"Thiol derivatives with metallopeptidase inhibitory activity"

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The present invention relates to thiol derivatives with metallopeptidase inhibitory activity and, more particularly, it relates to N-mercaptoacyl phenylalanine derivatives useful in the treatment of cardiovascular diseases.

The pharmacologic interest towards the study of metallopeptidase inhibitory molecules derives from the role that said enzymes exert on the level of the cardiocirculatory system.

It is well-known in fact that compounds with angiotensin converting enzyme (ACE) inhibitory activity are mainly useful in the treatment of hypertension, heart failure and post-infarct in that they inhibit the formation of angiotensin II, a substance which produces several effects among which the increase of the blood pressure.

Compounds with endothelin converting enzyme (ECE) inhibitory activity are useful as anti-vasoconstrictors because they inhibit the formation of endothelin, a 21 amino acid peptide with vasoconstrictor activity.

Instead, compounds with inhibitory activity of the neutral endopeptidase (NEP) enzyme, also called enkephalinase, are useful as vasodilators since the NEP enzyme is responsible for the inactivation, not only of endogenous enkephaline, but also of some natriuretic factors such as, for instance, the atrial factor (ANF), a hormone secreted by heart which increases the vasodilatation and, on the renal level, increases diuresis and natriuresis.

Therefore, even exerting their action on the cardiovascular system with different mechanisms of action, the compounds with metallopeptidase inhibitory activity are generally used, alone or in combination, in the treatment of hypertension, renal failure, congestive heart failure and ischemic cardiopathologies.

Among the thiol derivatives inhibitors of metallopeptidases, Thiorphan [(DL-(3-mercapto-2-benzylpropanoyl)glycine], described for the first time by Roques et al. in Nature, Vol. 288, pages 286-288, (1980), and Captopril (The Merck Index, XI ed., No. 1773, page 267) are considered the parent compounds for NEP-inhibitors and ACE-inhibitors, respectively.

Other molecules having thiol structure endowed with metallopeptidase inhibitory activity are described in the literature.

The US patents No. 4,401,677 and No. 4,199,512 (both in the name of E.R. Squibb & Sons,

Inc.) describe mercaptoalkanoyl amino acids endowed with enkephalinase inhibitory activity and ACE-inhibitory activity, respectively.

The European patent application No. 0419327 (Société Civile Bioproject) describes amino acid derivatives endowed with enkephalinase and ACE inhibitory activity, respectively.

The European patent application No. 0449523 (E.R. Squibb & Sons, Inc.) describes mercapto or acylthio trifluoromethylamides with NEP-inhibitory activity.

The international patent application No. WO 93/08162 [Rhone-Poulenc Rorer S.A. - Institut National de la Santé et de la Recherche Médicale (INSERM)] describes ß,ß-disubstituted α-mercaptomethylpropionylamides endowed with mixed ACE/NEP inhibitory activity.

The European patent application No. 0524553 [Institut National de la Santé et de la Recherche Médicale (INSERM)] describes acylmercaptoalkanoyldipeptides endowed with neutral endopeptidase and peptidyldipeptidase A inhibitory activity.

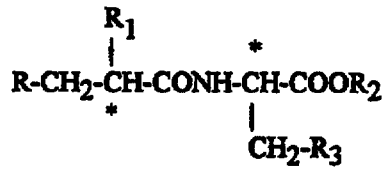
The European patent application No. 0566157 (Schering Corporation) describes thiol derivatives such as, for instance, N-mercaptoacyl amino acids, endowed with NEP-inhibitory activity.

α-Mercaptoacyl dipeptides endowed with ACE-inhibitory and NEP-inhibitory activity are also described by S.S. Bhagwat et al. in Bioorganic & Medicinal Chemistry Letters, 7, 735-738, 1995.

In this last work the authors conclude that, while the presence of a biphenylmethyl group confers an interesting mixed ACE/NEP-inhibitory activity at the molecules having an α-mercaptoacyl dipeptide structure, the substitution of the biphenyl group with groups such as α- or β-naphthyl causes a significant loss of activity.

Now we have found N-mercaptoacyl phenylalanine derivatives which are endowed with a remarkable inhibitory activity on the angiotensin converting enzyme as well as on the neutral endopeptidase enzyme (mixed or dual ACE/NEP inhibitory activity) which makes them particularly useful in the therapy of cardiovascular pathologies.

