

NEW 3-PHENYLPROPIONIC ACID DERIVATIVES AND THEIR USE AS PPAR- GAMMA REZEPTOR
LIGANDENField of the invention

The present invention relates to new compounds, being 3-phenylpropionic acid derivatives, pharmaceutical compositions comprising the same, and their use for the treatment and/or prevention of peroxysome proliferator-activated receptor gamma (PPAR γ) mediated diseases and conditions. The compounds show the ability to bind to PPAR γ receptor and modify its activity.

The state of the art

More than 20 years ago, the thiazolidinedione group of compounds was discovered, showing the activity in rodent models of type 2 diabetes and insulin resistance. Although their mechanism of action was not known, the compounds have been successfully used in therapy of type 2 diabetes. Publications demonstrating that they exerted their effect via the nuclear PPAR gamma receptor were published only in the middle of 90's. Now, it is well known that intracellular receptor proteins of the PPAR family control the expression of genes involved in the regulation of lipid-carbohydrate metabolism.

Diseases such as hyperlipidemia, atherosclerosis, obesity, and type 2 diabetes become the serious concern not only for developed industrial societies. It is estimated that more than 150 million people worldwide suffer from type 2 diabetes, and this number is expected to double by 2025. In Poland, currently about 2 million people suffer from this disease, and the same number is at risk of developing it. Costs of medical care in diabetic patients reach 6 to 8 percent of total medical care budgets. At the initial stage, diabetes can be symptomless, and may begin at any age; however, most often occurs at middle age and in elderly persons. The progress of type 2 diabetes is a result of overlapping of physiological disorders such as: tissue insulin resistance, insufficient pancreatic insulin production, elevated insulin production following intensified gluconeogenesis. Most often diabetic complications are microvascular changes in the retina, kidneys and nervous system, what

leads to increased risk of blindness, renal insufficiency and neuropathy. Diabetes is also the main causative factor of heart infarct and brain stroke.

PPAR γ receptors, belonging to the family of nuclear receptors, play the
5 role in the regulation of lipid metabolism and storage. They are
expressed in adipose tissue and large intestine, and are involved in the
lipogenesis process. Ligands activating PPAR γ receptor can enhance
insulin effect and lower the plasma glucose level. They can be also
10 useful in the management and therapy of lipid metabolism and energy
balance disorders.

There are known compounds being L-tyrosine derivatives or analogues,
which exert their action via modulation of the PPAR γ receptor response,
thus acting on the glucose metabolism, lipid hemostasis and energy
balance.

15 In the international patent applications Nos. WO03/011834 and
WO03/011814 there are disclosed N-(2-benzoylphenyl)-L-tyrosine
derivatives, which have a partial PPAR γ agonist activity and may be
useful in the treatment and prophylaxis of *inter alia* impaired insulin
tolerance, type 1 and 2 diabetes, dyslipidemia, disorders associated with
20 syndrome X, such as hypertension, obesity, insulin resistance,
hyperglycemia, atherosclerosis, myocardial ischemia, coronary heart
disease, renal diseases, as well as for improving cognitive functions and
for treating diabetic complications. The disclosed compounds represent
L-tyrosine derivatives wherein tyrosine hydroxyl group is substituted with
25 vinyl group and nitrogen in tyrosine amino group is substituted with 2-
benzoylphenyl group.

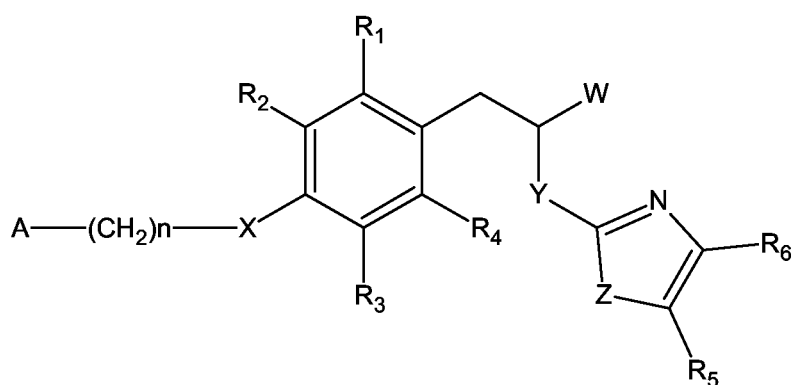
In the international patent application No. WO01/17994 there are
disclosed oxazole compounds as PPAR γ antagonists, which may be useful
in the treatment of diabetes, obesity, metabolic syndrome, impaired
30 insulin tolerance, syndrome X and cardiovascular diseases, including
dyslipidemia. The compounds represent L-tyrosine derivatives wherein
tyrosine carboxyl group is substituted with a 5-membered heterocyclic
group, tyrosine hydroxyl group is substituted with (5-methyl-2-
phenyloxazol-4-yl)ethyl group, and nitrogen in tyrosine amino group is
35 substituted with 2-benzoylphenyl group.

In the international patent application No. WO97/31907 there are disclosed 4-hydroxyphenylalcanoic acid derivatives with agonistic activity to PPAR γ . Among others, there are disclosed L-tyrosine derivatives wherein tyrosine hydroxyl group is substituted with a 5-membered heterocyclic group, which itself can be substituted, and nitrogen in tyrosine amino group is substituted with 2-substituted phenyl group, including 2-benzoylphenyl group.

In the art still exists a need for new compounds - ligands of PPAR γ , which may be useful in the treatment and/or prophylaxis of diabetes and complications resulting from or associated with diabetes, especially lipid metabolism disorders and cardiovascular diseases.

Summary of the invention

The present invention relates to 3-phenylpropionic acid derivatives of formula (I)



(I)

wherein:

W represents COOH group or its bioisosters, or -COO-C₁-C₄-alkyl group;

Y represents NH, N-C₁-C₁₀-alkyl, O, or S;

Z represents NH, N-C₁-C₁₀-alkyl, N-aryl, N-heteroaryl, S, or O;

X represents O, S, NH, N-C₁-C₁₀-alkyl, N-aryl, NSO₂-C₁-C₁₀-alkyl, N-SO₂-aryl, or N-SO₂-heteroaryl;

R₁ to R₆ each independently represents hydrogen atom or a substituent selected from the group consisting of:

- C₁-C₄-alkyl, C₁-C₄-alkoxy, C₃-C₇-cycloalkyl, C₃-C₇-cycloalkoxy, C₁-C₄-thioalkoxy, C₃-C₇-cyclothioalkoxy, halogen atom, halogen-substituted C₁-C₄-alkyl, halogen-substituted C₃-C₇-cycloalkyl, -NO₂, -CN, -SO₂-NH₂, -SO₂-NH-(C₁-C₄)-alkyl, -SO₂-N(C₁-C₄-alkyl)₂, -CO-(C₁-C₄)-alkyl, -O-CO-(C₁-C₄)-alkyl, -CO-O-(C₁-C₄)-alkyl, -CO-aryl, -CO-NH₂, -CO-NH-(C₁-C₄)-alkyl, -CO-N(C₁-C₄-alkyl)₂, aryl and heteroaryl, said aryl and heteroaryl being optionally substituted with one or more substituents independently selected from the group consisting of C₁-C₄-alkyl, C₁-C₄-alkoxy, C₃-C₇-cycloalkyl, C₃-C₇-cycloalkoxy, C₁-C₄-thioalkoxy, C₃-C₇-cyclothioalkoxy, halogen atom; halogen-substituted C₁-C₄-alkyl, halogen-substituted C₃-C₇-cycloalkyl; -NO₂, -CN, -SO₂-NH₂, -SO₂-NH-(C₁-C₄)-alkyl, -SO₂-N(C₁-C₄-alkyl)₂, -CO-(C₁-C₄)-alkyl, -O-CO-(C₁-C₄)-alkyl, -CO-O-(C₁-C₄)-alkyl, -CO-aryl, -CO-NH₂, -CO-NH-(C₁-C₄)-alkyl, -CO-N(C₁-C₄-alkyl)₂;

A represents C₁-C₄-alkyl, C₃-C₇-cycloalkyl, halogen-substituted C₁-C₄-alkyl, halogen-substituted C₃-C₇-cycloalkyl, aryl, heteroaryl, heterocyclyl, -NH-CO-(C₁-C₄)-alkyl, -N(C₁-C₄-alkyl)-CO-(C₁-C₄)-alkyl, -NH-CO-aryl, -N(C₁-C₄-alkyl)-CO-aryl, -N(C₁-C₄-alkyl)-CO-C₃-C₇-cycloalkyl, -NH-CO-NH₂, -NH-CO-NH-(C₁-C₄)-alkyl, -NH-CS-NH-(C₁-C₄)-alkyl, -NH-CO-NH-aryl, -NH-CS-NH-aryl, -SO₂-(C₁-C₄)-alkyl, -SO₂-aryl, or -SO₂-heteroaryl, wherein aryl, heteroaryl and heterocyclyl are optionally substituted with one or more substituents independently selected from the group consisting of C₁-C₄-alkyl, C₁-C₄-alkoxy and halogen atom; and

n represents an integer from 0 to 4, inclusive;

and pharmaceutically acceptable salts thereof.

One group of compounds of the invention comprises those compounds wherein W represents COOH.

Another group of compounds of the invention comprises those compounds wherein W represents -COO-C₁-C₄-alkyl, -COO-CH₃ group being preferred.

Another group of compounds of the invention comprises those compounds wherein Y represents NH.

Another group of compounds of the invention comprises those compounds wherein Y represents O.

- 5 Another group of compounds of the invention comprises those compounds wherein Y represents N-C₁-C₄-alkyl, N-CH₃ being preferred.

Still another group of compounds of the invention comprises those compounds wherein Z represents O.

- 10 Still another group of compounds of the invention comprises those compounds wherein Z represents S.

Still another group of compounds of the invention comprises those compounds wherein Z represents N-C₁-C₄-alkyl, especially N-CH₃.

Still another group of compounds of the invention comprises those compounds wherein Z represents N-phenyl.

- 15 Still another group of compounds of the invention comprises those compounds wherein X represents O.

Still another group of compounds of the invention comprises those compounds wherein X represents S.

- 20 Still another group of compounds of the invention comprises those compounds wherein X represents NSO₂-C₁-C₄-alkyl, especially NSO₂-CH₃.

Still another group of compounds of the invention comprises those compounds wherein W represents COOH, Y represents NH, Z represents S and X represents O.

- 25 Still another group of compounds of the invention comprises those compounds wherein W represents -COO-C₁-C₄-alkyl, especially -COO-CH₃, Y represents NH, Z represents S, and X represents O.

Still another group of compounds of the invention comprises those compounds wherein W represents COOH, Y represents NH, Z represents O, and X represents O.

Still another group of compounds of the invention comprises those compounds wherein W represents COOH, Y represents NH, Z represents O, and X represents NSO₂-C₁-C₄-alkyl, especially NSO₂-CH₃.

- 5 Still another group of compounds of the invention comprises those compounds wherein W represents COOH, Y represents NH, Z represents S, and X represents NSO₂-C₁-C₄-alkyl, especially NSO₂-CH₃.

A particular embodiment of the compounds of formula (I) as defined above are those compounds, wherein each of R₁ to R₆ represents hydrogen atom.

- 10 Another particular embodiment of the compounds of formula (I) as defined above are those compounds, wherein n is equal to 1 or 2.

- Another group of compounds of the invention comprises those compounds wherein A represents aryl or heteroaryl, said aryl or heteroaryl being optionally substituted with one or more substituents independently selected from the group consisting of C₁-C₄-alkyl, C₁-C₄-alkoxy, C₁-C₄-thioalkoxy, CN, halogen atom, and phenyl.
- 15

Within the above group, A preferably represents represents isoxazolyl, optionally substituted with one or more substituents independently selected from C₁-C₄-alkyl, especially -CH₃.

- 20 Also preferably, A represents phenyl, said phenyl being optionally substituted with one or more substituents independently selected from the group consisting of C₁-C₄-alkyl, C₁-C₄-alkoxy, C₁-C₄-thioalkoxy, CN, halogen atom, and phenyl, preferably with CN or -CH₃.

