirror images of each other are termed "**enantiomers**". When a compound has an asymmetric center, for example, it is bonded to four different groups, a pair of enantiomers is possible. An enantiomer can be characterized by the absolute configuration of its asymmetric center and is described by the R- and S-sequencing rules of Cahn, Ingold, and Prelog, or by the manner in which the molecule rotates the plane of polarized light and designated as dextrorotatory or levorotatory (i.e., as (+) or (-)-isomers respectively). A chiral compound can exist as either individual enantiomer or as a mixture thereof. A mixture containing equal proportions of the enantiomers is called a "**racemic mixture**".

Asymmetric or chiral centers may exist in the compounds of the present invention. Unless indicated otherwise, the description or naming of a particular compound in the specification and claims is intended to include all individual enantiomers and mixtures, racemic or otherwise, thereof. The methods for the determination of stereochemistry and the separation of stereoisomers are well-known in the art (see discussion in Chapter 4 of "Advanced Organic Chemistry", 4th edition J. March, John Wiley and Sons, New York, 1992). Individual stereoisomers of compounds or the present invention are prepared synthetically from commercially available starting materials that contain asymmetric or chiral centers or by preparation of mixtures of enantiomeric compounds followed by resolution well-known to those of ordinary skill in the art. These methods of resolution are exemplified by (1) attachment of a racemic mixture of enantiomers, designated (+/-), to a chiral auxiliary, separation of the resulting diastereomers by recrystallization or chromatography and liberation of the optically pure product from the auxiliary or (2) direct separation of the mixture of optical enantiomers on chiral chromatographic columns. Enantiomers are designated herein by the symbols "*R*" or "*S*," depending on the configuration of substituents around the chiral carbon atom, or are drawn by conventional means with a bolded line defining a substituent above the plane of the

page in three-dimensional space and a hashed or dashed line defining a substituent beneath the plane of the printed page in three-dimensional space.

As generally understood by those skilled in the art, an optically pure compound is one that is enantiomerically pure. As used herein, the term "**optically pure**" is intended to mean a compound that comprises at least a sufficient amount of a single enantiomer to yield a compound having the desired pharmacological activity. Preferably, "optically pure" is intended to mean a compound that comprises at least 90% of a single isomer (80% enantiomeric excess, i.e. "e.e."), preferably at least 95% (90% e.e.), more preferably at least 97.5% (95% e.e.), and most preferably at least 99% (98% e.e.). Preferably, the compounds of the invention are optically pure.

The terms "**isomer(s) of 4-hydroxyisoleucine**", "**isomer(s) of 4-OH**", "**isomer(s) of the invention**" or "**compound(s) of the invention**" as used herein, refer to the diastereoisomers of (2*S*,3*R*,4*S*)-4-hydroxyisoleucine as defined herein and include pharmaceutically acceptable lactones, salts, crystal forms, metabolites, solvates, esters, and prodrugs thereof.

The term "**pharmaceutically acceptable salt**" as use herein, represents those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and animals without undue toxicity, irritation, allergic response and the like and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge et al. describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences* 66:1-19, 1977. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention or separately by reacting the free base group with a suitable organic acid. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphersulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to

ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like.

The term "**pharmaceutically acceptable ester**" as used herein, represents esters that hydrolyze *in vivo* and include those that break down readily in the human body to leave the parent compound or a salt thereof. Suitable ester groups include, for example, those derived from pharmaceutically acceptable aliphatic carboxylic acids, particularly alkanoic, alkenoic, cycloalkanoic and alkanedioic acids, in which each alkyl or alkenyl group preferably has not more than 6 carbon atoms. Examples of particular esters include formates, acetates, propionates, butyates, acrylates and ethylsuccinates.

The term "**prodrug**" as used herein, represents compounds that are rapidly transformed *in vivo* to a parent compound of the above formula, for example, by hydrolysis in blood. A thorough discussion is provided in T. Higuchi and V. Stella, Pro-drugs as Novel Delivery Systems, Vol. 14 of the A.C.S. Symposium Series, Edward B. Roche, ed., Bioreversible Carriers in Drug Design, American Pharmaceutical Association and Pergamon Press, 1987, and Judkins et al., *Synthetic Communications* 26(23):4351-4367, 1996, each of which is incorporated herein by reference.

Prodrugs of isomers according to the invention are prepared by modifying functional groups in such a way that the modifications may be cleaved *in vivo* to release the parent isomer. Prodrugs include modified isomers wherein a hydroxy or amino group in any of said isomer is bonded to any group that may be cleaved *in vivo* to regenerate the free hydroxyl or amino group, respectively. Examples of prodrugs include, but are not limited to esters (e.g., acetate, formate, and benzoate derivatives), carbamates (e.g., N,N-dimethylaminocarbonyl) of hydroxy functional groups in compounds of Formulae (I), (II), or (III), and the like.

The term "**pharmaceutically acceptable prodrugs**" as used herein, represents those prodrugs of the compounds of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and animals without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention.

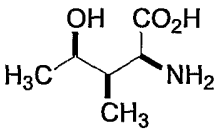
A "**pharmaceutically acceptable active metabolite**" is intended to mean a pharmacologically active product produced through metabolism in the body of an isomer of 4-OH.

A "**pharmaceutically acceptable solvate**" is intended to mean a solvate that retains the biological effectiveness and properties of the biologically active components of isomers according to the invention. Examples of pharmaceutically acceptable solvates include, but are not limited to water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid, and ethanolamine.

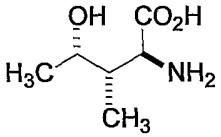
B) Compounds according to the invention

As will be described in detail hereinafter, the inventors have prepared series of isomers of 4-hydroxyisoleucine. According to preferred embodiments of the invention, these isomers are active for stimulating glucose uptake and/or stimulating insulin secretion in mammals, and can therefore be useful for preventing and/or treating disorders in which elevated glucose levels are problematic. Consequently, providing such isomers is not only desirable for the treatment of diabetes, but also for the treatment of other disorders of carbohydrate or lipid metabolism.

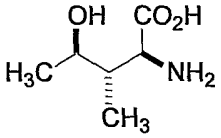
According to a first aspect, the present invention features isomers of *2S,3R,4S* 4-hydroxyisoleucine and pharmaceutically acceptable lactones, salts, crystal forms, prodrugs, esters, metabolites, or solvates thereof. In certain embodiments, the isomers of the present invention are selected from the group consisting of:



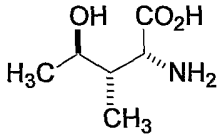
[8] (*2S,3R,4R*),



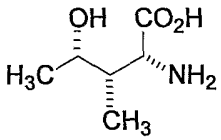
[10] (*2S,3S,4S*),



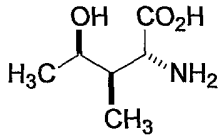
[13] (*2S,3S,4R*),



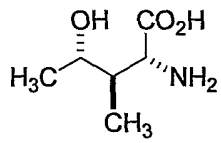
[17] (*2R,3S,4R*),



[20] (*2R,3S,4S*),

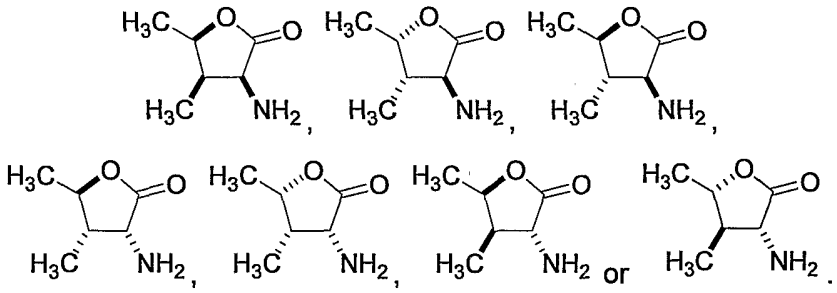


[22] (*2R,3R,4R*), and

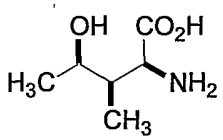


[25] (*2R,3R,4S*).

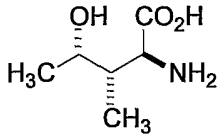
Exemplary prodrugs include those compounds in which the carboxylate group and the hydroxyl group are condensed to form one of the following lactones:



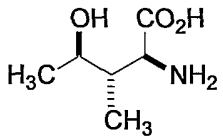
In preferred embodiments, the isomers of the present invention are selected from the group consisting of:



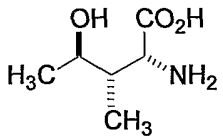
[8] (*2S,3R,4R*),



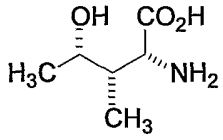
[10] (*2S,3S,4S*),



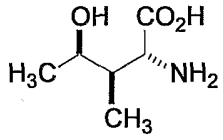
[13] (*2S,3S,4R*),



[17] (*2R,3S,4R*),

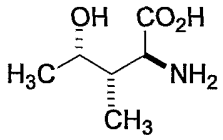


[20] (*2R,3S,4S*), and



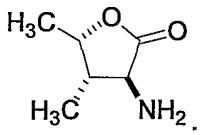
[22] (*2R,3R,4R*).

According to a related aspect, the invention features the following compound:



[10] (*2S,3S,4S*),

and pharmaceutically acceptable lactones, salts, crystal forms, prodrugs, esters, metabolites, or solvates thereof. In a preferred embodiment, the lactone is a lactone prodrug having the following structure:



The isomers and compositions (see hereinafter) of the invention may be prepared by employing techniques available in the art using starting materials that

are readily available. For instance, methods for the preparation of (*2S,3R,4S*)-4-hydroxyisoleucine have been described, see for example U.S. Patent Application Publication No. US 2003/0219880; Rolland-Fulcrand et al., *Eur. J. Org. Chem.* 873-877, 2004; and Wang et al., *Eur. J. Org. Chem.* 834-839, 2002. In addition, this compound can be isolated from the seeds of fenugreek (*Trigonella foenum-graecum*). Methods for making additional configurational isomers of 4-hydroxyisoleucine, or prodrug thereof, have also been described in PCT/FR2005/02805 filed Nov. 10, 2005 (WO 2006/_____published on May ____, 2006) which is incorporated herein by reference.

An additional aspect of the invention concerns new methods for the synthesis of isomers according to the invention. Certain novel and exemplary methods of preparing the inventive compounds are described in the Exemplification section. Such methods are within the scope of this invention.