Therefore, object of the present invention are the compounds of formula



(I)

wherein

R is a mercapto group or a R_4COS group convertible in the organism to mercapto group;

R_1 is a straight or branched C_2-C_4 alkyl group or an aryl or arylalkyl group having from 1 to 6 carbon atoms in the alkyl moiety wherein the aryl is a phenyl or a 5 or 6 membered aromatic heterocycle with one or two heteroatoms selected from the group consisting of nitrogen, oxygen and sulphur, optionally substituted with one or more substituents, the same or different, selected from the group consisting of halogen atoms, hydroxy groups, alkoxy, alkyl, alkylthio, alkylsulphonyl or alkoxycarbonyl groups having from 1 to 6 carbon atoms in the alkyl moiety, C_1-C_3 alkyl groups containing one or more fluorine atoms, carboxy groups, nitro groups, amino or aminocarbonyl groups, acylamino groups, aminosulphonyl groups, mono- or di-alkylamino or mono- or di-alkylaminocarbonyl groups having from 1 to 6 carbon atoms in the alkyl moiety;

R_2 is a hydrogen atom, a straight or branched C_1-C_4 alkyl or a benzyl group;

R_3 is a phenyl group substituted with a 5 or 6 membered aromatic heterocycle with one or two heteroatoms selected from the group consisting of nitrogen, oxygen and sulphur, being the phenyl and the heterocyclic groups optionally substituted with one or more substituents, the same or different, as indicated for R_1;

R_4 is a straight or branched C_1-C_4 alkyl group or a phenyl group;

the carbon atoms marked with an asterisk are stereogenic centers;

and pharmaceutically acceptable salts thereof.

The compounds of formula I contain two stereogenic centers and can thus exist in the form of stereoisomers.

Therefore, object of the present invention are the compounds of formula I in the form of stereoisomeric mixture as well as in the form of single stereoisomers.

The compounds of formula I object of the present invention are endowed with a mixed ACE/NEP-inhibitory activity and are useful in the treatment of cardiovascular diseases.

Although both terms mixed and dual are indifferently used, the term dual is the one more

commonly accepted in the literature for compounds endowed contemporaneously with ACE- and NEP-inhibitory activity.

In the present context, the terms mixed and dual are to be considered equivalent.

In the present description, unless otherwise specified, with the term straight or branched alkyl group we intend an alkyl such as methyl, ethyl, n.propyl, isopropyl, n.butyl, sec-butyl, tert-butyl, isobutyl, n.pentyl, 2-pentyl, 3-pentyl, isopentyl, tert-pentyl, n.hexyl and isohexyl group; with the term straight or branched alkoxy group we intend an alkoxy such as methoxy, ethoxy, n.propoxy and isopropoxy group; with the term halogen atom we intend a fluorine, chlorine, bromine or iodine atom; with the term acyl we intend an acyl group deriving from an aliphatic or aromatic carboxylic acid such as acetic, propionic, butyric and benzoic acid; with the term 5 or 6 membered aromatic heterocycle containing 1 or 2 heteroatoms selected among nitrogen, oxygen and sulphur we intend a group such as thiazole, isoxazole, oxazole, isothiazole, pyrazole, imidazole, thiophene, pyrrole, pyridine, pyrimidine, pyrazine, pyridazine and furan, optionally benzocondensed.

Examples of pharmaceutically acceptable salts of the compounds of formula I are the salts with alkali or alkali-earth metals and the salts with pharmaceutically acceptable organic bases.

Preferred compounds of formula I are the compounds wherein R_3 is a phenyl group substituted in position 4 with a heterocyclic group.

More preferred compounds, in this class, are the compounds of formula I wherein R_1 represents an arylalkyl group optionally substituted with one or more substituents, the same or different, selected from the group consisting of halogen atoms, hydroxy, alkyl or alkoxy.

Still more preferred compounds, in this class, are the compound of formula I wherein R_1 represents a phenylalkyl group optionally substituted with one or more substitutents, the same or different, selected from the group consisting of halogen atoms, hydroxy, alkyl or alkoxy.

Preferred examples of pharmaceutically acceptable salts of the compounds of formula I are the salts with alkali metals such as sodium, lithium and potassium.

It is clear to the man skilled in the art that the compounds of formula I wherein R is a R_4COS group convertible in the organism to mercapto group, as well as the compounds of formula I wherein R_2 is an alkyl or benzyl group, are biologic precursors (pro-drugs) of the corresponding compounds of formula I wherein R is a mercapto group (R=SH) or R_2 is a hydrogen atom

(R_2=H), respectively.

Specific examples of preferred compounds of formula I, object of the present invention, are:

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(2-thiazolyl)-phenylalanine methyl ester;

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(2-pyridyl)-phenylalanine methyl ester;

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(3-pyridyl)-phenylalanine benzyl ester;

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(2-furyl)-phenylalanine methyl ester;

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(5-pyrimidinyl)phenylalanine ethyl ester;

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(2-pyrazinyl)-phenylalanine methyl ester;

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(2-thienyl)-phenylalanine methyl ester;

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(3-thienyl)-phenylalanine methyl ester;

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(3-furyl)-phenylalanine methyl ester;

N-[3-phenylcarbonylthio-2-(3-pyridylmethyl)propionyl]-4-(2-thiazolyl)-phenylalanine methyl ester;

N-[3-acetylthio-2-(3-methoxyphenyl)methyl-propionyl]-4-(2-thiazolyl)-phenylalanine methyl ester;

N-[3-acetylthio-2-(2-fluorophenyl)methyl-propionyl]-4-(2-thiazolyl)-phenylalanine methyl ester;