- Further group of compounds of the invention comprises those compounds wherein A represents -N(C₁-C₄-alkyl)-CO-C₃-C₇-cycloalkyl, especially -N(CH₃)-CO-cyclohexyl.
- 25

- Further group of compounds of the invention comprises those compounds wherein one of R₅ and R₆ represents phenyl, optionally substituted with a substituent selected from the group consisting of C₁-C₄-alkyl, C₁-C₄-alkoxy, C₃-C₇-cycloalkyl, C₃-C₇-cycloalkoxy, C₁-C₄-thioalkoxy, C₃-C₇-cyclothioalkoxy, halogen atom, halogen-substituted -C₁-C₄-alkyl, halogen-substituted -C₃-C₇-cycloalkyl, -NO₂, -CN, -SO₂-NH₂, -SO₂-NH-C₁-
- 30

C₄-alkyl, -SO₂-N(C₁-C₄-alkyl)₂, -CO-C₁-C₄-alkyl, -O-CO-C₁-C₄-alkyl, -CO-O-C₁-C₄-alkyl, -CO-aryl, -CO-NH₂, -CO-NH-C₁-C₄-alkyl, and -CO-N(C₁-C₄-alkyl)₂, and the other of R₅ and R₆ represents hydrogen atom.

Preferably, one of R₅ and R₆ represents phenyl, optionally substituted
5 with a substituent selected from CN and C₁-C₄-alkyl, especially CH₃.

As examples of specific compounds of the invention, the following can be mentioned:

methyl (2S)-3-{4-[(3,5-dimethylisoxazol-4-yl)methylenoxy]phenyl}-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionate,

10 (2S)-3-{4-[(3,5-dimethylisoxazol-4-yl)methylenoxy]phenyl}-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionic acid,

methyl (2S)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionate,

15 (2S)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionic acid,

methyl (2S)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(4-cyanophenyl-1,3-thiazol-2-yl)amino]propionate,

(2S)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(4-cyanophenyl-1,3-thiazol-2-yl)amino]propionic acid,

20 methyl (2S)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(4-methylphenyl-1,3-thiazol-2-yl)amino]propionate,

(2S)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(4-methylphenyl-1,3-thiazol-2-yl)amino]propionic acid,

25 methyl 3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(5-phenyl-1,3-oxazol-2-yl)oxy]propionate,

3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(5-phenyl-1,3-oxazol-2-yl)oxy]propionic acid,

methyl 3-[4-(benzyloxy)phenyl]-2-(5-phenyl-1*H*-imidazol-2-ylthio)-propionate, and

30 3-[4-(benzyloxy)phenyl]-2-(5-phenyl-1*H*-imidazol-2-ylthio)propionic acid,
and pharmaceutically acceptable salts thereof.

The compounds of the invention have high affinity to peroxisome proliferator-activated receptors gamma (PPAR γ). Thus the compounds demonstrate the ability to bind to PPAR γ and to modulate its activity.

5 The compounds of the invention, wherein W represents -COO-C₁-C₄-alkyl, are the prodrugs of the compounds of the invention, wherein W represents COOH group.

10 The invention relates also to a pharmaceutical composition, comprising at least one compound of formula (I) as defined above, or its pharmaceutically acceptable salt, in combination with optional other pharmacologically active ingredients, together with one or more pharmaceutically acceptable carriers and/or excipients.

The invention relates also to a compound of formula (I) as defined above, for use as a medicament.

15 The invention further relates to a use of a compound of formula (I) as defined above or its pharmaceutically acceptable salt, for the preparation of a medicament for the treatment and/or prophylaxis of the diseases and conditions, mediated by peroxisome proliferator-activated receptors gamma (PPAR γ).

20 Such PPAR γ -mediated diseases and conditions include in particular impaired insulin tolerance, insulin resistance, type 1 and type 2 diabetes, complications resulting from or associated with diabetes, such as peripheral neuropathy, renal insufficiency, retinopathy, dyslipidemia, syndrome X associated disorders, such as hypertension, obesity, hyperglycemia, atherosclerosis, myocardial ischemia, coronary heart disease, and other cardiovascular diseases, and renal diseases.

25 The compounds of the invention can be also useful for improving cognitive functions, such as in dementia.

Detailed disclosure of the invention

30 Definitions

The term „bioisoster” as used herein relates to a chemical moiety, which replaces another moiety in a molecule of an active compound without

significant influence on its biological activity. Other properties of the active compound, such as for example its stability as a medicament, can be affected in this way.

As bioisoster moieties for carboxy (COOH) group can be mentioned especially 5-membered heterocyclic groups having from 1 to 4 heteroatoms selected from nitrogen, oxygen and sulphur, such as for example 1,3,4-oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,5-oxadiazolyl, 1,2,4-oxadiazolyl, 1,3,4-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,3-thiadiazolyl, 1,2,5-thiadiazolyl, furyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, isoxazolyl, isothiazolyl, and N-substituted tetrazolyl. 5-Membered heterocyclic groups can be optionally substituted with 1 or 2 substituents selected from the group comprising phenyl, pyridinyl, straight or branched alkyl group, amino group, hydroxy group, fluoro, chloro, bromo, iodo, trifluoromethyl, trifluoromethoxy, trifluorothiomethoxy, alkoxy, and thioalkoxy.

As bioisoster moieties for carboxy (COOH) group can be also mentioned phenyl and 6-membered heterocyclic groups having from 1 to 4 heteroatoms selected from nitrogen, oxygen and sulphur, such as for example pyridinyl, pyrazinyl, pyridazinyl, pyrimidinyl, triazinyl, tetrazinyl, and others. Phenyl and 6-membered heterocyclic groups can be optionally substituted with 1 or 2 substituents selected from the group comprising phenyl, pyridinyl, straight or branched alkyl group, amino group, hydroxy group, fluoro, chloro, bromo, iodo, trifluoromethyl, trifluoromethoxy, trifluorothiomethoxy, alkoxy, and thioalkoxy.

The term "halogen" relates to an atom selected from F, Cl, Br and I.

The term "alkyl" relates to a saturated, straight or branched hydrocarbon group, having indicated number of carbon atoms. As specific alkyl substituents, the following can be mentioned: methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl, pentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 2,2-dimethylpropyl, 1-ethylpropyl, hexyl, 1-methylpentyl, 2-methylpentyl, 1-ethylbutyl, 2-ethylbutyl, 3,3-dimethylbutyl, heptyl, 1-ethylpentyl, octyl, nonyl, and decyl.

The term "aryl" relates to a mono- or bicyclic aromatic group, having from 6 to 14 carbon atoms. The examples of aryl groups are phenyl, tolyl, xyl, naphthyl, such as naphth-1-yl, naphth-2-yl, 1,2,3,4-tetrahydronaphth-5-yl, and 1,2,3,4-tetrahydronaphth-6-yl.

- 5 The term "heteroaryl" relates to a mono- or bicyclic heteroaromatic group, having from 5 to 13 carbon atoms and 1 to 4 heteroatoms selected from N, O, and S. The examples of heteroaryl groups are pyrrol-1-yl, pyrrol-2-yl, pyrrol-3-yl, furyl, thienyl, imidazolyl, oxazolyl, thiazolyl, isoxazolyl, 1,2,4-triazolyl, oxadiazolyl, thiadiazolyl, tetrazolyl, pyridinyl, pyrimidinyl, 1,3,5-triazinyl, indolyl, benzo[b]furyl, benzo[b]thienyl, indazolyl, benzimidazolyl, azaindolyl, cynnolyl, isoquinolinyl, and carbazolyl.

- 15 The term "cycloalkyl" relates to a saturated or partially unsaturated cyclic hydrocarbon group, having from 3 to 7 carbon atoms. The examples of cycloalkyl groups are cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclopentadienyl, cyclohexyl, cyclohexenyl, and cycloheptyl.

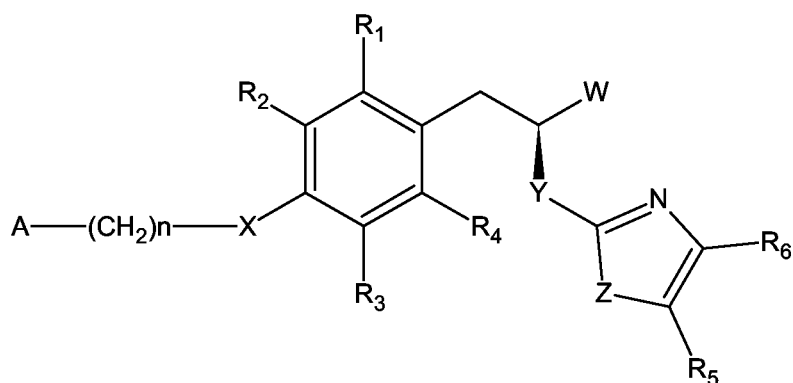
- 20 The term "heterocyclyl" relates to a saturated or partially unsaturated 5- to 6-membered cyclic hydrocarbon group, having from 1 to 4 heteroatoms, selected from N, O and S. Preferred saturated or partially unsaturated cyclic hydrocarbon is monocyclic and includes 4 or 5 carbon atoms and 1 to 3 heteroatoms. The examples of heterocyclyl groups are piperidinyl, piperazinyl, morpholinyl, and pyrrolidinyl.

- 25 The compounds of the invention possess chiral center at the carbon atom bearing W group and can exist in the form of the respective enantiomers, enantiomer mixtures as well as racemic mixtures.

- Therefore, the R and S enantiomers, enantiomer mixtures as well as racemic mixtures of the compounds of formula (I) form the part of the invention.

Thus in one specific embodiment, the invention relates to compounds of formula (I) having the stereochemical configuration such as shown in formula (IA):

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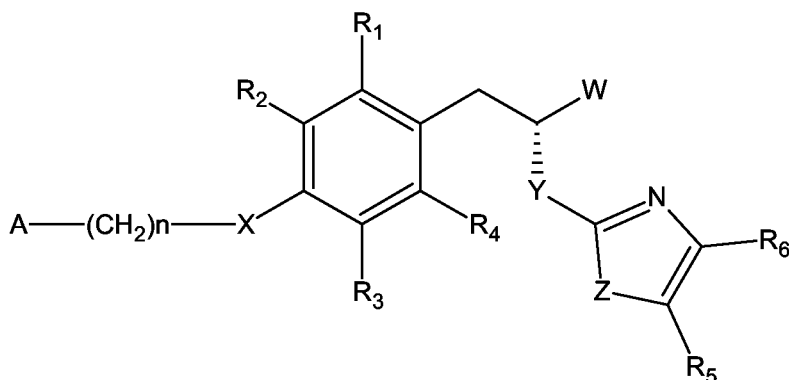


(IA)

wherein W, X, Y, Z, A, n, and R₁ to R₆ have the same meanings as defined above for formula (I),

- 5 and pharmaceutically acceptable salts thereof.

In the second specific embodiment, the invention relates to compounds of formula (I) having the stereochemical configuration such as shown in formula (IB):



(IB)

10

wherein W, X, Y, Z, A, n, and R₁ to R₆ have the same meanings as defined above for formula (I),

and pharmaceutically acceptable salts thereof.

- 15 The compounds of formula (I), bearing a basic group, can be converted into salts with inorganic or organic acids in a conventional and known manner, by the treatment with suitable acid in organic solvent, such as alcohol, ketone, ether or chlorinated solvent, and the recovery of a salt in a conventional manner. Examples of such salts are those with

pharmaceutically acceptable inorganic or organic acids. As examples of inorganic acid salts hydrochloride, hydrobromide, nitrate, sulfate, hydrogensulfate, pyrosulfate, sulfite, pyrosulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, and pyrophosphate, can be mentioned. As examples of organic acid salts acetate, propionate, acrylate, 4-hydroxybutyrate, caprylate, capronate, decanoate, oxalate, malonate, succinate, glutarate, adipate, pimelate, maleate, fumarate, citrate, tartrate, lactate, phenylacetate, mandelate, sebacate, suberate, benzoate, phthalate, alkyl- and arylsulfonates, such as methanesulfonate, propanesulfonate, p-toluenesulfonate, xylenesulfonate, salicylate, cinnamate, glutamate, aspartate, glucuronate, and galacturonate can be mentioned.

The compounds of formula (I) bearing an acidic group can be converted into salts with inorganic or organic base in a conventional and known manner by the reaction of a compound of formula (I) with suitable organic or inorganic base. Salts with pharmaceutically acceptable bases include alkaline or alkaline earth metal salts, such as Li, Na, K, Mg or Ca, ammonium salts, and salts with basic organic compounds, such as for example arginine, histidine, piperidine, morpholine, piperazine, ethylenediamine or triethylamine, as well as quaternary ammonium salts.

The present invention relates also to pharmaceutical compositions, comprising a compound of formula (I) with pharmaceutical excipients, depending on the selected route of administration.