C) Methods for stimulating glucose uptake and methods for stimulating insulin secretion

The isomers of the invention preferably stimulate glucose uptake by muscle tissues or adipose tissues and/or stimulate insulin secretion by pancreatic β-cells. The biological activity of the isomers of the invention may be measured by any of the methods available to those skilled in the art, including *in vivo* and *in vitro* assays. Some examples of suitable assays for such measurement are described herein in the Exemplification section. Additional examples of suitable art-recognized assays for such measurement are well known.

Accordingly, a related aspect, the invention provides a method of stimulating glucose uptake by muscle and or adipose tissues, the method comprising:

- providing at least one isomer according to the invention as defined herein;
- providing a functional *in vitro* cell-based assay in which glucose uptake stimulation is assessable; and
- introducing an effective amount of said isomer(s) into the assay for stimulating glucose uptake activity.

In one embodiment, the *in vitro* cell-based assay comprises 3T3-L1 adipocytes cells and is carried out in presence of about 10 µM 2-deoxy-D-glucose and about 16 µM ^{3}H-deoxy-D-glucose.

Accordingly, a related aspect, the invention provides a method of stimulating insulin secretion by β-cells, the method comprising:

- providing at least one isomer according to the invention as defined herein;

- providing a functional *in vitro* cell-based assay in which stimulation of insulin secretion is assessable; and
- introducing an effective amount of said isomer(s) into the assay for stimulating insulin secretion.

In one embodiment, the *in vitro* cell-based assay comprises INS-1 cells and is carried out in presence of a glucose concentration of about 2 mM to about 10 mM.

D) Pharmaceutical Compositions and Therapeutic Applications

Without wishing to be bound by any particular theory, the inventors have demonstrated that the isomers of the invention are suitable for stimulating glucose uptake, and/or stimulating insulin secretion. Therefore, present invention pertains to methods of using isomers of 4-OH and pharmaceutical compositions thereof for treatment or prevention purposes. In preferred embodiments, the method compromises administering any of the individual isomers described herein, or any combination thereof.

According to preferred embodiments of the invention, the mammal is a human subject in need of treatment by the methods and/or isomers of the invention, and is selected for treatment based on this need. A human in need of treatment, especially when referring to type 2 diabetes is art-recognized and includes subjects that have been identified as having abnormally high blood glucose levels, a reduced glucose tolerance, a disregulation of fat metabolism, and may have a surplus of weight (e.g. the subject may be obese). Humans in need of treatment may also be at risk of such a disease or disorder, and would be expected based on diagnosis, e.g., medical diagnosis, to benefit from treatment (e.g., curing, healing, preventing, alleviating, relieving, altering, remedying, ameliorating, improving, or affecting the disease or disorder, the symptom of the disease or disorder, or the risk of the disease or disorder).

Therefore, a related aspect of the invention concerns the use of isomers of the invention as an active ingredient in a pharmaceutical composition for treatment or prevention purposes. As used herein, "**treating**" or "**treatment**" is intended to mean at least the mitigation of a disease condition associated with a disorder of carbohydrate or lipid metabolism, and more particularly type 2 diabetes in a mammal, such as a human, that is alleviated by a stimulation of insulin secretion and/or by a stimulation of glucose uptake, and includes curing, healing, inhibiting (e.g., arresting or reducing the development of the disease or its clinical symptoms), relieving from,

improving and/or alleviating, in whole or in part, the disease condition (e.g., causing regression of the disease or its clinical symptoms).

As used herein, "**prophylaxis**" or "**prevent**" or "**prevention**" is intended to mean at least the reduction of likelihood of a disease condition associated with a disorder of carbohydrate or lipid metabolism, and more particularly type 2 diabetes in humans. Type 2 diabetes predisposing factors identified or proposed in the scientific literature include, among others, (i) a genetic predisposition to having the disease condition but not yet diagnosed as having it, (ii) being obese, (iii) having a disregulation of fat metabolism and/or (iv) having a sedentary life style. For example, it is likely that one can prevent or treat type 2 diabetes in a human by administering an isomer of the invention or a composition comprising the same, when the human is at a pre-diabetic state, when the human is overweight, when the human shows abnormally high blood glucose levels, and/or when the human exhibits a reduced tolerance to glucose.

The subject may be a female human or a male human, and it may be a child, a teenager, or an adult.

According to a specific aspect, the invention features a method for treating a mammal, such as a human, having diabetes mellitus (type 1 or type 2 diabetes), pre-diabetes, or Metabolic Syndrome, that includes administering to the mammal an isomer of the invention, and/or a composition comprising the same, in an amount sufficient to decrease its circulating glucose level.

According to certain embodiments, the isomers, compositions, and methods of the invention are administered at a therapeutically effective dosage sufficient to reduce the glucose levels in a subject's plasma, from about at least 5, 10, 15, 20 25, 30, 40, 50, 75, or 100 percent, when compared to original levels prior to treatment.

According to certain embodiments, the isomers, compositions, and methods of the invention are administered at a therapeutically effective dosage sufficient to increase insulin levels in a subject's plasma from about at least 5, 10, 15, 20 25, 30, 40, 50, 75, or 100 percent, when compared to original levels prior to treatment.

Typically, the isomers of the invention are given until glucose and/or insulin levels go back to normal. Due to the nature of the disorders and conditions targeted by the isomers of the invention, it is likely that a chronic or lifetime administration is going to be required. In preferred embodiments, isomers and pharmaceutical composition according to the invention are administered once to thrice a day.

The amount of glucose or insulin in the blood, or plasma of a subject can be evaluated by using techniques and methods well known to those skilled in the art,

including but not limited, to hand-held glucometer, enzymatic assays (e.g., glucose oxidase or hexokinase bases assays), enzyme-linked immunosorbent assay (ELISA), quantitative immunoblotting test methods, and radiolabeled immunoassay (RIA).

Therefore, the present invention provides pharmaceutical compositions comprising a therapeutically effective amount of an isomer of 4-OH as described herein in combination with a pharmaceutically acceptable carrier or excipient. Suitable carriers or excipients include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, parenteral, oral, anal, intravaginal, intravenous, intraperitoneal, intramuscular, intraocular, subcutaneous, intranasal, intrabronchial, or intradermal routes among others.

Acceptable methods of preparing suitable pharmaceutical forms of the pharmaceutical compositions are known to those skilled in the art. For example, pharmaceutical preparations may be prepared following conventional techniques of the pharmaceutical chemist involving steps such as mixing, granulating, and compressing when necessary for tablet forms, or mixing, filling, and dissolving the ingredients as appropriate, to give the desired products for various routes of administration.

Toxicity and therapeutic efficacy of the isomers according to the invention can be evaluated by standard pharmaceutical procedures in cell cultures or experimental animals. The therapeutic efficacy of the isomers according to the invention can be evaluated in an animal model system that may be predictive of efficacy in human diseases. For instance, animal models for evaluating efficacy in glucose uptake include animal models for diabetes or other relevant animal models in which glucose infusion rate can be measured. Animal models for evaluating insulinotropic efficacy include animal models for diabetes or other relevant animal models in which secretion of insulin can be measured. Examples of suitable animal models for diabetes include, but are not limited to DIO mice, ob/ob mice, db/db mice, and Zucker fa/fa rats. Alternatively, the ability of an isomer can be evaluated *in vitro*, by examining the ability of the compound to stimulate glucose uptake using differentiated 3T3-L1 adipocyte cells (see Example 2) or using L6 myocytes, by examining the ability of the compound to stimulate insulin secretion using INS-1 cells (see Example 3) or using perfused pancreas. While agents that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets

such agents to the site of affected tissue in order to minimize potential damage to unaffected cells and, thereby, reduce side effects, whenever possible.

A wide range of drugs can be used with the isomers, compositions, and methods of the present invention. Such drugs may be selected from antidiabetic agents, antihypertensive agents, anti-inflammatory agents, antiobesity agents, etc.

A non-limiting list of useful antidiabetic agents that can be used in combination with an isomer of the invention include insulin, biguanides, such as, for example metformin (Glucophage®, Bristol-Myers Squibb Company, U.S.; Stagid®, Lipha Santé, Europe); sulfonylurea drugs, such as, for example, gliclazide (Diamicron®), glibenclamide, glipizide (Glucotrol® and Glucotrol XL®, Pfizer), glimepiride (Amaryl®, Aventis), chlorpropamide (e.g., Diabinese®, Pfizer), tolbutamide, and glyburide (e.g., Micronase®, Glynase®, and Diabeta®); glinides, such as, for example, repaglinide (Prandin® or NovoNorm®; Novo Nordisk), ormitiglinide, nateglinide (Starlix®), senaglinide, and BTS-67582; insulin sensitizing agents, such as, for example, glitazones, a thiazolidinedione such as rosiglitazone maleate (Avandia®, Glaxo Smith Kline), pioglitazone (Actos®, Eli Lilly, Takeda), troglitazone, ciglitazone, isaglitazone, darglitazone, englitazone, CS-011/CI-1037, T 174, GI 262570, YM-440, MCC-555, JTT-501, AR-H039242, KRP-297, GW-409544, CRE-16336, AR-H049020, LY510929, MBX-102, CLX-0940, GW-501516, and the compounds described in WO 97/41097 (DRF-2344), WO 97/41119, WO 97/41120, WO 98/45292, WO 99/19313 (NN622/DRF-2725), WO 00/23415, WO 00/23416, WO 00/23417, WO 00/23425, WO 00/23445, WO 00/23451, WO 00/41121, WO 00/50414, WO 00/63153, WO 00/63189, WO 00/63190, WO 00/63191, WO 00/63192, WO 00/63193, WO 00/63196, and WO 00/63209; glucagon-like peptide 1 (GLP-1) receptor agonists, such as, for example, Exendin-4 (1-39) (Ex-4), Byetta™ (Amylin Pharmaceuticals Inc.), CJC-1131 (Conjuchem Inc.), NN-2211 (Scios Inc.), and those GLP-1 agonists described in WO 98/08871 and WO 00/42026; agents that slow down carbohydrate absorption, such as, for example, α-glucosidase inhibitors (e.g., acarbose, miglitol, voglibose, and emiglitate); agents that inhibit gastric emptying, such as, for example, glucagon-like peptide 1, cholescystokinin, amylin, and pramlintide; glucagon antagonists, such as, for example, quinoxaline derivatives (e.g., 2-styryl-3-[3-(dimethylamino)propylmethylamino]-6,7-dichloroquinoxaline; Collins et al., *Bioorganic and Medicinal Chemistry Letters* 2(9):915-918, 1992), skyrin and skyrin analogs (e.g., those described in WO 94/14426), 1-phenyl pyrazole derivatives (e.g., those described in U.S. Patent No. 4,359,474), substituted disilacyclohexanes (e.g., those described in U.S. Patent No. 4,374,130), substituted

pyridines and biphenyls (e.g., those described in WO 98/04528), substituted pyridyl pyrroles (e.g., those described in U.S. Patent No. 5,776,954), 2,4-diaryl-5-pyridylimidazoles (e.g., those described in WO 98/21957, WO 98/22108, WO 98/22109, and U.S. Patent No. 5,880,139), 2,5-substituted aryl pyrroles (e.g., those described in WO 97/16442 and U.S. Patent No. 5,837,719), substituted pyrimidinone, pyridone, and pyrimidine compounds (e.g., those described in WO 98/24780, WO 98/24782, WO 99/24404, and WO 99/32448), 2-(benzimidazol-2-ylthio)-1-(3,4-dihydroxyphenyl)-1-ethanones (see Madsen et al., *J. Med. Chem.* 41:5151-5157, 1998), alkylidene hydrazides (e.g., those described in WO 99/01423 and WO 00/39088), and other compounds, such as those described in WO 00/69810, WO 02/00612, WO 02/40444, WO 02/40445, and WO 02/40446; and glucokinase activators, such as, for example, those described in WO 00/58293, WO 01/44216, WO 01/83465, WO 01/83478, WO 01/85706, and WO 01/85707.

Other examples of antidiabetic agents that can be used in combination with one or more isomers according to the invention include imidazolines (e.g., efaroxan, idazoxan, phentolamine, and 1-phenyl-2-(imidazolin-2-yl)benzimidazole); glycogen phosphorylase inhibitors (see, e.g., WO 97/09040); oxadiazolidinediones, dipeptidyl peptidase-IV (DPP-IV) inhibitors, protein tyrosine phosphatase (PTPase) inhibitors, inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis and/or glycogenolysis, glucose uptake modulators, glycogen synthase kinase-3 (GSK-3) inhibitors, compounds that modify lipid metabolism (e.g., antihyperlipidemic agents and antilipidemic agents), peroxisome proliferator-activated receptor (PPAR) agonists or antagonists in general, retinoid X receptor (RXR) agonists (e.g., ALRT-268, LG-1268, and LG-1069), and antihyperlipidemic agents or antilipidemic agents (e.g., cholestyramine, colestipol, clofibrate, gemfibrozil, lovastatin, pravastatin, simvastatin, probucol, and dextrothyroxine). Other suitable antidiabetic agents are listed in **Table 1** provided elsewhere herein.

Examples of antihypertensive agents that can be used with the isomers of the invention include β-blockers (e.g., alprenolol, atenolol, timolol, pindolol, propranolol, and metoprolol), angiotensin converting enzyme (ACE) inhibitors (e.g., benazepril, captopril, enalapril, fosinopril, lisinopril, quinapril, and ramipril), calcium channel blockers (e.g., nifedipine, felodipine, nicardipine, isradipine, nimodipine, diltiazem, and verapamil), and α-blockers (e.g., doxazosin, urapidil, prazosin, and terazosin).

Examples of anti-inflammatory agents that can be used with the isomers of the invention include anti-histamines, and anti-TNFα.

Examples of anti-obesity agents that can be used with the isomers of the invention include Xenical™ (Roche), Meridia™ (Abbott), Acomplia™ (Sanofi-Aventis), Pramlintide (Amylin), and sympathomimetic phentermine.

The isomers, compositions, and methods of the present invention may also be used with analogs of 4-OH, such as those decribed in the PCT application entitled "ANALOGS OF 4-HYDROXYISOLEUCINE AND USES THEREOF" which claims priority of US Provisional Application 60/654,342 filed February 18, 2005.

Accordingly, another aspect of relates to a pharmaceutical kit or pharmaceutical composition that includes any of the isomers of 4-OH described herein, or any combination thereof, and a second antidiabetic agent. The pharmaceutical kit or composition can include a 4-hydroxyisoleucine isomer and a second antidiabetic agent that is formulated into a single composition, such as, for example, a tablet or a capsule. The invention also provides methods of treating diabetes (type 1 diabetes or type 2 diabetes), pre-diabetes, or Metabolic Syndrome in patients, which include administering to a patient one or more isomers of 4-hydroxyisoleucine such as those described herein, in combination with one or more antidiabetic agents. The combination of agents can be administered at or about the same time as one another or at different times.

The combinations of the invention provide several advantages. For example, because the drug combinations described herein can be used to obtain an improved (e.g., additive or synergistic) effect, it is possible to consider administering less of each drug, leading to a decrease in the overall exposure of patients to drugs, as well as any untoward side effects of any of the drugs. In addition, greater control of the disease may be achieved, because the drugs can combat the disease through different mechanisms.

Administration

With respect to the therapeutic methods of the invention, it is not intended that the administration of compounds to a mammal be limited to a particular mode of administration, dosage, or frequency of dosing; the present invention includes all modes of administration, including oral, intraperitoneal, intramuscular, intravenous, intra-articular, intralesional, subcutaneous, by inhalation, or any other route sufficient to provide a dose adequate to prevent or treat diabetes (type 1 diabetes or type 2 diabetes) and other disorders of carbohydrate or lipid metabolism, such as those described herein. One or more compounds may be administered to the mammal in a single dose or multiple doses. When multiple doses are administered, the doses may

be separated from one another by, for example, several hours, one day, or one week. It is to be understood that, for any particular subject, specific dosage regimes should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. Exemplary mammals that can be treated using the isomers, compositions, and methods of the invention include humans, primates such as monkeys, animals of veterinary interest (e.g., cows, pigs, sheep, goats, buffaloes, and horses), and domestic pets (e.g., dogs and cats). The isomers and compositions of the invention can also be administered to rodents (e.g., mice, rats, gerbils, hamsters, guinea pigs, and rabbits) for treatment purposes and/or for experimental purposes (e.g., studying the compounds' mechanism(s) of action, screening and testing efficacy of the isomers, structural design, etc.)

For clinical applications in therapy or as a prophylactic, isomers or compositions of the present invention can generally be administered, e.g., orally, subcutaneously, parenterally, intravenously, intramuscularly, colonically, nasally, intraperitoneally, rectally, by inhalation, or buccally. Compositions containing at least one isomer of 4-hydroxyisoleucine according to the invention that is suitable for use in human or veterinary medicine can be presented in a form permitting administration by a suitable route. These compositions can be prepared according to customary methods, using one or more pharmaceutically acceptable carriers or excipients. The carriers comprise, among other things, diluents, sterile aqueous media, and various non-toxic organic solvents. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical field, and are described, for example, in *Remington: The Science and Practice of Pharmacy* (20th ed.), ed. A.R. Gennaro, Lippincott Williams & Wilkins, 2000, Philadelphia, and *Encyclopedia of Pharmaceutical Technology*, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York. The compositions can be presented in the form of tablets, pills, granules, powders, aqueous solutions or suspensions, injectable solutions, elixirs, or syrups, and the compositions may optionally contain one or more agents chosen from the group comprising sweeteners, flavorings, colorings, and stabilizers in order to obtain pharmaceutically acceptable preparations.

The choice of vehicle and the content of active substance in the vehicle are generally determined in accordance with the solubility and chemical properties of the product, the particular mode of administration, and the provisions to be observed in pharmaceutical practice. For example, excipients such as sodium citrate, calcium carbonate, and dicalcium phosphate and disintegrating agents such as starch, alginic

acids, and certain complex silicates combined with lubricants (e.g., magnesium stearate, sodium lauryl sulfate, and talc) can be used for preparing tablets. To prepare a capsule, it is advantageous to use high molecular weight polyethylene glycols. When aqueous suspensions are used, they can contain emulsifying agents that facilitate suspension. Diluents such as ethanol, polyethylene glycol, propylene glycol, glycerol, chloroform, or mixtures thereof can also be used. In addition, low calorie sweeteners, such as, for example, isomalt, sorbitol, xylitol, can be used in a formulation of the invention.

For parenteral administration, emulsions, suspensions, or solutions of the compositions of the invention in vegetable oil (e.g., sesame oil, groundnut oil, or olive oil), aqueous-organic solutions (e.g., water and propylene glycol), injectable organic esters (e.g., ethyl oleate), or sterile aqueous solutions of the pharmaceutically acceptable salts can be used. The solutions of the salts of the compositions of the invention are especially useful for administration by intramuscular or subcutaneous injection. Aqueous solutions that include solutions of the salts in pure distilled water can be used for intravenous administration with the proviso that (i) their pH is adjusted suitably, (ii) they are appropriately buffered and rendered isotonic with a sufficient quantity of sodium chloride, and (iii) they are sterilized by heating, irradiation, or microfiltration. Suitable compositions containing the isomers of the invention can be dissolved or suspended in a suitable carrier for use in a nebulizer or a suspension or solution aerosol, or can be absorbed or adsorbed onto a suitable solid carrier for use in a dry powder inhaler. Solid compositions for rectal administration include suppositories formulated in accordance with known methods. It is understood that the appropriate doses and concentrations of the agent(s) in the formulations (i.e., isomer(s) of 4-hydroxyisoleucine alone and/or in combination with other drug(s)) will vary, depending on a number of factors including the dosages of the agents to be administered, the route of administration, the nature of the agent(s), the frequency and mode of administration, the therapy desired, the form in which the agent(s) are administered, the potency of the agent(s), the sex, age, weight, and general condition of the subject to be treated, the nature and severity of the condition treated, any concomitant diseases to be treated, and other factors that will be apparent to those of skill in the art. A dose of the pharmaceutical composition contains at least a therapeutically effective amount of an isomer according to the invention and is preferably made up of one or more pharmaceutical dosage units. The selected dose can be administered to a human subject in need of treatment. A "**therapeutically effective amount**" is intended to mean that amount of isomer(s) of

the invention that confers a therapeutic effect on the subject treated. The therapeutic effect may be objective (i.e., measurable by some test or marker (e.g., insulin or glucose levels)) or subjective (i.e., the subject gives an indication of or feels an effect).