One of the embodiments of the invention are pharmaceutical compositions suitable for oral administration. Pharmaceutical compositions suitable for oral administration can be in the form of tablets, capsules, pills, lozenges, powders or granules, or solutions or dispersions in a liquid, or similar. Each of said forms will comprise a predetermined amount of a compound of the invention as an active ingredient. The composition in the form of a tablet can be prepared employing any pharmaceutical excipients known in the art for that purpose, and conventionally used for the preparation of solid pharmaceutical compositions. The examples of such excipients are starch, lactose, microcrystalline cellulose, magnesium stearate and binders, for example polyvinylpyrrolidone. Furthermore, an active

compound can be formulated as controlled-release preparation, such as tablets comprising hydrophilic or hydrophobic matrix.

Pharmaceutical composition in the form of a capsule can be formulated using conventional procedures, for example by incorporation of a mixture of an active compound and excipients into hard gelatin capsules. Alternatively, a semi-solid matrix of an active compound and high molecular weight polyethylene glycol can be formed and filled into hard gelatin capsules, or soft gelatin capsules can be filled with a solution of an active compound in polyethylene glycol or dispersion thereof in an edible oil. Powder forms for reconstitution before use (for example lyophilized powders) are also contemplated. Alternatively, oily vehicles for injection formulation can be used as well.

Liquid forms for parenteral administration may be formulated for administration by injection or continuous infusion.

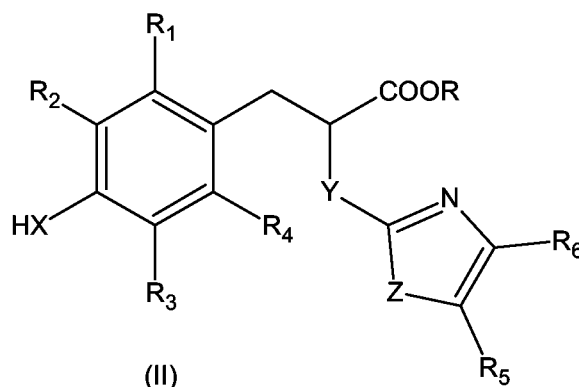
Acceptable routes of administration by injection are intravenous, intraperitoneal, intramuscular and subcutaneous, intravenous injections being usually preferred. A typical composition for intravenous injection comprises a sterile isotonic aqueous solution or dispersion, including, for example, an active compound and dextrose or sodium chloride. Other examples of suitable excipients are lactated Ringer solution for injections, lactated Ringer solution for injections with dextrose, Normosol-M with dextrose, acylated Ringer solution for injections. The injection formulation can optionally include a co-solvent, for example polyethylene glycol, chelating agent, for example ethylenediaminetetraacetic acid; stabilizing agent, for example cyclodextrin; and antioxidant, for example sodium pyrosulfate.

A dosage administered will depend on the patient condition and selected route of administration, and will be adjusted by the physician.

The compounds of the invention can be prepared using the processes described below and exemplified in the Examples.

The compounds of the invention of formula (I) wherein W represents -COOH or -COO-C₁-C₄-alkyl, and X, Y, Z, A, n, and R₁ to R₆ have the meanings as defined above for formula (I), can be prepared by:

- 5 a) a substitution of hydrogen atom at X with A(CH₂)_n- group in a compound of formula (II)

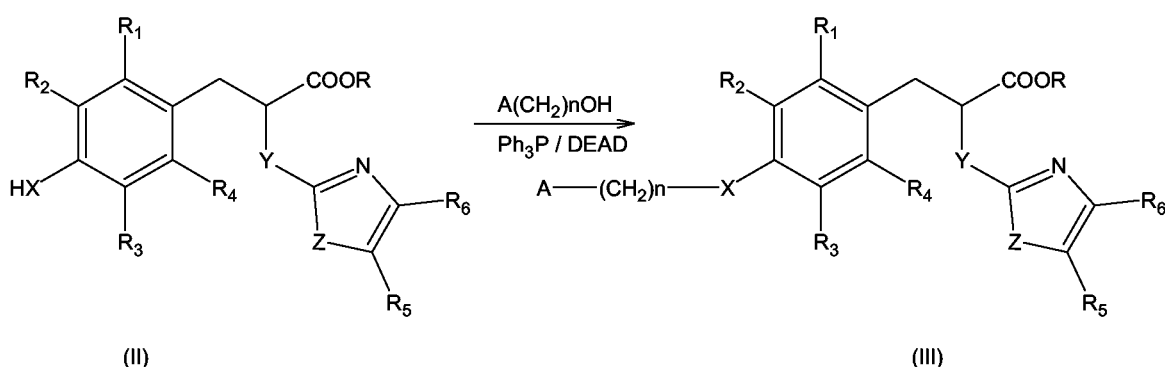


- 10 wherein R represents C₁-C₄ alkyl and X, Y, Z, and R₁ to R₆ have the meanings as defined for formula (I) above to form a compound of formula (II) wherein R represents C₁-C₄ alkyl and X, Y, Z, and R₁ to R₆ have the meanings as defined for formula (I) above, and then

- 15 b) optionally, a basic hydrolysis of the ester group -COOR to -COOH group to form a compound of formula (I) wherein W represents -COOH.

- 20 Said substitution in step a) can be performed by Mitsunobu reaction of a compound of formula (II) wherein R represents C₁-C₄ alkyl and X, Y, Z, and R₁ to R₆ have the meanings as defined for formula (I) above, with a compound of formula A(CH₂)_n-OH wherein A and n have the meanings as defined above for formula (I), according to the scheme 1:

Scheme 1

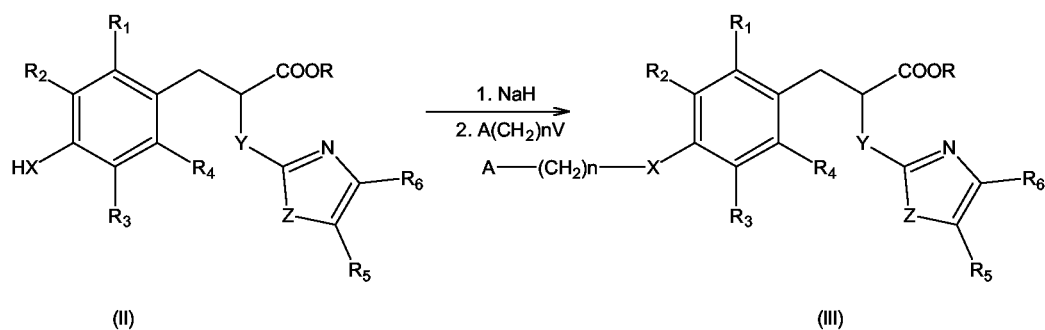


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Mitsunobu reaction can be carried out in anhydrous solvents such as ether or halogenated alkane, in the presence of diazo compounds such as DEAD, DIAD, ADDP, and triphenylphosphine, usually at -20 to $20^\circ C$.

Alternatively, said substitution of hydrogen atom at X can be carried out by alkylating a compound of formula (II) wherein R represents C_1 - C_4 alkyl, and X, Y, Z, and R_1 to R_6 have the meanings as defined for formula (I) above, with a compound of formula $A(CH_2)_n-V$ wherein $A(CH_2)_n-$ has the meaning as defined above for formula (I), and V represents a leaving group selected from halogens and alkylsulfonyl or arylsulfonyl groups, in the presence of a strong base capable of generating an anion from the compound (II), such as sodium hydride, according to the scheme 2:

Scheme 2



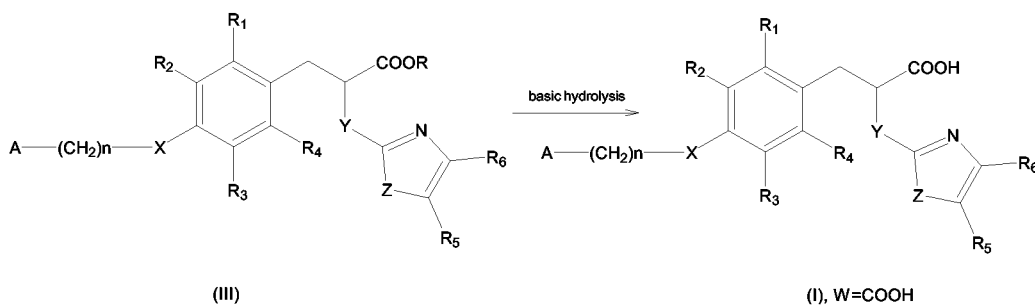
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Alkylation reaction can be performed in an inert organic solvent, such as anhydrous DMF, THF, DMSO. The strong base capable of generating the anion can be sodium hydride. Sodium hydride can be used dry or as a suspension in mineral oil. Generating of the anion is carried out at room temperature until the completion of the evolution of hydrogen. Then in the second stage the alkylating agent $A(CH_2)_n-V$ is added, neat or as a solution in an inert organic solvent such as DMF, THF, DMSO. The second step of alkylation can be carried out at 0 to 100°C.

The hydrolysis of the ester group in step b) can be carried out in basic conditions, in the manner known in the art. As the examples of the base, alkaline metal hydroxides can be mentioned, such as sodium, potassium and lithium hydroxides. For preparing single enantiomers of a compound of formula (I), it is preferable to carry out the hydrolysis with lithium hydroxide, which allows for the retention of the configuration.

Basic hydrolysis in step b) can be for example carried out in a three-solvent system consisting of THF (tetrahydrofuran), methanol and water, which allows to obtain homogenous reaction mixture. At the end of the hydrolysis, the reaction mixture can be neutralized with hydrochloric acid and, if desired, the free acid product can be extracted, for example with ethyl acetate, according to the scheme 3 shown below:

Scheme 3



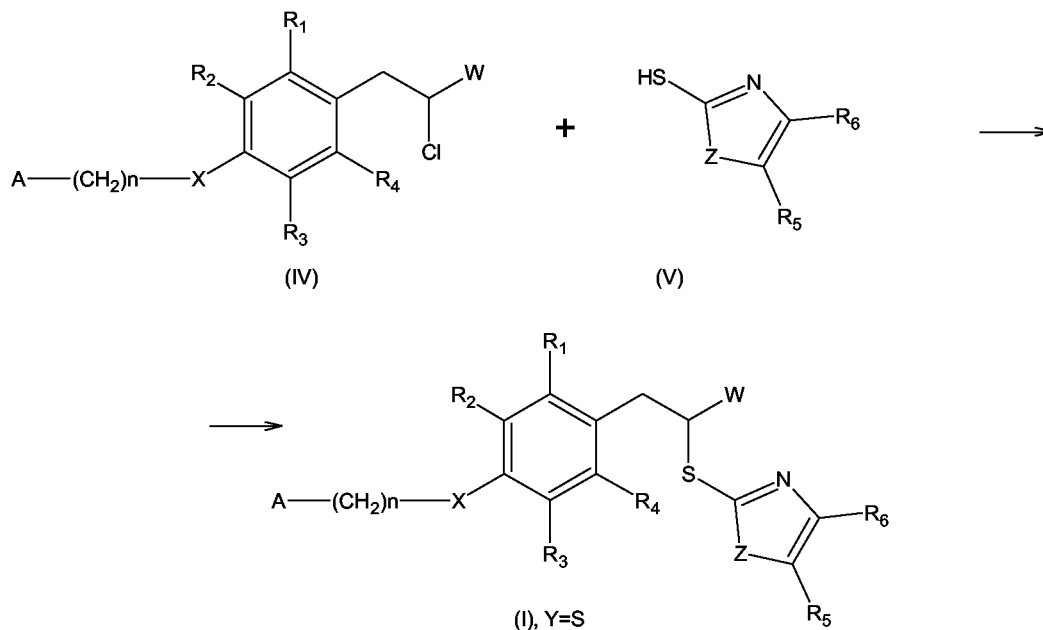
25

Compounds of formula (I) wherein $Y = S$ and X, W, Z, A, n , and R_1 to R_6 have the meanings as defined above can be prepared by reaction of a compound of formula (IV) wherein W, X, A, n , and R_1 to R_4 have the meanings as defined above for formula (I), with a compound of formula

(V) wherein Z and R₅ to R₆ have the meanings as defined above for formula (I), in the presence of a base in an alcoholic solution, according to the scheme 4.