A dose of the pharmaceutical composition contains at least a therapeutically effective amount of an isomer according to the invention and is preferably made up of one or more pharmaceutical dosage units. The selected dose can be administered to a mammal, for example, a human patient, in need of treatment. As is noted above, a "therapeutically effective amount" is intended to mean that amount of isomer(s) according to the invention that, when administered to a subject for treating a disease, confers a therapeutic effect on the subject treated. The therapeutic effect may be objective (i.e., measurable by some test or marker (e.g., insulin or glucose blood levels)) or subjective (i.e., the subject gives an indication of or feels an effect). For instance, in one embodiment relating to type 2 diabetes, a "therapeutically effective" amount will increase glucose uptake by muscle and/or adipose tissues, and/or it will stimulate insulin secretion by pancreatic β-cells. In another embodiment relating to type 2 diabetes, a "therapeutically effective" amount reduces glucose levels and/or increase insulin levels in the subject's blood by, for example, at least about 20%, or by at least about 40%, or even by at least about 60%, or by at least about 80% relative to untreated subjects.

The amount that will correspond to a "therapeutically effective amount" will vary depending upon factors such as the particular compound, the route of administration, excipient usage, the disease condition and the severity thereof, the identity of the subject in need thereof, the age, weight, etc., of the subject to be treated and the possibility of co-usage with other agents for treating a disease. Nevertheless the therapeutically effective amount can be readily determined by one of skill in the art.

For administration to mammals, and particularly humans, it is expected that in the treatment of an adult dosages from about 0.1 mg to about 50 mg (e.g., about 5 mg to about 100 mg, about 1 mg to about 50 mg, or about 5 mg to about 25 mg) of each active compound per kg body weight per day can be used. A typical oral dosage can be, for example, in the range of from about 50 mg to about 5 g per day, (e.g., about 100 mg to about 4 g, 250 mg to 3 g, or 500 mg to 2 g), administered in one or more dosages, such as 1 to 3 dosages. Dosages can be increased or decreased as needed, as can readily be determined by those of skill in the art. For example, the amount of a particular agent can be decreased when used in

combination with another agent, if determined to be appropriate. In addition, reference can be made to standard amounts and approaches that are used to administer the agents mentioned herein.

Examples of dosages for antidiabetic agents mentioned herein are provided in **Table 1**, below. The antidiabetic agents can be used in these dosages when combined with a configurational isomer of 4-hydroxyisoleucine, which generally is administered in an amount in the range of, for example, 250 mg - 1 g/day (e.g., 350-900, 450-800, or 550-700 mg/day). Alternatively, due to the potential additive or synergistic effects obtained when using drug combinations of the invention, the amounts in Table 1 and/or the amount of isomer administered can be decreased (by, e.g., about 10-70%, 20-60%, 30-50%, or 35-45%), as determined to be appropriate by those of skill in this art.

The physician in any event will determine the actual dosage that will be most suitable for an individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

As for dosing, it is understood that duration of a treatment using any of the compounds or compositions of the invention will vary depending on several factors, such as those listed herein before for dosing. Nevertheless, appropriate duration of administration can be readily determined by one of skill in the art. According to certain embodiments, the compounds of the invention are administered on a daily, weekly or on a continuous basis.

Table 1: List of well-known antidiabetic agents

Antidiabetic agent	Recommended dosage and/or administration
Insulin	400 IU per vial - 40 IU per day (mean value)
Gliclazide (Diamicron)	80 mg/tablet - 1 to 4 tablets per day
Glibenclamide (Daonil) or Glyburide (Micronase, Glynase, Diabeta)	5 mg/tablet - 1 to 3 tablets per day (Glibenclamide); 1.25 to 6 mg/tablet - 1 to 2 tablets per day (Glyburide)
Glipizide (Glucotrol, Glibenese)	5 mg/tablet - 1 to 4 tablets per day
Glimepiride (Amaryl, Amarel)	1 to 4 mg/tablet - 6 mg per day maximum
Chlorpropamide (Diabinese)	250 mg/tablet - 125 to 1000 mg per day per day
Tolbutamide	500 mg/tablet - 1 to 4 tablets per day
Repaglinide (Prandin)	0.5 to 16 mg per day

Antidiabetic agent	Recommended dosage and/or administration
Nateglinide, Senaglinide (Starlix)	60 to 120 mg/tablet - 3 tablets per day
Tolazamide	100 to 500 mg/tablet
Rosiglitazone	2 to 8 mg/tablet - 8 mg per day maximum
Pioglitazone	15 to 45 mg/tablet - 15 to 45 mg per day
Troglitazone	200 to 400 mg/tablet - 200 to 600 mg per day
Ciglitazone	0.1 mg/tablet
Exenatide (Amylin)	0.09 to 0.270 mg per day
Acarbose	50 to 100 mg/tablet - 150 to 600 mg per day
Miglitol	50 to 100 mg/tablet - 150 to 300 mg per day
Voglibose	0.1 to 0.9 mg per day
Phentolamine	50 mg - 4 to 6 times per day
Cholestyramine (Colestipol)	4 g/unit - 12 to 16 g per day
Clofibrate	500 mg/capsule - 1 to 4 capsules per day
Gemfibrozil (Lipur)	450 mg/tablet - 2 tablets per day
Lovastatin	10 and 20 mg/tablet
Pravastatin	20 mg/tablet - 10 to 40 mg per day
Simvastatin (Zocor, Lodales)	5 and 20 mg/tablet - 5 to 40 mg per day
Probucol	250 mg/tablet - 1 g per day
Dextrothyroxine	2 to 6 mg per day
Alprenolol	50 mg/tablet - 4 to 8 tablets per day
Atenolol	50 to 100 mg / tablet - 100 to 200 mg per day
Timolol	10 mg/tablet - 10 to 20 mg per day
Pindolol	5 and 15 mg/tablet - 5 to 60 mg per day
Propranolol	40 mg/tablet - 80 to 160 mg per day
Metoprolol	100 and 200 mg/tablet - 50 to 200 mg per day
Captopril	25 and 50 mg/tablet - 12.5 to 150 mg per day
Enalapril	5 and 20 mg/tablet - 5 to 40 mg per day
Nifedipine	10 mg/capsule - 30 to 60 mg per day
Diltiazem	60 mg/tablet - 3 to 6 tablets per day
Verapamil	120 and 240 mg/capsule - 240 to 360 mg per day
Doxazosin	2 to 8 mg per day
Prazozin	2.5 and 5 mg/tablet - 2.5 to 20 mg per day

The isomers and compositions of the invention are conceived to be effective primarily in the treatment of disorders of carbohydrate metabolism, particularly type 2 diabetes. However, it is conceivable that the isomers and compositions according to the present invention may also be useful in connection with disorders of fat metabolism, including but not limited to lipodystrophy associated with HIV and lipidemia, because they may influence fat distribution.

It is also conceivable to use isomers of the invention for others related or unrelated applications. For instance, it might be useful to provide in-dwelling devices such as catheters coated with the isomers of the invention, for improving cardiovascular functions.

EXAMPLES

The Examples set forth herein below provide exemplary syntheses of the compounds of the invention. Also provided are exemplary methods for assaying the compounds of the invention for their activity as stimulators of glucose uptake and as stimulators of insulin secretion. These examples are given to enable those skilled in the art to more closely understand and to practice the present invention and are not intended to either define or limit its scope.

Example 1: General procedure for the *preparation of isomers of 4-hydroxyisoleucine*

A) General Experimental Procedures

A scheme for the synthesis of the eight different configurational isomers (*SRS*, *SRR*, *SSS*, *SSR*, *RSR*, *RSS*, *RRR*, and *RRS*) of 4-hydroxyisoleucine is given in Figure 1. Imine intermediate **1** was prepared from *p*-anisidine and ethyl glyoxalate (Cordova et al., A highly enantioselective amino acid-catalyzed route to functionalized alpha-amino acids, *J. Am. Chem. Soc.* 124:1842-43, 2002). The reaction of imine **1** with 2-butanone in the presence of L-proline as a catalyst followed by silica gel chromatography yielded 2*S*,3*S* isomer **2**. Epimerization at C-3 was achieved with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) to yield 2*S*,3*R* isomer **3**. The (2*S*,3*R*,4*S*); (2*S*,3*R*,4*R*); (2*S*,3*S*,4*S*); and (2*S*,3*S*,4*R*) isomers of 4-hydroxyisoleucine are obtained from either **2** or **3** as follows:

Deprotection of amine moiety of **3** (removal of p-methoxyphenyl group) with ceric ammonium nitrate (CAN) and subsequent reduction with KBH_4 in water and concomitant cyclization provided lactone **4**, which upon base hydrolysis with lithium hydroxide and recrystallization from absolute ethanol gave pure (2*S*,3*R*,4*S*)-4-

hydroxyisoleucine **5**. Alternatively, deprotection of the amine moiety of **3** with CAN was followed by isolation of amine intermediate **6**, which was subsequently reduced with potassium borohydride in methanol to give the lactone intermediate **7**, which upon base hydrolysis with lithium hydroxide and recrystallization from ethanol gave (2*S*,3*R*,4*R*) 4-hydroxyisoleucine (compound **8**). Further purification of compound **8** was carried out using preparative HPLC.

Similar reactions starting from compound **2**, using sodium borohydride instead of potassium borohydride for preparation of lactone **12** from aminoketone **11** lead to the isolation of (2*S*,3*S*,4*S*) 4-hydroxyisoleucine (compound **10**) and (2*S*,3*S*,4*R*) 4-hydroxyisoleucine (compound **13**).

When compound **1** was reacted with 2-butanone in the presence of a catalytic amount of D-proline, compound **14**, which is the enantiomer of compound **2**, was formed. As above, epimerization of the C-3 of compound **14** was achieved with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) to yield *2R,3S* isomer **15**. By reaction sequences identical to those used for the preparation of compounds **5**, **8**, **10**, and **13**, the (*2R,3S,4R*); (*2R,3S,4S*); (*2R,3R,4R*); and (*2R,3R,4S*) isomers (compounds **17**, **20**, **22**, and **25**, respectively) were obtained from compounds **14** and **15**.

Detailed reaction conditions used in the preparation of compounds **1** through **25** are as follows. ^{1}H and ^{13}C NMR spectra are of D_2O solutions, and chemical shifts are reported in ppm using methanol (δ 3.34 for ^{1}H and δ 49.50 for ^{13}C) as the internal standard.

B) Detailed Experimental Procedures

Synthesis of compound **1**

To a stirred solution of *p*-anisidine (50 g, 406 mmol) in toluene (400 mL) in a 1 liter, round-bottomed flask was added sodium sulfate (200 g, ~2.5 eq). Ethyl glyoxalate (82 mL, 50% in toluene, 406 mmol) was added slowly to the above reaction mixture, and the mixture was stirred for 30 min. After this time, the sodium sufate was filtered off using celite and toluene was removed under reduced pressure. Compound **1** (80 g, 95%) was isolated after drying and used as is for the next reaction.

Synthesis of compound **2**

A mixture of 2-butanone (800 mL, 22 eq) and L-proline (15.8 g, 0.35 eq) in dry DMF (600 mL) was stirred at room temperature under nitrogen. To this reaction mixture was slowly added a solution of compound **1** in dry DMF (200 mL) and Et_3N

(22.4 mL, 0.40 eq). After stirring the reaction mixture at room temperature for 8 h, L-proline was filtered off, excess 2-butanone was removed under reduced pressure, and DMF was removed in vacuo at 50°C. The crude amine (compound **2**) was purified by column chromatography (SiO_2, 85:15 hexanes/EtOAc).

Synthesis of compound **3**

Compound **2** was dissolved in *t*-BuOMe (15 mL) and to the stirred reaction mixture was added 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) (1 mL, ~0.04 eq). The reaction mixture was stirred under nitrogen for 2 h. A solid cake was obtained after overnight evaporation of the solvent at room temperature, which upon recrystallization from hot ethanol gave compound **3** (48 g, 43% yield).

Synthesis of (2*S*,3*R*,4*S*)-4-Hydroxyisoleucine (compound **5**)

To a solution of compound **3** (11.6 g, 40 mmol) in CH_3CN (20 mL) was added a solution of ammonium cerium (IV) nitrate (CAN) (65.6 g, 3 eq) in water (120 mL) with stirring at 0°C. The color gradually changed from blue to green upon addition of CAN. The reaction mixture was stirred for 2.5 h, and the progress of the reaction followed by TLC analysis. After completion, the reaction mixture was extracted with EtOAc (4 x 150 mL) and the aqueous phase used for the next step.

The aqueous phase was neutralised to pH 7 with saturated Na_2CO_3, and cooled to -15°C and stirred. After cooling for 30 min, KBH_4 (3.2 g, 60 mmol, 1.5 eq) was added to the reaction mixture. The reaction was allowed to warm to 0°C for about 45 min and followed by TLC. The reaction mixture was then made basic with 2 N Na_2CO_3 to a pH of 8-9 and extracted with CH_2Cl_2 (5 x 400 mL). The organic phase was washed with water, dried over Na_2SO_4 and evaporated under reduced pressure to obtain a 90:10 mixture of lactones (compound **4** (3*S*,4*R*,5*S*) to compound **7** (3*S*,4*R*,5*R*); 3.73 g, 62.6%).

To a solution of the 90:10 lactone mixture in water (96 mL, 0.3 M) was added LiOH (1.1 g, 43.3 mmol, 1.5 eq), and the mixture was stirred at room temperature for 2 h. After the reaction was complete, it was acidified by careful addition of AcOH (43.3 mmol, 2.4 mL). The reaction mixture was concentrated under reduced pressure and last traces of water were removed by repeated addition and removal of ethanol. The crude product was crystallised from absolute EtOH to give 1.56 g of 98% pure (2*S*,3*R*,4*S*) 4-hydroxyisoleucine (compound **5**). Further purification by preparative

HPLC gave compound **5** as white shiny powder: mp 215-222 (subl.); $[\alpha]_D^{H2O}$ +30.7 (c,1); ^{1}H NMR (200 MHz) δ 3.90 (m, 1H), 3.84 (m, 1H), 1.91(m, 1H), 1.23 (d, J = 5.6 Hz, 3H) 0.95 (d, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz) δ 174.32, 70.46, 57.54, 41.90, 21.30, 12.70.

Synthesis of (2*S*,3*R*,4*R*)-4-Hydroxyisoleucine (compound **8**)

To a solution of compound **3** (11.6 g, 40 mmol) in CH_3CN (20 mL) was added a solution of ceric ammonium nitrate (CAN) (65.6 g, 3 eq) in water (120 mL) with stirring at 0°C. The color gradually changed from blue to green upon addition of CAN. The reaction mixture was stirred for 45 min, and the progress of the reaction followed by TLC. After completion, the reaction mixture was extracted with EtOAc (4 x 150 mL) and the aqueous phase was carefully neutralised with saturated Na_2CO_3 solution to slightly basic pH (~8). The aqueous phase was extracted with CH_2Cl_2 (4 x 150 mL) and organic extracts were combined, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield 5.52 g (79.7%) of compound **6** as a brownish oil.

To a solution of compound **6** in methanol (15 mL), cooled to 0°C, was quickly added KBH_4 (2.58 g, 47.8 mmol). The reaction mixture was stirred at 0°C for 45 min and then gradually warmed to room temperature. The solvent was removed in vacuo, and the mixture was diluted with water. The aqueous phase was extracted with CH_2Cl_2 (4 x 150 mL). The organic phase was washed with brine, dried over anhydrous Na_2SO_4 and evaporated in vacuum to give a 75:25 mixture of compound **7** (3*S*,4*R*,5*R*) to compound **4** (3*S*,4*R*,5*S*) (2.9 g, 70.2%).

The solution of compound **7**/compound **4** mixture in water (100 mL) was treated with LiOH (805 mg, 33.7 mmol) and stirred at room temperature for 1 h before carefully acidifying with AcOH (1.91 mL, 33.72 mmol). After concentrating under reduced pressure, the traces of water were removed by repeated addition and removal of absolute ethanol. A crude greyish solid was obtained from a cold solution of 90% ethanol. Further recrystallization from 90% ethanol yielded 1.4 g of 75:25 diastereomeric ratio of compound **8** to compound **5**. Repeated crystallisations improved the purity of compound **8** to 90%, and further purification using preparative HPLC gave pure (2*S*,3*R*,4*R*) 4-hydroxyisoleucine (compound **8**) as a white shiny material: mp 202-204°C (subl.); $[\alpha]_D^{H2O}$ - 21.6 (c, 0.5); ^{1}H-NMR (300 MHz) δ 4.05 (m, 1H), 3.80 (d, J = 4.2 Hz, 1H), 2.13 (m, 1H) 1.20 (d, J = 6.3 Hz, 3H), 1.05 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz) δ 174.49, 69.13, 59.97, 39.12, 20.71, 9.38.

Synthesis of (2*S*,3*S*,4*S*)-4-Hydroxyisoleucine (compound **10**)

Compound **2** (5.6 g, 20 mmol) was dissolved in acetonitrile (10 mL), and to this was added a solution of ceric ammonium nitrate (CAN) (33 g, 60 mmol) in water (60 mL) with stirring at 0°C. The reaction mixture color gradually changed from blue to green upon addition of CAN. The reaction mixture was stirred for 45 min and extracted with ethyl acetate (4 x 150 mL). The aqueous phase was neutralized with saturated Na_2CO_3 and pH was carefully adjusted to 7. After cooling the reaction mixture to -15°C for 90 min, KBH_4 (1.6 g, 30 mmol, 1.5 eq) was added. The reaction was allowed to warm up to 0°C for about 45 min and then treated with 2 N Na_2CO_3 to a pH of 8-9, followed by extraction with CH_2Cl_2 (5 x 400 mL). The organic phase was washed with water, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to obtain 1.42 g of a 75:25 mixture of lactones (compound **9** (3*S*,4*S*,5*S*) to compound **12** (3*S*,4*S*,5*R*)).

To the mixture of lactones in water (35 mL) was added LiOH (395 mg, 16.5 mmol, 1.5 eq) and the mixture was stirred at room temperature for 2 h. After this time, the reaction mixture was carefully acidified with AcOH (16.5 mmol, 0.9 mL). The solvent was removed under vacuum, and repeated addition and removal of absolute ethanol led to complete removal of water. The crude material obtained was dissolved in 90% EtOH and left overnight. The separated white solid was filtered and washed several times with EtOH, and recrystallized from 90% EtOH to obtain white crystals of (2*S*,3*S*,4*S*)-4-hydroxyisoleucine (compound **10**, 500 mg). Further purification using preparative HPLC led to pure shiny material: mp 253-255°C; $[\alpha]_D^{H2O}$ +28 (c, 0.25);^{1}H NMR (300 MHz) δ 4.11 (m, 1H), 3.87 (d, J = 2.7 Hz, 1H), 2.21 (m, 1H), 1.23 (d, J = 6.3 Hz, 3H), 0.92 (d, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz) δ 174.64, 71.39, 60.39, 38.97, 21.11, 6.19.

Synthesis of (2*S*,3*S*,4*R*)-4-Hydroxyisoleucine (compound **13**)

To a solution of compound **2** (11.6 g, 40 mmol) in acetonitrile (20 mL) was added a solution of ammonium cerium (IV) nitrate (CAN) (65.6g, 120 mmol) in water (120 mL) with stirring at 0°C. The reaction mixture color gradually changed from blue to green upon addition of CAN. The reaction mixture was stirred for 45 min and extracted with ethyl acetate (4 x 150 mL). The aqueous phase was carefully neutralised with saturated Na_2CO_3 solution to a pH of 8, followed by extraction with CH_2Cl_2 (4 x 150 mL). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield 4 g of compound **11** as brown oil.

To a solution of **11** in MeOH (15 mL) at 0°C was quickly added $NaBH_4$ (962 mg, 1.1 eq, 25.43 mmol). The reaction mixture was vigorously stirred at 0°C for 45 min and gradually warmed to room temperature. The solvent was removed under reduced pressure, the residue diluted with water, and the aqueous phase extracted with CH_2Cl_2 (4 x 150 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and evaporated in vacuum to give 2 g of a mixture of compound **12** (3*S*,4*S*,5*R*) and compound **9** (3*S*,4*S*,5*S*).

The mixture was dissolved in water (40 mL) and LiOH (556.9 mg, 18.6 mmol) was added. The reaction mixture was stirred at room temperature for 1 h and carefully acidified with AcOH (1.31 mL). The solvent was removed under vacuum. The crude product was dissolved in a minimum amount of water and the compound was loaded on a column packed with dowex 50w x 8 (H^+) resin (50 g). The column was first eluted with water 4 x 50 mL and then fractions were collected by eluting with 2 M NH_4OH. The isolated product was dissolved in 90% EtOH and left standing over night. The separated solid (250 mg) was filtered, washed with cold EtOH, and recrystalised from 90% EtOH to obtain a mixture of diastereoisomers.