Scheme 4

5

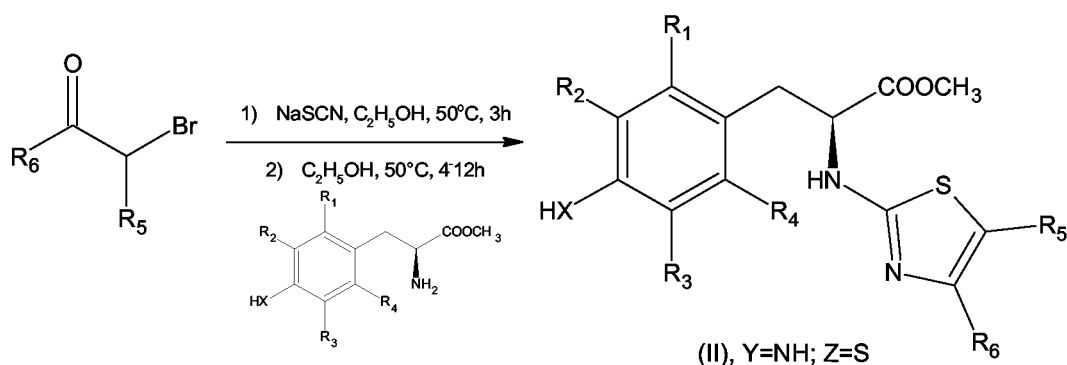


In the case of the preparation of compounds of formula (I) wherein W represents COOH, the starting compound in the above process is a compound of formula (V) wherein W is an ester-protected COOH group. At the end of the reaction, COOH group is deprotected by basic hydrolysis.

Compounds of formula (I) can be prepared both in a racemic form and in a form of a single enantiomer, when starting from optically active materials. Alternatively, racemic compounds of formula (I) can be resolved into enantiomers, using conventional techniques known in the art.

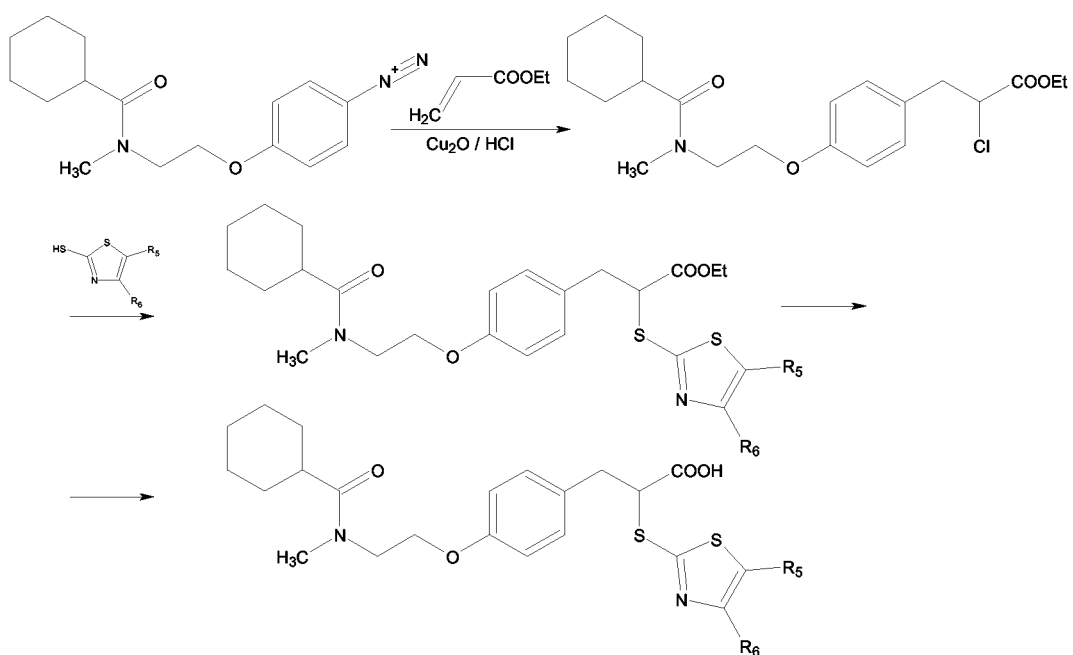
Starting materials of formula (II) wherein Y = NH can be prepared by using or adapting a method described in Joachim Rudolph, *Facile Access to N-Thiazolyl α -Amino Acids from α -bromo ketones and α -Amino Acids*, Tetrahedron, 56 (2000) 3161-3165, according to the scheme 5 shown below.

Scheme 5



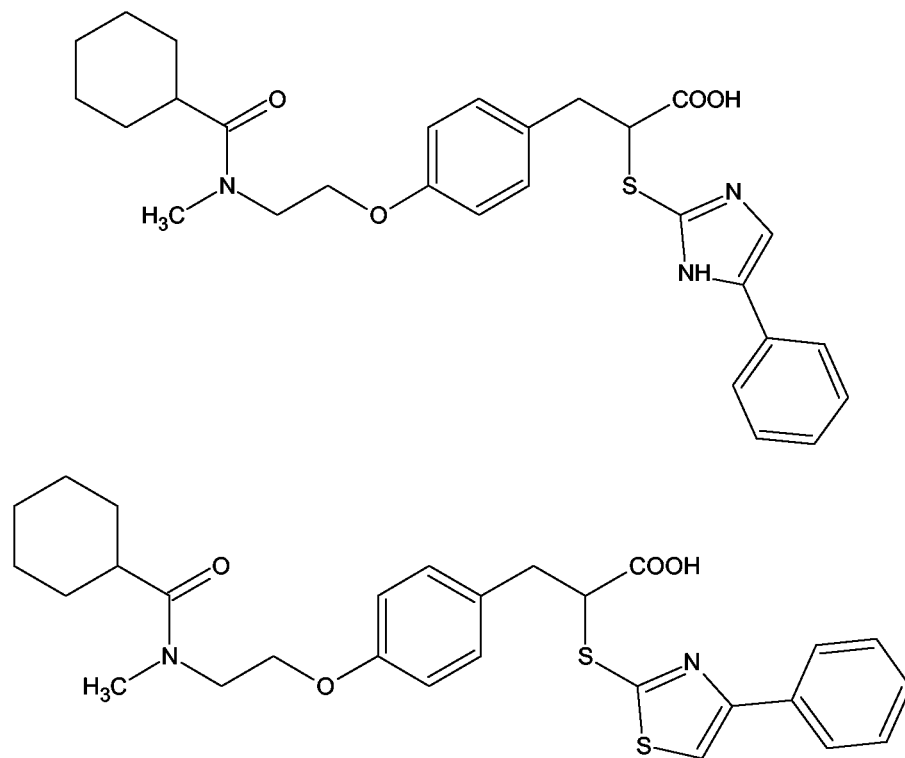
- 5 Starting ethyl 2-chloro-3-phenylpropionate derivatives of formula IV can be prepared by using or adapting a method described in Y.Kawamatsu, H. Asakawa, T. Saraie, E. Imamiya, K. Nishikawa, Y. Hamuro, *Arzneim. Forsch. Drug Res.*, 30 (I), 4, 1980, 585-589. The method was exemplified on the scheme 6. According to the scheme 6, chloroester obtained in the
- 10 Meerwein reaction is reacted with 1,3-thiazole-2-thiol derivatives, in the presence of a base in an alcoholic solution, to give corresponding ethyl α -(1,3-thiazol-2-ylthio)ester. This ester is hydrolyzed in the NaOH or KOH aqueous-alcoholic solution. Free acids are released from salts with diluted hydrochloric acid.

Scheme 6



In this manner, the following exemplary compounds were obtained.

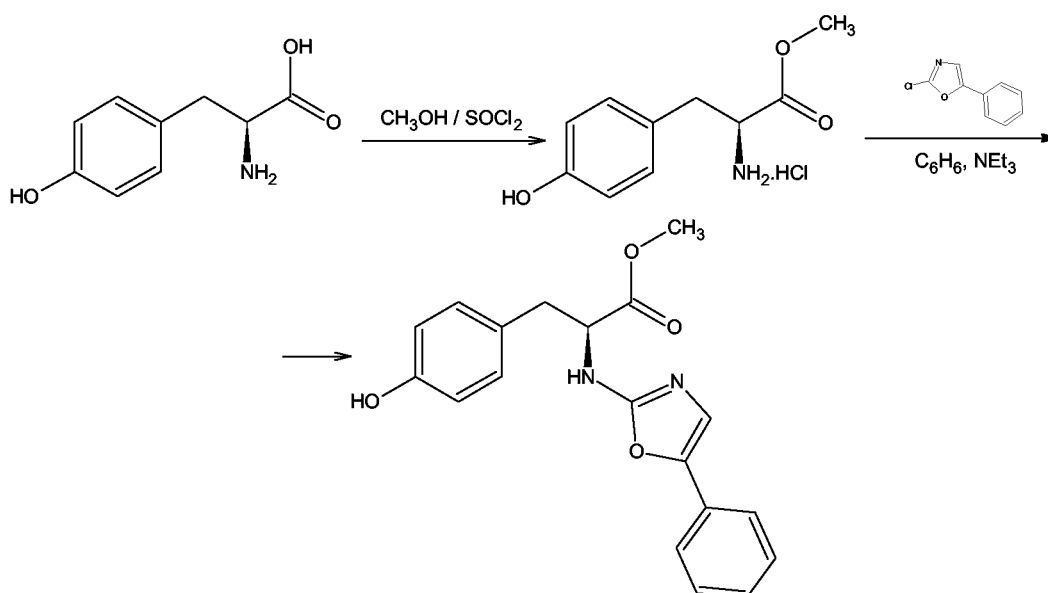
5



Starting tyrosine derivatives of formula (II) wherein X = O, Y = NH, and Z = O, were obtained according to Shyam B. Advani, Joseph Sam, Journal of Pharmaceutical Sciences, Vol. 57, 10, 1968. For example, according to the scheme 7, L-tyrosine methyl ester hydrochloride was obtained by esterification of L-tyrosine with methanol in the presence of thionyl chloride, followed by the reaction of L-tyrosine methyl ester hydrochloride with 2-chloro-5-phenyl-1,3-oxazole in benzene in the presence of triethylamine. Similar procedures were used in the case of D-tyrosine and D,L-tyrosine.

10

Scheme 7



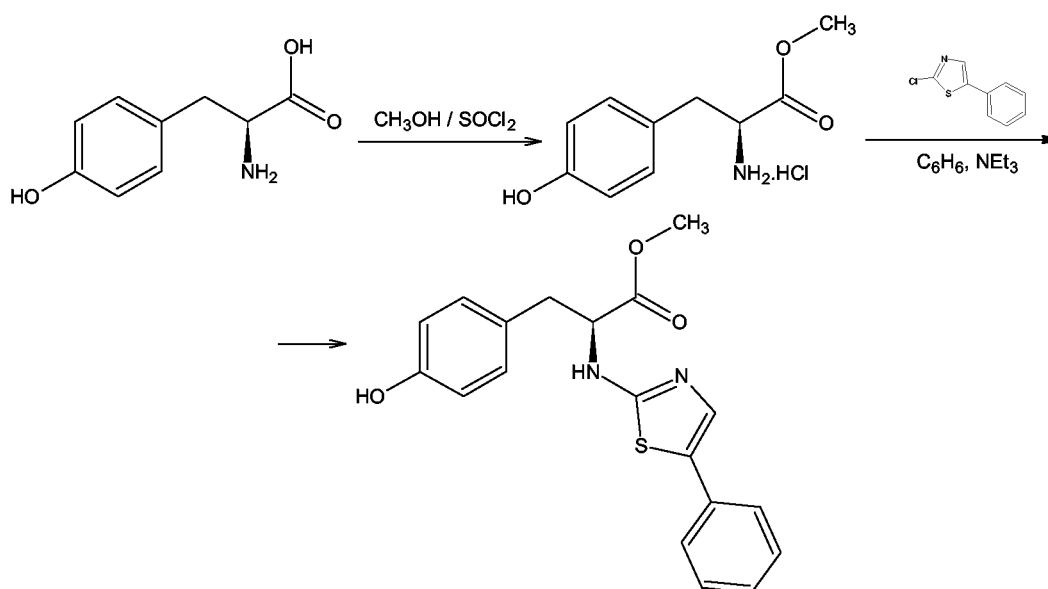
15 Tyrosine compounds of formula (II) wherein X = O, Y = NH, and Z = NH, N-alkyl, N-aryl, N-heteroaryl or S, can be prepared by adapting the method of Shyam B. Advani, Joseph Sam, Journal of Pharmaceutical Sciences, Vol. 57, 10, 1968, described above.

20 Tyrosine derivatives of formula (II) wherein X = O, Y = NH, and Z = S, can be prepared according to the method described in Edward S. Lazer, Clara K. Miao, Hin-Chor Wong, Rondla Sorcek, Denice M. Spero, Alex Galman, Kollol Pal, Mark Behnke, Anne G. Graham, Jane M. Watrous, Carol A. Homon, Juergen Nagle, Arvind Shah, Yvan Guindon, Peter R.

Farina, Julian Adams, J.Med.Chem., 1994,37,913-923, according to the scheme 8.

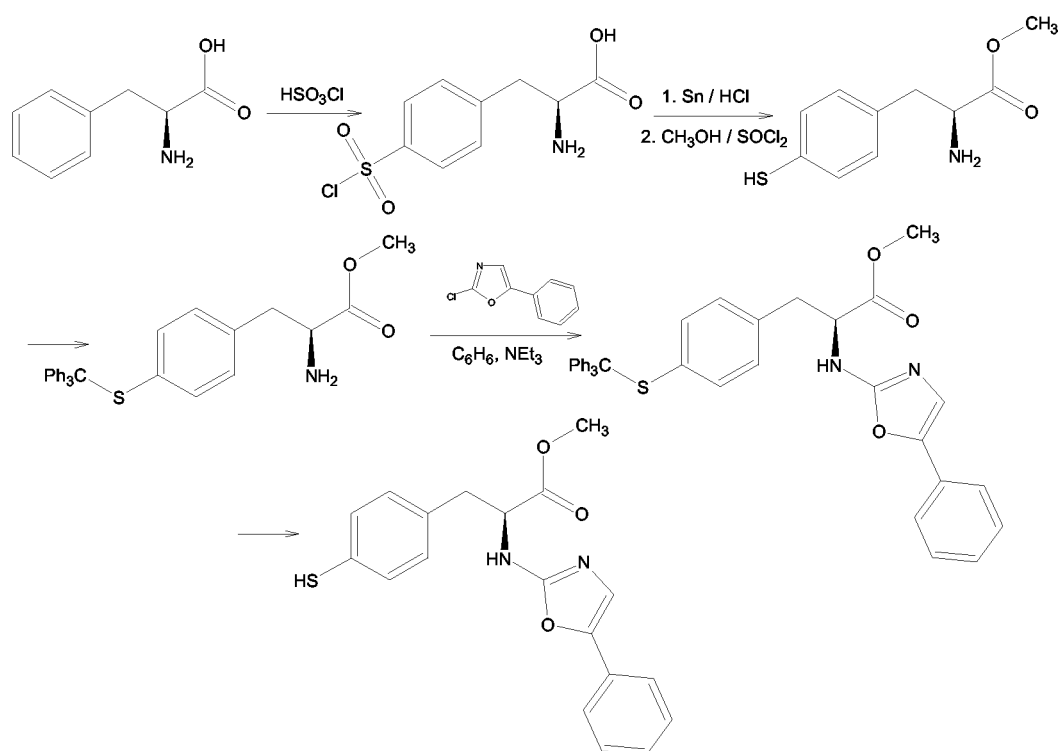
Scheme 8

5



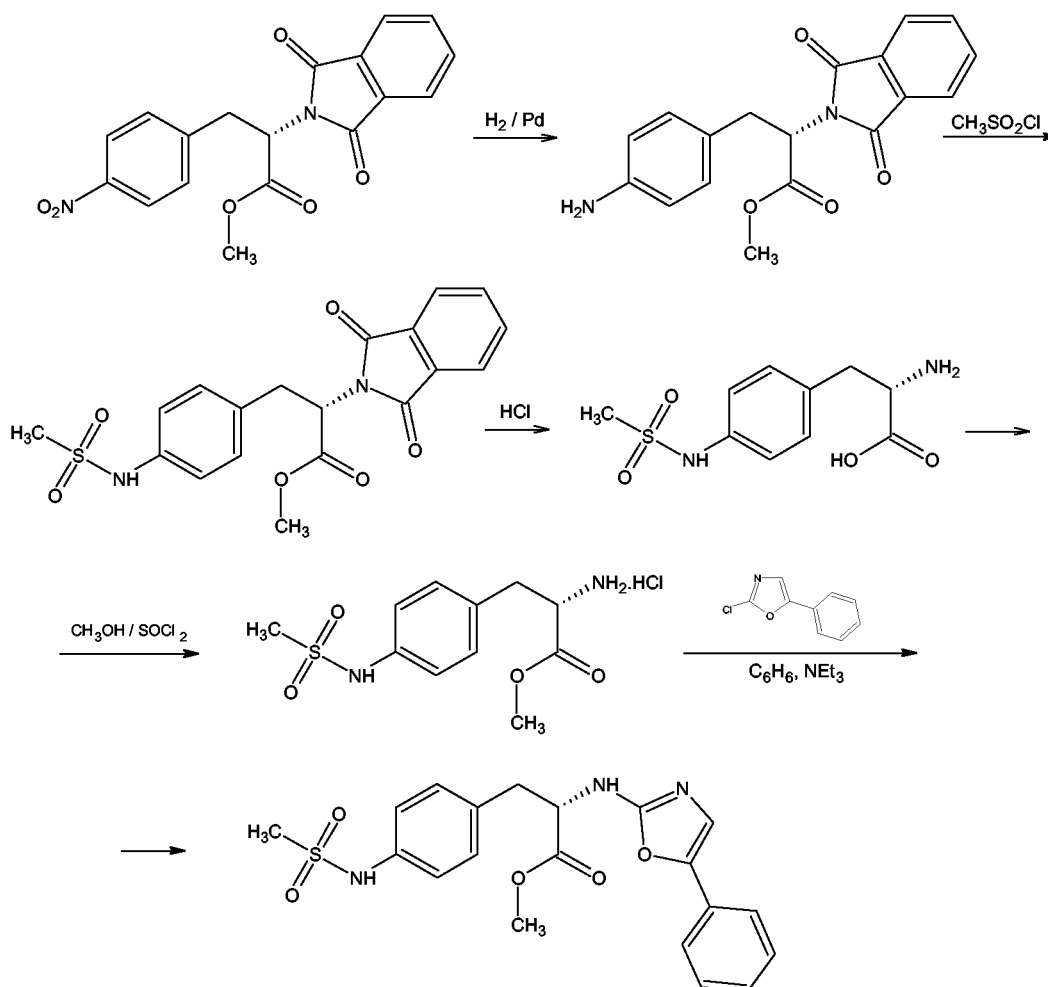
Starting 4-mercaptophenylalanine derivatives of formula (II) wherein Y = NH, Z = O, and X = S, were prepared according to the scheme 9, from 4-mercaptophenylalanine, which was obtained according to Helen S.M. Lu, Martin Volk, Yuriy Kholodenko, Edward Gooding, Robin M. Hochstrasser, William F. DeGrado, Journal of the American Chemical Society, 119,31,1997,7173-7180. The mercapto (SH) group in 4-mercaptophenylalanine was protected with trityl group, followed by substitution of one hydrogen atom at α -amino nitrogen atom with 5-phenyl-1,3-oxazol-2-yl. The final step of the synthesis is deprotection of the SH group.

Scheme 9



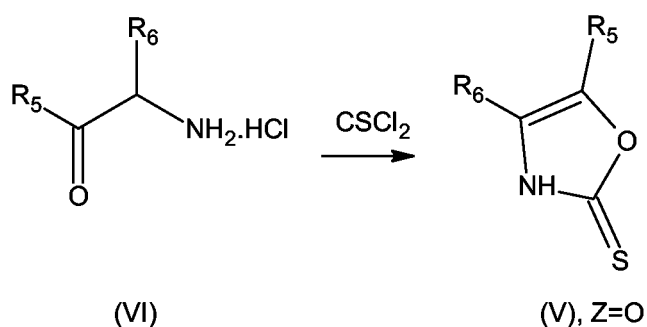
- 5 The 4-aminophenylalanine derivatives of formula (II) wherein Y = NH, Z = O, and X = NSO₂-CH₃, were obtained according to the scheme 10 from 4-nitro-N-phthaloylphenylalanine methyl ester. The first step of the synthesis was performed according to F. Bergel, J.A. Stock, Journal of Organic Chemistry, 1956, 90-96. 4-Amino-N-phthaloylphenylalanine methyl ester thus obtained was mesylated with mesyl chloride in pyridine in the presence of catalytic amounts of DMAP. The subsequent step was the removal of phthaloyl group, by heating with 6M aqueous HCl. Thus obtained 4-methanesulfonylaminophenylalanine was converted into methyl ester hydrochloride by esterification in methanol in the presence of thionyl chloride. The subsequent step was the reaction of 4-methanesulfonylaminophenylalanine methyl ester hydrochloride with 2-chloro-5-phenyl-1,3-oxazole in the presence of triethylamine in benzene.

Scheme 10



- 5 Starting compounds of formula (V) wherein $Z = \text{O}$, i.e. substituted 1,3-oxazole-2(3H)-thiones, can be prepared according to the description in G. Kjellin, J. Sandstroem Acta.Chem.Scand. 23, 2879, 1969, by reaction of a compound of formula (VI) wherein R_5 and R_6 have the meanings as in formula (I), according to the scheme 11.

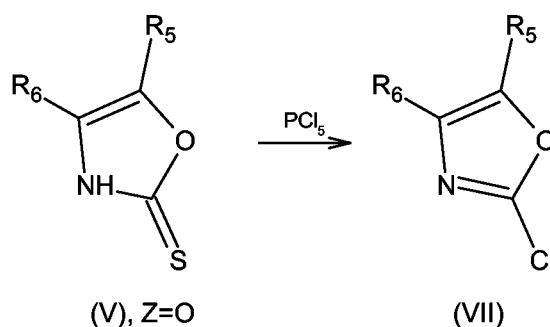
Scheme 11



- 5 Starting compounds of formula (VII), i.e. substituted 2-chloro-1,3-oxazoles can be obtained using or adapting procedures described in Roger Garick Harrison, FR 2313372, by reaction of a compound of formula (V) wherein Z=O and R_5 and R_6 have the meanings as in formula (I), with phosphorus pentoxide according to the scheme 11.

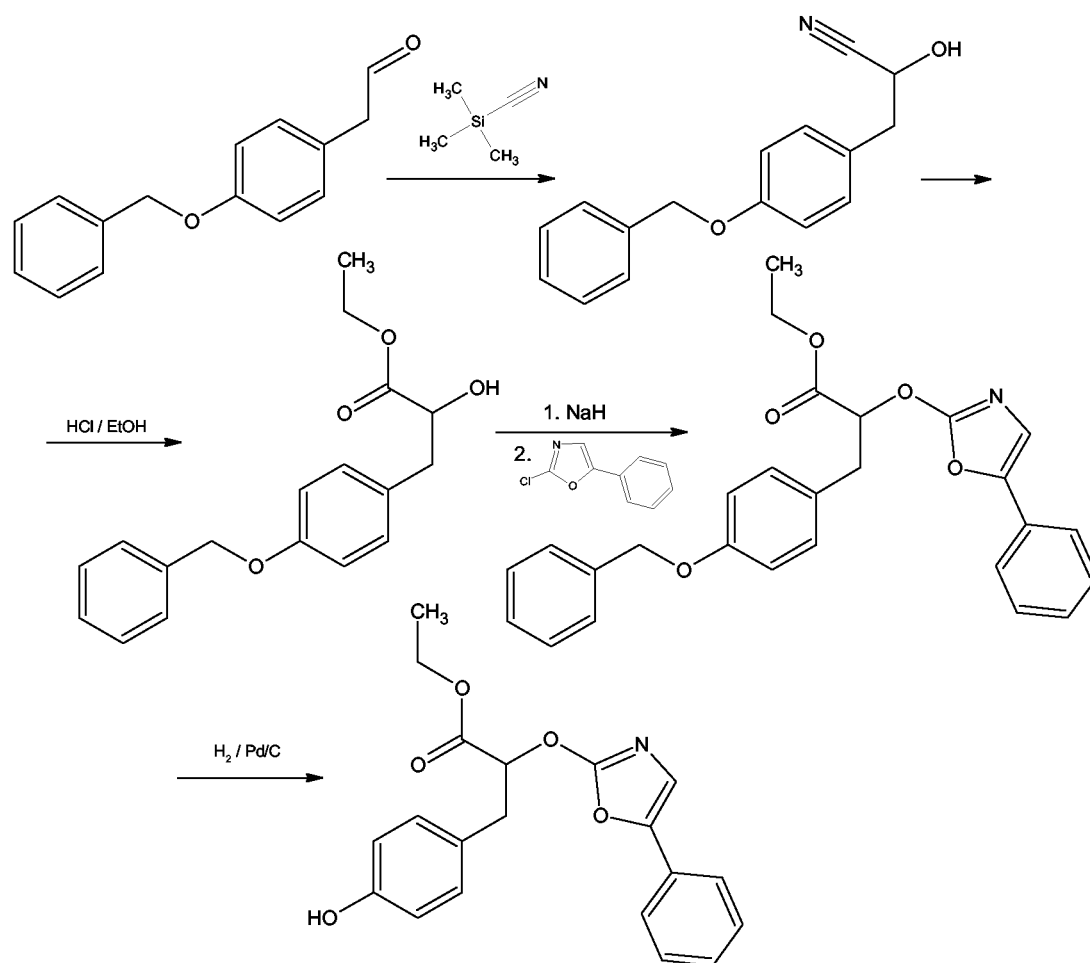
10

Scheme 11



- 15 3-[4-(Benzyloxy)phenyl]-2-hydroxypropionic acid ethyl ester was obtained according to Takamura Makoto, Yanagisawa Hiroaki, Kanai Motoru, Shibasaki Masakatsu, Efficient Synthesis of Antihyperglycemic (S)- α -Aryloxy- β -phenylpropionic Amides Using a Bifunctional Asymmetric Catalyst, *Chem.Pharm.Bull.*, 50, 8, 2002,1118-1121. Subsequently, the
 20 ester was treated with sodium hydride and then with 2-chloro-5-phenyl-1,3-oxazole, according to the scheme 12.