This diastereoisomer mixture of compounds **10** and **13** was purified by preparative HPLC to produce (2*S*,3*S*,4*R*) 4-Hydroxyisoleucine (compound **13**) as a white shiny powder: mp 173-175°C; $[\alpha]_D^{H2O}$ + 6.0 (c, 0.25); ^1H NMR (300 MHz) δ 4.02 (d, J = 3 Hz, 1H), 3.81 (m, 1H), 2.12 (m, 1H) 1.28 (d, J = 6.6 Hz, 3H), 0.97 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz) δ 174.93, 70.18, 56.34, 40.46, 21.24, 12.15.

<u>Syntheses of (2*R*,3*S*,4*R*)-4-Hydroxyisoleucine (compound **17**), (2*R*,3*S*,4*S*)-4-Hydroxyisoleucine (compound **20**), (2*R*,3*R*,4*R*)-4-Hydroxyisoleucine (compound **22**), and (2*R*,3*R*,4*S*)-4-Hydroxyisoleucine (compound **25**)</u>

The procedures used in the syntheses of compounds **17**, **20**, **22**, and **25** were identical to those used for compounds **5**, **8**, **10**, and **13**, except that compound **1** was reacted with 2-butanone in the presence of D-proline to produce compound **14** (the antipode of compound **12**). The physical and NMR data of compounds **17**, **20**, **22**, and **25** are as follows:

<u>(2*R*,3*S*,4*R*)-4-Hydroxyisoleucine (compound **17**)</u>: mp 217-225°C (subl.); $[\alpha]_D^{H2O}$ -31 (c, 1); ^1H NMR (200 MHz) δ 3.89 (m, 1H), 3.84 (m, 1H), 1.90 (m, 1H) 1.23 (d, J = 6.4 Hz, 3H), 0.95 (d, J =7 Hz, 3H); ^{13}C NMR (50 MHz) δ 174.36, 70.43, 57.51, 41.91, 21.30, 12.6.

(2*R*,3*S*,4*S*)-4-Hydroxyisoleucine (compound **20**): mp 200-204°C (subl.); $[\alpha]_D^{H2O}$ +22 (c, 0.5); ^{1}H NMR (200 MHz) δ 4.04 (m, 1H), 3.80 (m, 1H), 2.12 (m, 1H), 1.19 (d, J = 6.2 Hz, 3H) 1.05 (d, J = 7.2 Hz, 3H); ^{13}C NMR (50 MHz) δ 174.55, 69.12, 59.97, 39.12, 20.73, 9.40.

(2*R*,3*R*,4*R*)-4-Hydroxyisoleucine (compound **22**): mp 250-254°C; $[\alpha]_D^{H2O}$ -30 (c, 0.25); ^{1}H-NMR (200 MHz) δ 4.10 (m, 1H), 3.87 (d, J = 2.6 Hz 1H), 2.23 (m, 1H) 1.23 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 7.2 Hz,3H); ^{13}C NMR (50 MHz) δ 174.64, 71.29, 60.35, 38.96, 21.12, 6.22.

(2*R*,3*R*,4*S*)-4-Hydroxyisoleucine (compound **25**): mp 173°C; $[\alpha]_D^{H2O}$ -5.6 (c, 0.25); ^{1}H NMR (300 MHz) δ 4.01 (d, J = 2.7 Hz, 1H), 3.80 (m, 1H), 2.11 (m, 1H) 1.27 (d, J = 6.3 Hz, 3H), 0.97 (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz) δ 174.96, 70.18, 56.35, 40.44, 21.23, 12.10.

Example 2: Stimulation of glucose uptake by differentiated 3T3-L1 adipocyte cells by configurational isomers of (2S,3R,4S)-4-hydroxyisoleucine

3T3-L1 adipocyte cells (ATCC; CI-173) were cultured in 12 well tissue culture plates for 3 days in order to reach confluence (Lakshmanan et al., Analysis of insulin-stimulated glucose uptake in differentiated 3T3-L1 adipocytes. *Diabetes Mellitus: Methods and Protocols*, Saire Ozcna, Ed., Humana Press Inc., Tonowa, New Jersey 97-103, 2003). The culture medium was removed and replaced with differentiation medium (Green and Meuth, *Cell* 3:127-133, 1974; Madsen et al., *Biochem. J.* 375:539-549, 2003) and the cells were incubated for an additional 9 days. The state of differentiation was confirmed by visual examination. Cell starvation was conducted for 5 hours by replacing the differentiation medium with one lacking fetal calf serum. During the last 30 minutes of the starvation period, the cells were exposed to each of the configurational isomers of 4-hydroxyisoleucine (compounds **5**, **8**, **10**, **13**, **17**, **20**, **22**, and **25**) at a concentration of 0.5 mM. Cells exposed to insulin (0.0167 U/mL; Sigma; Cat. No. 15534) for the last 30 minutes of the starvation period were used as a positive control and cells exposed to 0.5 mM isoleucine were used as a control for background uptake. All treatments were performed in quadruplicate. Cells were washed, then fresh medium containing 16 µM ^{3}H-deoxy-D-glucose (0.5 µCi/mL) and 10 µM 2-deoxy-D-glucose was added and the cells were incubated for 10 min. Glucose uptake was stopped by washing the cells with ice cold PBS. The cells were

lysed and specific activity in the lysate was determined relative to background uptake of ^{3}H-deoxy-glucose. Results, which are shown in **Figure 2**, were standardized on the basis of protein content per well.

As expected, insulin strongly promoted glucose uptake while isoleucine did not. All configurational isomers of (2*S*,3*R*,4*S*)-4-hydroxyisoleucine (compound **5**) showed good stimulation of glucose uptake, with compounds **8**, **13**, **20**, and **25** having the best activity. Accordingly, the conformational isomers of (2*S*,3*R*,4*S*)-4-hydroxyisoleucine have the potential to be therapeutic agents for the treatment of diabetes and related conditions.

Example 3: Glucose-dependent stimulation of insulin secretion in INS-1 cells by configurational isomers of (2S,3R,4S)-4-hydroxyisoleucine

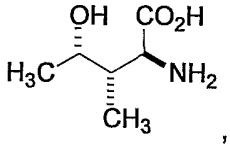
The configurational isomers of 4-hydroxyisoleucine were tested in a blinded fashion for insulinotropic effect on INS-1 cells. Briefly, the cells were plated at a density of 2 x 10^5 in 12 well plates and incubated for 2 days in RPMI with 10% fetal calf serum and 11 mM glucose. The medium was removed on the third day post-plating and replaced with RPMI containing 3 mM glucose with 10% fetal calf serum. The cells were incubated for an additional 24 hours. On the fourth day post-plating, the medium was removed and replaced with Krebs-Ringer bicarbonate buffer containing 2 mM glucose. The cells were incubated for 30 min and the buffer was removed and replaced with Krebs-Ringer bicarbonate buffer with 4.5 mM glucose containing an optical isomer at a concentration of 0.5 mM. The cells were incubated for 1 hour. Basal insulin secretion was determined by incubating the cells in the presence of buffer with 2 mM glucose. The presence of glucose at 4.5 and 10 mM stimulated insulin secretion and served as the reference control and positive control, respectively. A positive stimulatory response by a tested 4-hydroxyisoleucine configurational isomer was taken as the response above that elicited by 4.5 mM glucose. As shown in **Figure 3**, all of the configurational isomers of 4-hydroxyisoleucine except compound **13** (the *2S,3S,4R* isomer) exhibited insulinotropic activity, with compounds **8**, **10**, and **20** as active as compound **5** (the *2S,3R,4S* isomer known to have insulinotropic activity, see Broca et al., 4-Hydroxyisoleucine: effects of synthetic and natural analogues on insulin excretion, *Eur. J. Pharmacol.* 390:339-345, 2000; and Sauvaire et al., *Diabetes* 47:206-210, 1998).

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof

will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

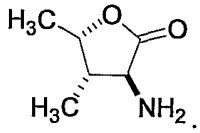
WHAT IS CLAIMED IS:

1. A compound having the following structure:



or a pharmaceutically acceptable salt, lactone, or prodrug thereof.

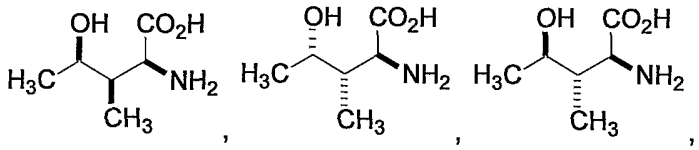
2. The compound of claim 1, wherein said lactone is

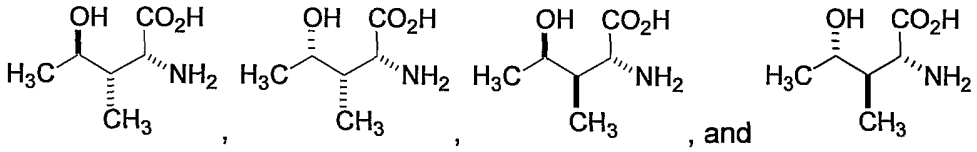


3. Use of compound according to claim 1, and/or use of a pharmaceutically acceptable salt, lactone, or prodrug of said compound, for the manufacture of a medicine for use in the prevention or treatment of a disorder of carbohydrate or lipid metabolism in a human.

4. A pharmaceutical composition comprising: (i) a compound according to claim 1,
and/or comprising a pharmaceutically acceptable salt, lactone, or prodrug of said compound; and (ii) a pharmaceutically acceptable carrier or excipient.