Scheme 12



5 The following abbreviations are used herein:

DIAD: diisopropyl azodicarboxylate

DEAD: diethyl azodicarboxylate

ADDP: azodicarbonyldipiperidine

EXAMPLES

10 Example 1

(2S)-3-{4-[(3,5-Dimethylisoxazol-4-yl)methoxy]phenyl}-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionic acid and its methyl ester

R_1 to $\text{R}_5 = \text{H}$, $\text{R}_6 = \text{C}_6\text{H}_5$, $\text{W} = \text{COOH}/\text{COOCH}_3$, $\text{X} = \text{O}$, $\text{Z} = \text{S}$, $\text{Y} = \text{NH}$, $n = 1$,
 $\text{A} = 3,5\text{-dimethylisoxazol-4-yl}$

Step A: methyl (2S)-3-(4-hydroxyphenyl)-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionate

15.40 g (0.1 mol) of phenacyl chloride and 8.66 g (0.107 mol) of dry sodium thiocyanate in ethanol (200 ml) were stirred for 3h at 50°C. The solution of 19.51 g (0.1 mol) of (S)-tyrosine methyl ester in ethanol (100 ml) was added in one portion and the reaction mixture was stirred for 12h. After removing ethanol by distillation, water and ethyl acetate were added. Aqueous phase was extracted twice with ethyl acetate, combined organic phases were dried over sodium sulfate, and the solvent was evaporated. The product was purified by chromatography. The yield was 20.54 g (58%). MS (ES) 354 (M⁺, 100%)

Step B: Methyl (2S)-3-{4-[(3,5-dimethylisoxazol-4-yl)methoxy]phenyl}-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionate

(3,5-Dimethylisoxazol-4-yl)methanol (0.28 g, 1.5 mmol), methyl (2S)-3-(4-hydroxyphenyl)-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionate from Step A (0.35 g, 1 mmol) and triphenylphosphine (0.79 g, 3 mmol) were dissolved in tetrahydrofuran (THF). After cooling the reaction mixture to 5°C, DEAD (0.52 g, 3 mmol) was added. The reaction was then stirred at room temperature for 18-24h. THF was evaporated to obtain crude methyl (2S)-3-{4-[(3,5-dimethylisoxazol-4-yl)methoxy]phenyl}-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionate.

Step C: (2S)-3-{4-[(3,5-Dimethylisoxazol-4-yl)methoxy]phenyl}-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionic acid

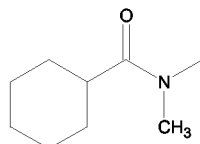
The crude product from Step B was dissolved in a THF/MeOH/H₂O mixture (6:0.1:1; 2 ml). Aqueous 1M LiOH solution (1.6 ml) was added and the mixture was stirred for 3 days at room temperature. Then the reaction mixture was neutralized with 1M HCl, a small amount of water was added, and the mixture extracted with ethyl acetate. The solvent was evaporated. The product was purified by chromatography (SiO₂, ethyl acetate/hexane) The yield was 35%. MS (ES) 463 (M⁺, 100%)

Example 2

(2S)-3-(4-{2-[(Cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionic acid and its methyl ester

R_1 to R_5 = H, R_6 = C_6H_5 , W = COOH/COOCH₃, Y = NH, X = O, Z = S, n = 1,

5 A = (cyclohexylcarbonyl)(methyl)amino of the formula:



Step A: Methyl (2S)-3-{4-[(methylsulfonyl)amino]phenyl}-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionate

29.1 g (0.1 mol) of *N*-[4-(2-bromoacetyl)phenyl]methanesulfonamide and
 10 8.66 g (0.107 mol) of dry sodium thiocyanate in ethanol (200 ml) were stirred for 3h at 50°C. The solution of 19.51 g (0.1 mol) of (S)-tyrosine methyl ester in ethanol (100 ml) was added in one portion and the reaction mixture was stirred for 12h. After removing ethanol by distillation, water and ethyl acetate were added. Aqueous phase was
 15 then extracted twice with ethyl acetate, combined organic phases were dried over sodium sulfate and the solvent was evaporated. The product was purified by chromatography (SiO₂, ethyl acetate/hexane). The yield was 20.12 g (45%). MS (ES) 447 (M^+ , 100%)

Step B Methyl (2S)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]-ethoxy}phenyl)-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionate
 20

N-(2-Hydroxyethyl)-*N*-methylcyclohexanecarboxamide (0.19 g, 1.5 mmol), methyl (2S)-3-(4-hydroxyphenyl)-2-[(4-phenyl-1,3-thiazol-2-yl)-amino]propionate from Step A (0.35 g, 1 mmol) and triphenylphosphine (0.79 g, 3 mmol) were dissolved in tetrahydrofuran (THF). After cooling
 25 the reaction mixture to 5°C, ADDP (0.76 g, 3 mmol) was added. The reaction was then stirred at room temperature for 18-24h. THF was evaporated to obtain crude methyl (2S)-3-(4-{2-[(cyclohexylcarbonyl)-(methyl)amino]ethoxy}phenyl)-2-[(4-phenyl-1,3-thiazol-2-yl)amino]-propionate.

30 Step C: (2S)-3-(4-{2-[(Cyclohexylcarbonyl)(methyl)amino]ethoxy}-phenyl)-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionic acid

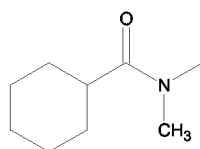
The crude product from Step B was dissolved in a THF/MeOH/H₂O mixture (6:0.1:1; 2 ml). Aqueous 1M LiOH solution (1.6 ml) was added and the mixture was stirred for 3 days at room temperature. Then the reaction mixture was neutralized with 1M HCl, a small amount of water was added and the mixture extracted with ethyl acetate. The solvent was evaporated. The product was purified by chromatography (SiO₂, ethyl acetate/hexane). The yield was 42%.

MS (ES) 507 (M⁺, 100%)

Example 3

10 (2S)-3-(4-{2-[(Cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(4-cyanophenyl-1,3-thiazol-2-yl)amino]propionic acid and its methyl ester

R₁ to R₅ = H, R₆ = 4-CN-C₆H₅, W = COOH/COOCH₃, X = O, Z = S, Y = NH, n = 2, A = (cyclohexylcarbonyl)(methyl)amino of the formula:



15 Step A: Methyl (2S)-2-{[4-(4-cyanophenyl)-1,3-thiazol-2-yl]amino}-3-(4-hydroxyphenyl)propionate

22.3 g (0.1 mol) of 4-(bromoacetyl)benzonitrile and 8.66 g (0.107 mol) of dry sodium thiocyanate in ethanol (200 ml) were stirred for 3h at 50°C. Then 19.51 g (0.1 mol) of (S)-tyrosine methyl ester in ethanol (100 ml) was added in one portion and the reaction mixture was stirred for 12h. After removing ethanol by distillation, water and ethyl acetate were added. Aqueous phase was extracted twice with ethyl acetate, combined organic phases were dried over sodium sulfate and the solvent was evaporated. The product was purified by chromatography (SiO₂, ethyl acetate/hexane). The yield was 53%. MS (ES) 379 (M⁺, 100%)

Step B: Methyl (2S)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(4-cyanophenyl-1,3-thiazol-2-yl)amino]propionate

N-(2-Hydroxyethyl)-N-methylcyclohexanecarboxamide (0.19 g, 1.5 mmol), methyl (2S)-2-{[4-(4-cyanophenyl)-1,3-thiazol-2-yl]amino}-3-(4-hydroxyphenyl)propionate from Step A (0.35 g, 1 mmol) and triphenylphosphine (0.79 g, 3 mmol) were dissolved in tetrahydrofuran (THF). After cooling the reaction mixture to 5°C, ADDP (0.76 g, 3 mmol) was

added. The reaction was then stirred at room temperature for 18-24h. THF was evaporated to obtain crude methyl (2S)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-{[4-(4-cyanophenyl-1,3-thiazol-2-yl)amino]}propionate.

- 5 Step C: (2S)-3-(4-{2-[(Cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-{[4-(4-cyanophenyl-1,3-thiazol-2-yl)amino]}propionic acid

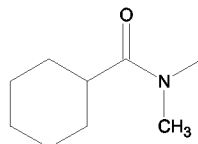
The crude product from Step B was dissolved in a THF/MeOH/H₂O mixture (6:0.1:1; 2 ml). Aqueous 1M LiOH solution (1.6 ml) was added and the mixture was stirred for 3 days at room temperature. Then the
 10 reaction mixture was neutralized with 1M HCl, a small amount of water was added and the mixture extracted with ethyl acetate. The solvent was evaporated. The product was purified by chromatography (SiO₂, ethyl acetate/hexane). The yield was 38%.

MS (ES) 532 (M⁺, 100%)

15 Example 4

(2S)-3-(4-{2-[(Cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(4-methylphenyl-1,3-thiazol-2-yl)amino]propionic acid and its methyl ester

R₁ to R₅ = H, R₆ = 4-CH₃-C₆H₅, W = COOH/COOCH₃, X = O, Z = S, Y = NH, n = 2, A = (cyclohexylcarbonyl)(methyl)amino of the formula:



20

Step A: Methyl (2S)-2-{[4-(4-methylphenyl)-1,3-thiazol-2-yl]amino}-3-(4-hydroxyphenyl)propionate

21.2 g (0,1 mol) of 2-bromo-1-(4-methylphenyl)ethanol and 8.66 g (0.107 mol) of dry sodium thiocyanate in ethanol (200 ml) were stirred for 3h at
 25 50°C. The solution of 19,51 g (0,1 mol) of (S)-tyrosine methyl ester in ethanol (100 ml) was then added in one portion and the reaction mixture was stirred for 12h. After removing ethanol by distillation, water and ethyl acetate were added. Aqueous phase was extracted twice with ethyl acetate, combined organic phases were dried over sodium sulfate
 30 and the solvent was evaporated. The product was purified by chromatography (SiO₂, ethyl acetate/hexane). The yield was 17.67 g (48%). MS (ES) 368 (M⁺, 100%)

Step B: 2-[(Cyclohexylcarbonyl)(methyl)amino]ethyl 4-toluene-sulfonate

4-Toluenesulfonyl chloride (1.9 g, 10 mmol) was added portionwise to the solution of *N*-(2-hydroxyethyl)-*N*-methylcyclohexanecarboxamide
5 (1.85 g, 10 mmol) in pyridine (30 ml) at room temperature. After stirring at room temperature for 5h, the reaction mixture was poured into 200 ml of water and extracted three times with 50 ml of dichloromethane. Combined extracts were washed with 1M HCl, aqueous sodium bicarbonate, and brine. The aqueous phase was dried over anhydrous
10 magnesium sulphate and the solvent was evaporated to obtain the product 2-[(cyclohexylcarbonyl)(methyl)amino]ethyl 4-toluenesulfonate with the yield of about 87%.

Step C: Methyl (2*S*)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(4-methylphenyl-1,3-thiazol-2-yl)amino]propionate

15 To the solution of 3.68 g of methyl (2*S*)-2-[[4-(4-methylphenyl)-1,3-thiazol-2-yl]amino]-3-(4-hydroxyphenyl)propionate from Step A in dimethylformamide (50 ml) at room temperature under argone atmosphere NaH (0.4 g, 60% dispersion in mineral oil) was added portionwise with stirring. When the evolution of the gas ceased, the
20 solution of 2-[(cyclohexylcarbonyl)(methyl)amino]ethyl 4-toluene-sulfonate from Step B (3.39 g, 10 mmol) w dimethylformamide (10 ml) was added dropwise. The mixture was heated with stirring at 80°C. After cooling, the mixture was poured into 1 l of water and extracted several times with ethyl acetate. Combined extracts were washed with brine,
25 dried over magnesium sulphate, and the solvent was evaporated to give crude methyl (2*S*)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}-phenyl)-2-[4-(4-methylphenyl-1,3-thiazol-2-yl)amino]propionate.

Step D: (2*S*)-3-(4-{2-[(Cyclohexylcarbonyl)(methyl)amino]ethoxy}-phenyl)-2-[4-(4-methylphenyl-1,3-thiazol-2-yl)amino]propionic acid

30 1 g of the crude product from Step C was dissolved in a THF/MeOH/H₂O mixture (6:0.1:1; 2 ml). Aqueous 1M LiOH solution (8 ml) was added and the mixture was stirred for 3 days at room temperature. Then the reaction mixture was neutralized with 1M HCl, a small amount of water was added and the mixture extracted with ethyl acetate. The solvent

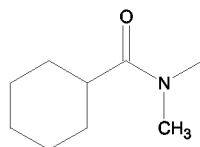
was evaporated. The product was purified by chromatography (SiO₂, ethyl acetate/hexane). The yield was 35%.

MS (ES) 521 (M⁺, 100%)

Example 5

- 5 3-(4-{2-[(Cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(5-phenyl-1,3-oxazol-2-yl)oxy]propionic acid and its methyl ester

R₁ to R₄ and R₆ = H, R₅ = C₆H₅, W = COOH/COOCH₃, X = O, Z = O, Y = O, n = 2, A = (cyclohexylcarbonyl)(methyl)amino of the formula:



- 10 Step A: Methyl 3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(5-phenyl-1,3-oxazol-2-yl)oxy]propionate

N-(2-hydroxyethyl)-*N*-methylcyclohexanecarboxamide (0.19 g, 1.5 mmol), methyl 3-(4-hydroxyphenyl)-2-[4-(5-phenyl-1,3-oxazol-2-yl)oxy]propionate (0.35 g, 1 mmol), and triphenylphosphine (0.79 g, 3 mmol) were dissolved in tetrahydrofuran (THF). The reaction mixture was cooled to 5°C and DEAD (0.52 g, 3 mmol) was added. The reaction was then stirred at room temperature for 18-24h. THF was evaporated to obtain crude methyl 3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(5-phenyl-1,3-oxazol-2-yl)oxy]propionate.

- 20 Step B: 3-(4-{2-[(Cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(5-phenyl-1,3-oxazol-2-yl)oxy]propionic acid

The crude product from Step B was dissolved in a THF/MeOH/H₂O mixture (6:0.1:1; 2 ml). Aqueous 1M LiOH solution (1.6 ml) was added and the mixture was stirred for 3 days at room temperature. Then the reaction mixture was neutralized with 1M HCl, a small amount of water was added and the mixture extracted with ethyl acetate. The solvent was evaporated. The product was purified by chromatography (SiO₂; ethyl acetate/hexane). The yield was 41%.

MS (ES) 492 (M⁺, 100%)

Example 6

3-[4-(Benzyloxy)phenyl]-2-(5-phenyl-1*H*-imidazol-2-ylthio)propionic acid and its methyl ester

5 R_1 to R_4 and R_6 = H, R_5 = C₆H₅, W = COOH/COOCH₃, X = O, Z = N, Y = S, n = 1, A = phenyl

Step A: Methyl 3-[4-(benzyloxy)phenyl]-2-(5-phenyl-1*H*-imidazol-2-ylthio)propionate

10 The solution of 0.3 g (0,001 mol) of methyl 3-[4-(benzyloxy)phenyl]-2-chloropropionate in methanol (2 ml) was added dropwise to the solution of 0.18 g (0,001 mol) 5-phenyl-1*H*-imidazol-2-thiol and 0.04 g (0.001 mol) of NaOH in methanol (3 ml). The solution then was heated at reflux for 5h. The crude product obtained after removing the solvent was used without purification in the next step of the synthesis.

15 Step B: 3-[4-(Benzyloxy)phenyl]-2-(5-phenyl-1*H*-imidazol-2-ylthio)-propionic acid

20 The crude product from Step A was dissolved in a MeOH/H₂O mixture (2:1, 4 ml). 0.7 g KOH was then added to the solution and the mixture was refluxed for 2h. Subsequently, the reaction mixture was neutralized with 1M HCl, a small amount of water was added and the mixture extracted with ethyl acetate. The solvent was evaporated. The product was purified by chromatography (SiO₂; ethyl acetate). The yield was 42%.

MS (ES) 430 (M⁺, 100%)

Biological tests

25 The ability of the compounds of the invention to bind to the PPAR gamma receptor and to modify its activity was determined using the following methods.

In vitro binding

30 The ability of the compounds to bind to the PPAR gamma receptor (*in vitro*) was determined according to the procedure described below, using the method of competitive radioligand displacement from the ligand-receptor complex. PPAR agonist ³H-rosiglitazone at final concentration 10nM was used as the radioligand. An excess of unlabelled test compounds at final concentration 20μM was also added to the

reaction. The source of the receptor in assays was human recombinant protein containing LBD (ligand binding domain) of the PPAR gamma. The separation of the radioligand unbound with the receptor was performed by dextran coated charcoal technique. The radioactivity was measured using LS 6500-Beckman Coulter scintillation counter. The obtained scintillation counts values were compared to the values obtained for samples incubated with the radioligand (assumed 0% displacement) and to the values obtained for samples containing both the radioligand and an excess of non-radiolabelled rosiglitazone (assumed 100% displacement). The obtained values were comprised in the 0-130% range.

References:

1. ADD1/SREBP1 activates PPAR gamma through the production of endogenous ligand. Proc. Natl. Acad. Sci. U S A. 1998 Apr 14;95(8):4333-7.
2. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). J. Biol. Chem. 1995 Jun 2;270(22):12953-6.
3. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. Proc. Natl. Acad. Sci. U S A. 1997 Apr. 29; 94(9):4318-23.

20 Binding in adipocytes

To confirm the ability of the tested molecules to bind *in vivo*, analogous experiments with the use of murine fibroblasts 3T3-L1 cell line differentiated into adipocytes were performed. Differentiation of fibroblasts cells was performed on 12-well plates during 10 days period. On the day of the experiment, the cells were washed twice with PBS solution prior to 1h incubation in DMEM medium containing tritium-labelled reference compound (rosiglitazone) at 30pM concentration and different concentrations of the tested compounds (in the 100 pM -20 µM concentration range) at 37°C. Then the cells were washed three times with PBS solution and solubilized in 1M NaOH solution. In the lysate prepared as described above, both radioactivity (using LS 6500 Beckman Coulter scintillation counter) and protein concentration (using Bradford method) were measured. Nonspecific binding was estimated in the presence of non-labelled reference compound (at 20µM concentration).

The obtained scintillation counts values were compared to the values obtained for samples incubated with the radioligand (assumed 0% displacement) and to the values obtained for samples containing both the radioligand and an excess of non-radiolabelled rosiglitazone (assumed 100% displacement). The obtained values were comprised in the 0-130% range.

References:

1. Identification of high-affinity binding sites for the insulin sensitizer rosiglitazone (BRL-49653) in rodent and human adipocytes using a radioiodinated ligand for peroxisomal proliferator-activated receptor gamma. J. Pharmacol. Exp. Ther. 1998 Feb;284(2):751-9.
2. Differential regulation of the stearoyl-CoA desaturase genes by thiazolidinediones in 3T3-L1 adipocytes. J. Lipid Res. 2000 Aug;41(8):1310-6.
3. Distinct stages in adipogenesis revealed by retinoid inhibition of differentiation after induction of PPARgamma. Mol Cell Biol. 1996 Apr;16(4):1567-75.
4. Differentiation Kinetics of *in vitro* 3T3-L1 Preadipocyte Cultures. Tissue Eng. 2002 Dec;8(6):1071-1081.
5. Role of PPARgamma in regulating a cascade expression of cyclin-dependent kinase inhibitors, p18(INK4c) and p21(Waf1/Cip1), during adipogenesis. J. Biol. Chem. 1999 Jun 11;274(24):17088-97.

Adipogenesis

3T3-L1 cell line cells (from ATCC) were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% Fetal Bovine Serum and antibiotics. Two days before the experiment, the cells were passaged into 12-well microplates (30 x 10⁴ cells/well) and maintained for subsequent 2 days to confluency. After this time, the medium was replaced with DMEM + FBS +antibiotics and tested compounds at final concentration of 50 µM were added to the cells. Under these conditions, the cells were maintained for 14 days, changing the medium with the test compounds every 2 days. After 10-14 days the differentiated cells were stained with Oil Red O prior to photographing.

References:

1. Differential regulation of the stearoyl-CoA desaturase genes by thiazolidinediones in 3T3-L1 adipocytes. J. Lipid Res. 2000 Aug;41(8):1310-6.

Glucose uptake

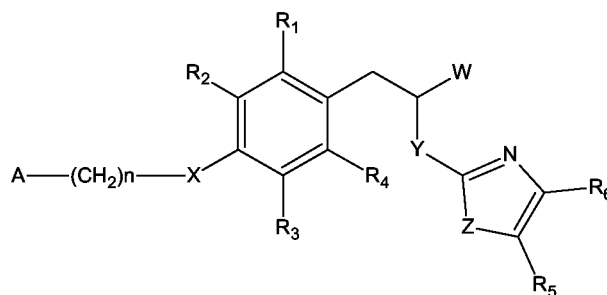
- 5 Differentiated 3T3-L1 fibroblasts were incubated in DMEM supplemented with 10% FBS and antibiotics with test compounds (at the concentration of 20µM) for 48h. After this time, the cells were washed with PBS, and then serum-free DMEM was added to the cells. The cells were kept in an incubator for 3h (37°C / 5% CO₂) and then medium was replaced with
10 KHR buffer (25 mM HEPES-NaOH; pH 7.4; 125 mM NaCl; 5 mM KCl; 1.2 mM MgSO₄; 1.3 mM CaCl₂; 1.3 mM KH₂PO₄) and the cells were incubated for 30 minutes at 37°C. Glucose uptake was initiated by the addition to each test well of 50µl KHR buffer containing 0,5mM 2 deoxy-D-[1,2-³H]glucose (0,5µCi) and 100nM insulin. After 10 min incubation at 37°C,
15 the medium was aspirated, and the cells were washed three times with ice-cold KHR buffer. Then the cells were dissolved in 1M NaOH. In the lysate prepared as described above, both radioactivity (using LS 6500 Beckman Coulter scintillation counter) and protein concentration (using Bradford method) were measured. Nonspecific binding was estimated in
20 the presence of non-labelled reference compound (at 20µM concentration).

References:

1. Role of peroxisome proliferator-activated receptor-gamma in maintenance of the characteristics of mature 3T3-L1 adipocytes. Diabetes. 2002 Jul;
25 51(7):2045-55.
2. Identification of high-affinity binding sites for the insulin sensitizer rosiglitazone (BRL-49653) in rodent and human adipocytes using a radioiodinated ligand for peroxisomal proliferator-activated receptor gamma. J. Pharmacol. Exp. Ther. 1998 Feb; 284(2):751-9.
- 30 3. Identification of bioactive molecules by adipogenesis profiling of organic compounds. J. Biol. Chem. 2003 Feb 28;278(9):7320-4. Epub 2002 Dec 19.
4. Evidence for the involvement of vicinal sulfhydryl groups in insulin-activated hexose transport by 3T3-L1 adipocytes. J. Biol. Chem. 1985 Mar 10;260(5):2646-52.