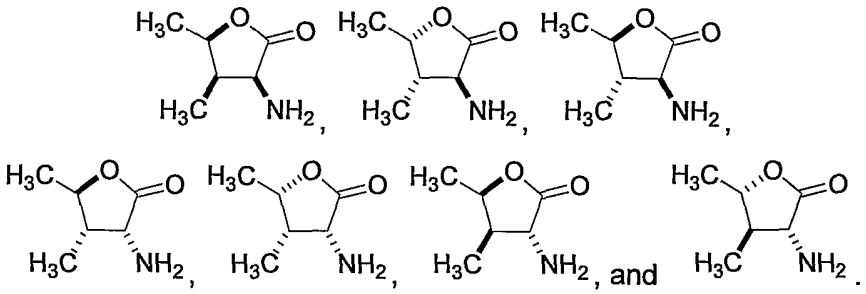
5. A pharmaceutical composition comprising: (i) a compound, selected from the group consisting of:





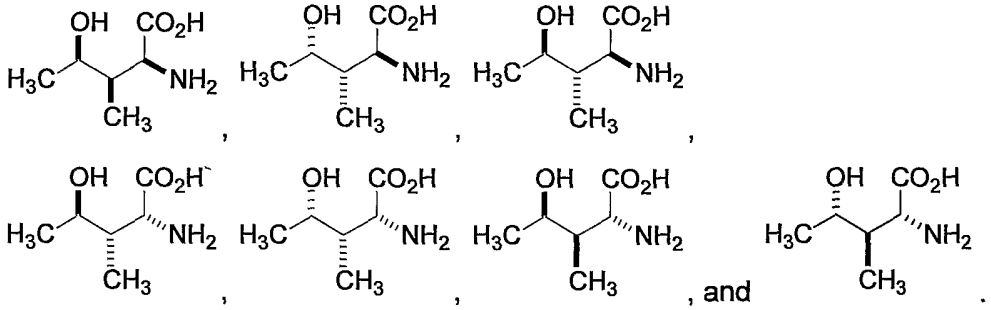
and/or a pharmaceutically acceptable salt, lactone or prodrug of said compound; and (ii) a pharmaceutically acceptable carrier or excipient.

6. The pharmaceutical composition of claim 5, wherein said lactone is selected from the group consisting of:



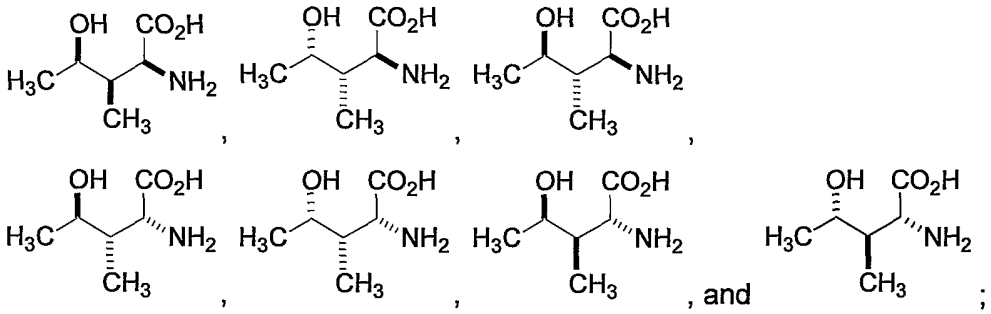
7. The pharmaceutical composition of claim 5, further comprising at least one antidiabetic agent selected from the list given in Table 1.

8. A pharmaceutical kit comprising: (i) a compound, selected from the group consisting of:



and/or a pharmaceutically acceptable salt, lactone, or prodrug of said compound; and (ii) instructions for the use of said compound for decreasing circulating glucose levels in a human patient.

9. Use of compound selected from the group consisting of:

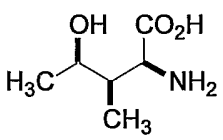
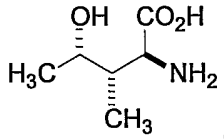
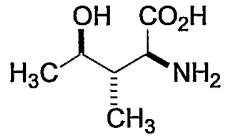
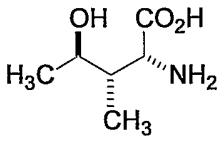
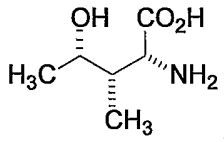
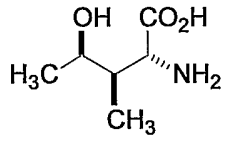
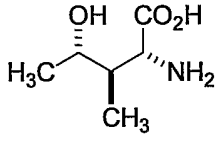


and/or use of a pharmaceutically acceptable salt, lactone, or prodrug of said compound, for the manufacture of a medicine for use in the prevention or treatment of a disorder of carbohydrate or lipid metabolism in a human.

10. The use according to claim 9, wherein said disorder of carbohydrate metabolism is diabetes mellitus.

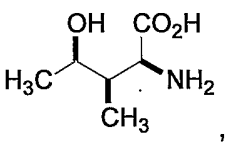
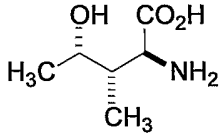
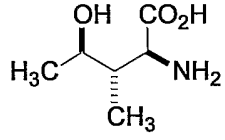
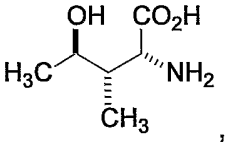
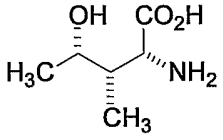
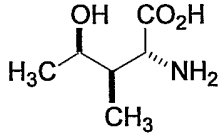
11. The use of claim 9, wherein said disorder of carbohydrate metabolism is type 2 diabetes mellitus.

12. Use of compound selected from the group consisting of:

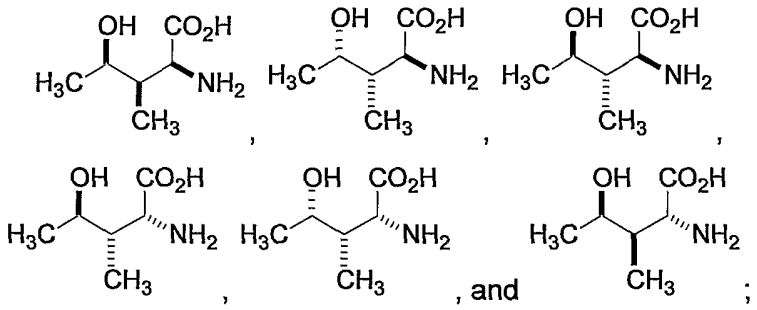
and/or use of a pharmaceutically acceptable salt, lactone, or prodrug of said compound, for the preparation of a medicine for the treatment of type 2 diabetes mellitus a human.

13. A method for stimulating glucose uptake by muscle cells and/or adipocyte cells, comprising contacting said cells with an effective amount of a compound selected from the group consisting of:

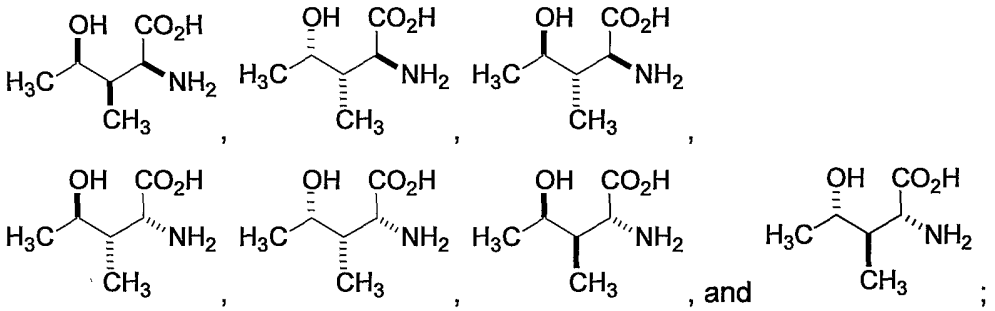
and/or with an effective amount of a pharmaceutically acceptable salt, lactone, or prodrug of said compound.

14. A method for stimulating insulin secretion by pancreatic β-cells, comprising contacting said cells with an effective amount of a compound selected from the group consisting of:



and/or with an effective amount of a pharmaceutically acceptable salt, lactone, or prodrug of said compound.

15. A method for treating a mammal having a disorder of carbohydrate or lipid metabolism, said method comprising administering to said mammal a compound selected from the group consisting of:



and/or administering to said mammal a pharmaceutically acceptable salt, lactone, or prodrug of said compound, wherein said compound, salt, lactone, or prodrug is(are) administered in an amount sufficient to decrease the circulating glucose level in said mammal.

16. The method of claim 15, wherein said mammal is selected from the group consisting of primates, animals of agricultural and veterinary interest, rodents, and domestic pets.