Claims

1. New 3-phenylpropionic acid derivatives of formula (I):



(I)

- 5 wherein:

W represents COOH group or its bioisosters, or -COO-C₁-C₄-alkyl group;

Y represents NH, N-C₁-C₁₀-alkyl, O, or S;

Z represents NH, N-C₁-C₁₀-alkyl, N-aryl, N-heteroaryl, S, or O;

- 10 X represents O, S, NH, N-C₁-C₁₀-alkyl, N-aryl, NSO₂-C₁-C₁₀-alkyl, N-SO₂-aryl, or N-SO₂-heteroaryl;

R₁ to R₆ each independently represents hydrogen atom or a substituent selected from the group consisting of:

- 15 C₁-C₄-alkyl, C₁-C₄-alkoxy, C₃-C₇-cycloalkyl, C₃-C₇-cycloalkoxy, C₁-C₄-thioalkoxy, C₃-C₇-cyclothioalkoxy, halogen atom, halogen-substituted C₁-C₄-alkyl, halogen-substituted C₃-C₇-cycloalkyl, -NO₂, -CN, -SO₂-NH₂, -SO₂-NH-C₁-C₄-alkyl, -SO₂-N(C₁-C₄-alkyl)₂, -CO-C₁-C₄-alkyl, -O-CO-C₁-C₄-alkyl, -CO-O-C₁-C₄-alkyl, -CO-aryl, -CO-NH₂, -CO-NH-C₁-C₄-alkyl, -CO-N(C₁-C₄-alkyl)₂, aryl, and heteroaryl, said aryl and heteroaryl being optionally substituted with one or more substituents independently selected from the group consisting of C₁-C₄-alkyl, C₁-C₄-alkoxy, C₃-C₇-cycloalkyl, C₃-C₇-cycloalkoxy, C₁-C₄-thioalkoxy, C₃-C₇-cyclothioalkoxy, halogen atom; halogen-substituted C₁-C₄-alkyl, halogen-substituted C₃-C₇-cycloalkyl; -NO₂, -CN, -SO₂-NH₂, -SO₂-NH-C₁-C₄-alkyl, -SO₂-N(C₁-C₄-alkyl)₂, -CO-C₁-C₄-alkyl, -O-CO-C₁-C₄-alkyl, -CO-O-C₁-C₄-alkyl, -CO-aryl,
- 20

-CO-NH₂, -CO-NH-C₁-C₄-alkyl, and -CO-N(C₁-C₄-alkyl)₂;

A represents C₁-C₄-alkyl, C₃-C₇-cycloalkyl, halogen-substituted C₁-C₄-alkyl, halogen-substituted C₃-C₇-cycloalkyl, aryl, heteroaryl, heterocyclyl, -NH-CO-C₁-C₄-alkyl, -N(C₁-C₄-alkyl)-CO-C₁-C₄-alkyl,
5 -NH-CO-aryl, -N(C₁-C₄-alkyl)-CO-aryl, -N(C₁-C₄-alkyl)-CO-C₃-C₇-cycloalkyl,
-NH-CO-NH₂, -NH-CO-NH-C₁-C₄-alkyl, -NH-CS-NH-C₁-C₄-alkyl,
-NH-CO-NH-aryl, -NH-CS-NH-aryl, -SO₂-C₁-C₄-alkyl, -SO₂-aryl, or -SO₂-heteroaryl wherein aryl, heteroaryl, and heterocyclyl are optionally substituted with one or more substituents independently selected from
10 the group consisting of C₁-C₄-alkyl, C₁-C₄-alkoxy, and halogen atom; and

n represents an integer from 0 to 4, inclusive;

and pharmaceutically acceptable salts thereof.

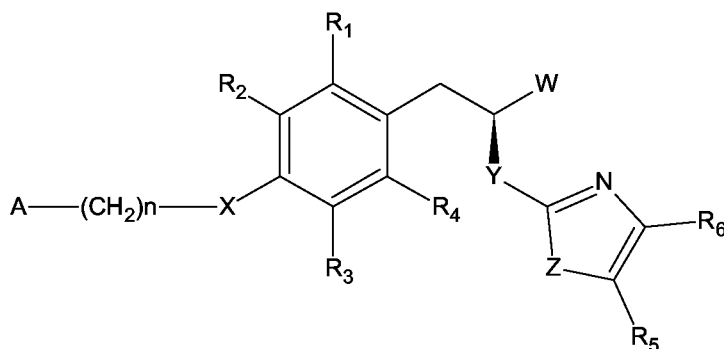
2. The compound of claim 1 wherein W represents COOH.
3. The compound of claim 1 wherein W represents -COO-C₁-C₄-alkyl.
- 15 4. The compound of any of claims 1 to 3 wherein Y represents NH.
5. The compound of any of claims 1 to 3 wherein Y represents O.
6. The compound of any of claims 1 to 3 wherein Y represents N-C₁-C₄-alkyl, especially N-CH₃.
7. The compound of any of claims 1 to 6 wherein Z represents O.
- 20 8. The compound of any of claims 1 to 6 wherein Z represents S.
9. The compound of any of claims 1 to 6 wherein Z represents N-C₁-C₄-alkyl, especially N-CH₃.
10. The compound of any of claims 1 to 6 wherein Z represents N-phenyl.
- 25 11. The compound of any of claims 1 to 10 wherein X represents O.
12. The compound of any of claims 1 to 10 wherein X represents S.

13. The compound of any of claims 1 to 10 wherein X represents $\text{NSO}_2\text{-C}_1\text{-C}_4\text{-alkyl}$, especially $\text{NSO}_2\text{-CH}_3$.
14. The compound of claim 1 wherein W represents COOH , Y represents NH , Z represents S , and X represents O .
- 5 15. The compound of claim 1 wherein W represents $\text{-COO-C}_1\text{-C}_4\text{-alkyl}$, especially -COO-CH_3 , Y represents NH , Z represents S , and X represents O .
16. The compound of claim 1 wherein W represents COOH , Y represents O , Z represents O , and X represents O .
- 10 17. The compound of any one of claims 1 to 16 wherein each of R_1 to R_6 represents hydrogen atom.
18. The compound of any one of claims 1 to 17 wherein n is equal to 1 or 2.
- 15 19. The compound of any one of claims 1 to 18 wherein A represents aryl or heteroaryl, said aryl and heteroaryl being optionally substituted with one or more substituents independently selected from the group consisting of $\text{C}_1\text{-C}_4\text{-alkyl}$, $\text{C}_1\text{-C}_4\text{-alkoxy}$, $\text{C}_1\text{-C}_4\text{-thioalkoxy}$, CN , halogen, and phenyl.
- 20 20. The compound of claim 19 wherein A represents isoxazolyl, optionally substituted with one or more substituents independently selected from $\text{C}_1\text{-C}_4\text{-alkyl}$, especially -CH_3 .
21. The compound of claim 19 wherein A represents phenyl.
22. The compound of any one of claims 1 to 18 wherein A represents $\text{-N(C}_1\text{-C}_4\text{-alkyl)-CO-C}_3\text{-C}_7\text{-cycloalkyl}$.
- 25 23. The compound of claim 22 wherein A represents $\text{-N(CH}_3\text{)-CO-cyclohexyl}$.
24. The compound of any one of claims 1 to 23 wherein one of R_5 and R_6 represents phenyl, optionally substituted with a substituent selected from the group consisting of $\text{C}_1\text{-C}_4\text{-alkyl}$, $\text{C}_1\text{-C}_4\text{-alkoxy}$, $\text{C}_3\text{-C}_7\text{-cycloalkyl}$,

C₃-C₇-cycloalkoxy, C₁-C₄-thioalkoxy, C₃-C₇-cyclothioalkoxy, halogen atom, halogen-substituted -C₁-C₄-alkyl, halogen-substituted -C₃-C₇-cycloalkyl, -NO₂, -CN, -SO₂-NH₂, -SO₂-NH-C₁-C₄-alkyl, -SO₂-N(C₁-C₄-alkyl)₂, -CO-C₁-C₄-alkyl, -O-CO-C₁-C₄-alkyl, -CO-O-C₁-C₄-alkyl, -CO-aryl, -CO-NH₂,
 5 -CO-NH-C₁-C₄-alkyl, and -CO-N(C₁-C₄-alkyl)₂, and the other of R₅ and R₆ represents hydrogen atom.

25. The compound of claim 24 wherein one of R₅ and R₆ represents phenyl, optionally substituted with a substituent selected from CN and C₁-C₄-alkyl, especially CH₃.

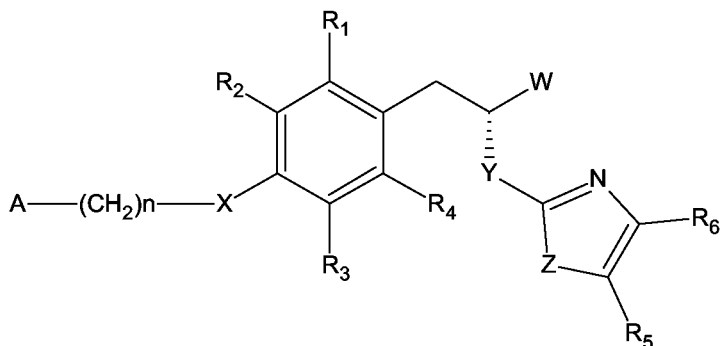
10 26. The compound of any one of claims 1 to 25 having stereochemical configuration as shown in formula (IA):



(IA)

and pharmaceutically acceptable salts thereof.

15 27. The compound of any one of claims 1 to 25 having stereochemical configuration as shown in formula (IB):



(IB)

and pharmaceutically acceptable salts thereof.

28. The compound of claim 1, said compound being selected from the following:

5 methyl (2S)-3-{4-[(3,5-dimethylisoxazol-4-yl)methylenoxy]phenyl}-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionate;

(2S)-3-{4-[(3,5-dimethylisoxazol-4-yl)methylenoxy]phenyl}-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionic acid;

methyl (2S)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionate;

10 (2S)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[(4-phenyl-1,3-thiazol-2-yl)amino]propionic acid;

methyl (2S)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(4-cyanophenyl-1,3-thiazol-2-yl)amino]propionate;

15 (2S)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(4-cyanophenyl-1,3-thiazol-2-yl)amino]propionic acid

methyl (2S)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(4-methylphenyl-1,3-thiazol-2-yl)amino]propionate;

(2S)-3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(4-methylphenyl-1,3-thiazol-2-yl)amino]propionic acid

20 methyl 3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(5-phenyl-1,3-oxazol-2-yl)oxy]propionate;

3-(4-{2-[(cyclohexylcarbonyl)(methyl)amino]ethoxy}phenyl)-2-[4-(5-phenyl-1,3-oxazol-2-yl)oxy]propionic acid

25 methyl 3-[4-(benzyloxy)phenyl]-2-(5-phenyl-1*H*-imidazol-2-ylthio)-propionate; and

3-[4-(benzyloxy)phenyl]-2-(5-phenyl-1*H*-imidazol-2-ylthio)propionic acid

and pharmaceutically acceptable salts thereof.

29. A pharmaceutical composition comprising a compound as defined in any one of claims 1 to 28 or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier and/or excipients.

5 30. A compound as defined in any one of claims 1 to 28 for use as a medicament.

31. The use of a compound as defined in any one of claims 1 to 28 for the preparation of a medicament for the treatment and/or prevention of diseases and conditions mediated by peroxysome proliferator-activated receptor gamma (PPAR γ).
10

32. The use of claim 31 wherein said disease or condition is selected from the group consisting of type 1 diabetes, type 2 diabetes, insulin resistance, metabolic syndrome, complications resulting from or associated with diabetes, cardiovascular disorders, atherosclerosis, obesity, cognitive disorders, and lipid metabolism disorders.
15

33. A method of treatment and/or prophylaxis of diseases and conditions mediated by peroxysome proliferator-activated receptor gamma (PPAR γ) in a mammal subject in need thereof, which method comprises administration to said mammal a compound as defined in any one of claims 1 to 28 in a therapeutically or prophylactically effective amount.
20

34. The method of claim 33 wherein said disease or condition is selected from the group consisting of type 1 diabetes, type 2 diabetes, insulin resistance, metabolic syndrome, complications resulting from or associated with diabetes, cardiovascular disorders, atherosclerosis, obesity, cognitive disorders, and lipid metabolism disorders.
25