17. The method of claim 15, wherein said mammal is a human.

18. The method of claim 15, wherein said disorder of carbohydrate metabolism is diabetes mellitus.

19. The method of claim 15, wherein said disorder of carbohydrate metabolism is type 2 diabetes mellitus.

20. The method of claim 15, wherein said disorder of carbohydrate metabolism is Metabolic Syndrome.

21. The method of claim 15, wherein said disorder of carbohydrate metabolism is pre-diabetes.

22. The method of claim 15, wherein said disorder of lipid metabolism is type 2 diabetes.

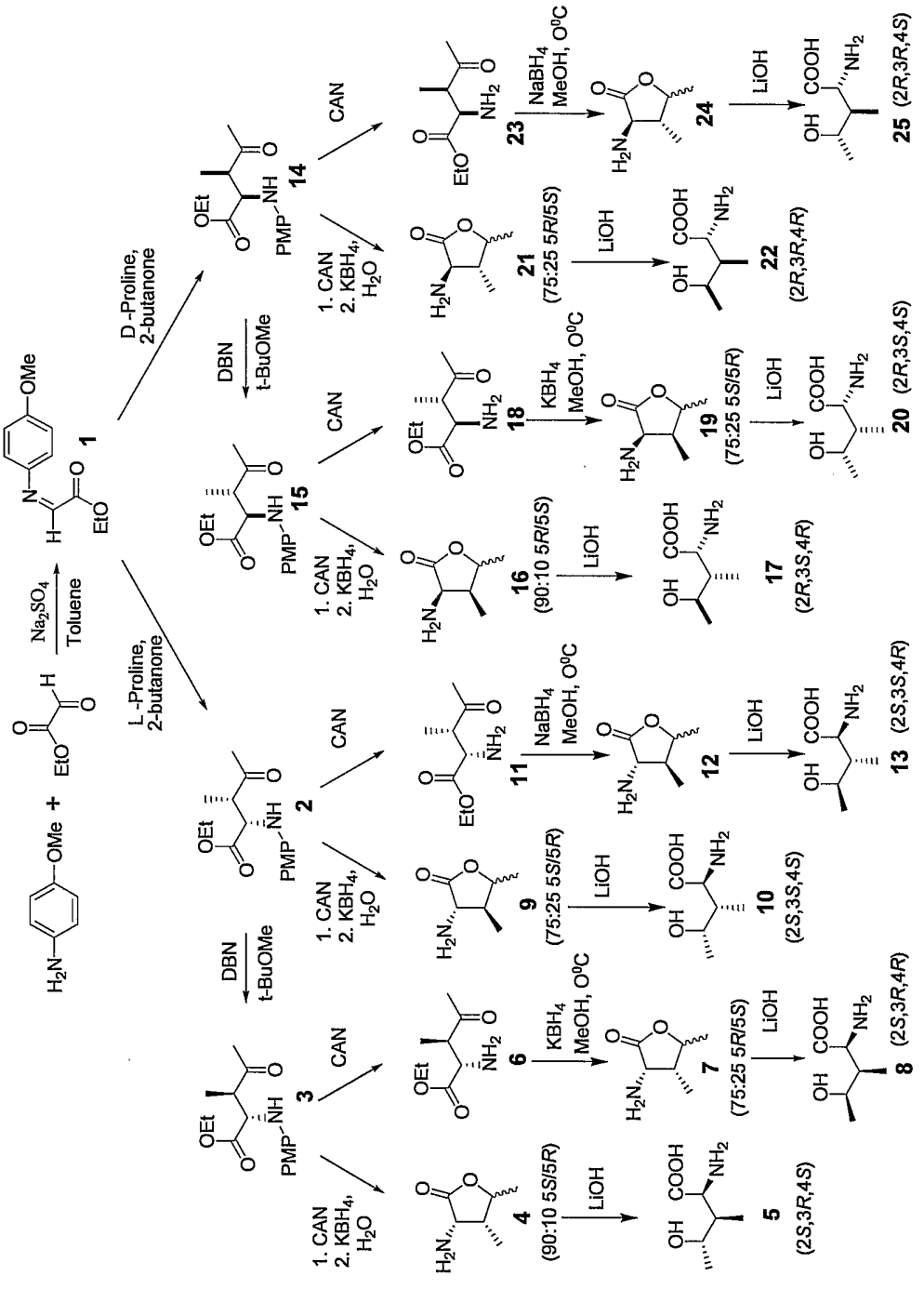

Na2SO4
Toluene
L -Proline,
2-butanone
D -Proline,
2-butanone
DBN
t-BuOMe
1. CAN
2. KBH4,
H2O
CAN
KBH4
MeOH, O°C
NaBH4
MeOH, O°C
LiOH
(90:10 5S/5R)
(75:25 5S/5R)
(75:25 5R/5S)
(90:10 5R/5S)
(2S,3R,4S)
(2S,3S,4S)
(2S,3S,4R)
(2S,3R,4R)
(2R,3S,4S)
(2R,3S,4R)
(2R,3R,4R)
(2R,3R,4S)

Figure 1

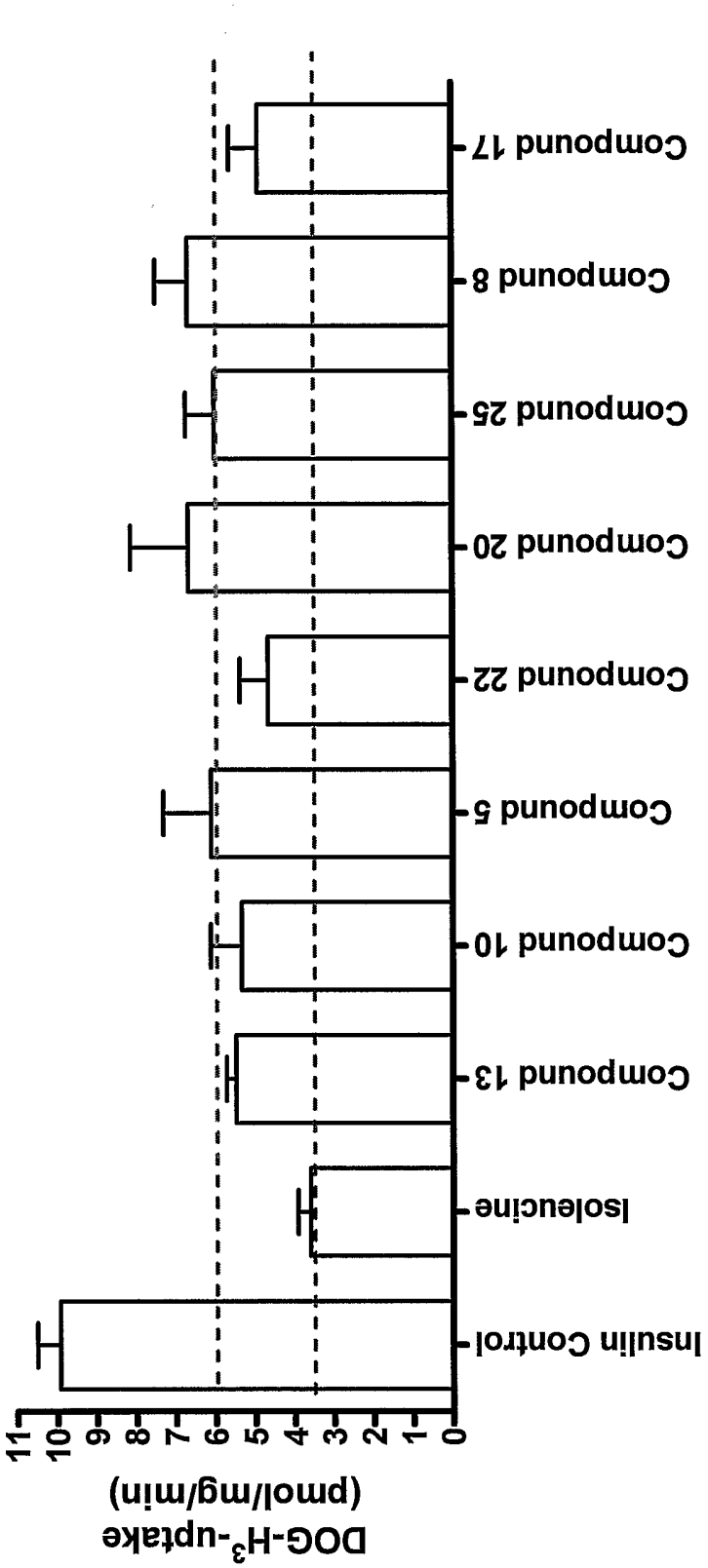

DOG-H3-uptake
(pmol/mg/min)
0
1
2
3
4
5
6
7
8
9
10
11
Insulin Control
Isoleucine
Compound 13
Compound 10
Compound 5
Compound 22
Compound 20
Compound 25
Compound 8
Compound 17

Figure 2

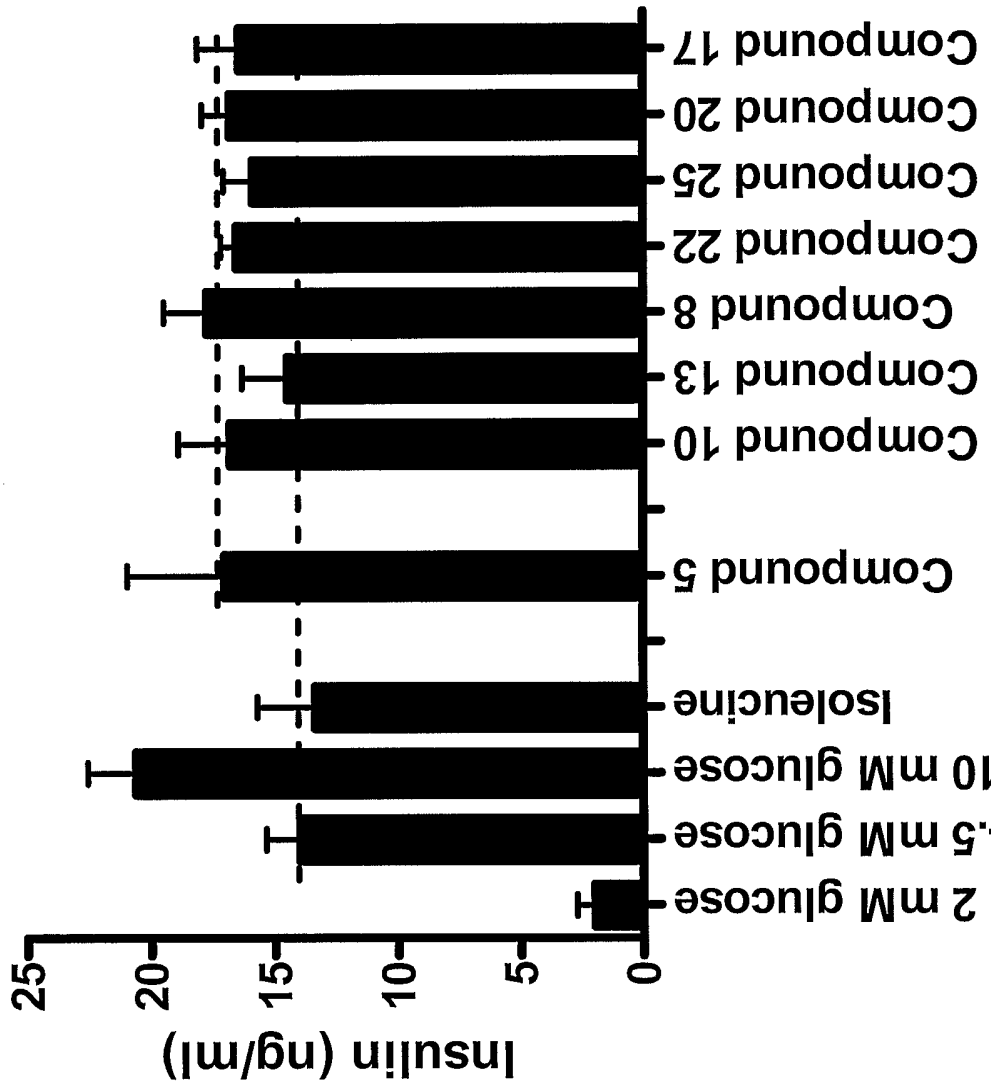

Insulin (ng/ml)
0
5
10
15
20
25
2 mM glucose
4.5 mM glucose
10 mM glucose
Isoleucine
Compound 5
Compound 10
Compound 13
Compound 8
Compound 22
Compound 25
Compound 20
Compound 17

Figure 3