N-[3-phenylcarbonylthio-2-(2-thienyl)methyl-propionyl]-4-(2-thiazolyl)-phenylalanine methyl ester;

N-[2-(2-furyl)methyl-3-phenylcarbonylthio-propionyl]-4-(2-thiazolyl)-phenylalanine methyl ester;

N-[2-(3-methyl-5-isoxazolyl)methyl-3-phenylcarbonylthio-propionyl]-4-(2-thiazolyl)-phenylalanine methyl ester;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(2-thiazolyl)-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(2-pyridyl)-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(3-pyridyl)-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(2-furyl)-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(5-pyrimidinyl)-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(2-pyrazinyl)-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(2-thienyl)-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(3-thienyl)-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(3-furyl)-phenylalanine;

N-[3-mercapto-2-(3-pyridylmethyl)propionyl]-4-(2-thiazolyl)-phenylalanine;

N-[3-mercapto-2-(3-methoxyphenyl)methyl-propionyl]-4-(2-thiazolyl)-phenylalanine;

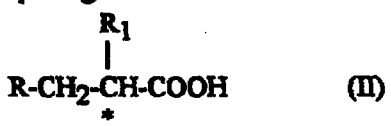
N-[3-mercapto-2-(2-thienyl)methyl-propionyl]-4-(2-thiazolyl)-phenylalanine;

N-[3-mercapto-2-(3-methyl-5-isoxazolyl)methyl-propionyl]-4-(2-thiazolyl)-phenylalanine;

N-[2-(2-fluorophenyl)methyl-3-mercapto-propionyl]-4-(2-thiazolyl)-phenylalanine;

N-[2-(2-furyl)methyl-3-mercapto-propionyl]-4-(2-thiazolyl)-phenylalanine.

The preparation of the compounds of formula I, object of the present invention, is carried out according to a synthetic process comprising the reaction between a compound of formula

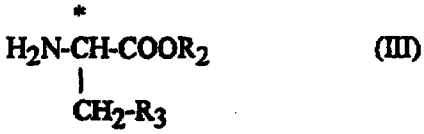


(II)

wherein

R and R_1 have the above reported meanings;

and a phenylalanine derivative of formula



(III)

wherein

R_2 and R_3 have the above reported meanings.

The condensation reaction is carried out according to conventional techniques of the chemistry of peptides.

Before carrying out the reaction it can be useful to properly protect the optional functional groups which could interfere in the reaction.

The optional protection is carried out according to conventional techniques.

In this respect the compounds wherein R is a R_4COS group are preferably used as intermediates of formula II, thus obtaining the corresponding compounds of formula I wherein

R=R_4COS from which, by hydrolysis, the compounds of formula I wherein R=SH can be obtained.

The evaluation of the usefulness of the optional protection as well as the selection of the kind of adopted protection, according to the reaction to be carried out and to the functional groups to be protected, are within the normal knowledge of the man skilled in the art.

The removal of the optional protective groups is carried out according to conventional techniques.

For a general reference to the use of protective groups in organic chemistry see Theodora W. Greene and Peter G.M. Wuts "Protective Groups in Organic Synthesis", John Wiley & Sons, Inc., II Ed., 1991.

The compounds of formula II are known or easily prepared according to conventional methods as described, for instance, in British patent No. 1576161 in the name of E.R. Squibb & Sons Inc.

Alternatively, the compounds of formula II can be prepared according to the synthetic methods described by M.C. Fournié-Zalusky et al. in J. Med. Chem. 1994, 37, 1070-1083.

Also the intermediate compounds of formula III are known or easily prepared according to known methods.

For instance, the compounds of formula III can be prepared according to the synthetic methods described by W. C. Shieh et al. in J. Org. Chem. 1992, 57, 379-381.

Alternatively, the compounds of formula III can be prepared by coupling methods (cross-coupling) starting from halogenated heterocyclic compounds and stannyl phenylalanine derivatives, as described by D.S. Wilbur et al. in Bioconjugate Chem., 1993, 4, 574-580.

The compounds of formula I in the form of single stereoisomers are prepared by stereoselective synthesis or by separation of the stereoisomeric mixture according to conventional techniques.

Also the preparation of the salts of the compounds of formula I, object of the present invention, is carried out according to conventional techniques.

The compounds of formula I, object of the present invention, are endowed with a mixed ACE/NEP-inhibitory activity and are useful in the treatment of cardiovascular diseases.

The inhibitory activity of the compounds of formula I was evaluated by means of in vitro tests

(example 8).

In particular, the inhibitory activity of the compounds of formula I was evaluated in comparison to the aforementioned Thiorphan and Captopril.

The in vitro inhibitory activity of the compounds of formula I, expressed as IC_{50} value, resulted at nM concentrations.

Said activity resulted to be comparable to that of Captopril, to what it concerns the ACE-inhibitory activity, and greater than that of Thiorphan, to what it concerns the NEP-inhibitory activity.

The inhibitory activity of the compounds of formula I, moreover, was also evaluated by means of ex-vivo tests in comparison to known compounds (example 9).

In particular, N-[3-mercapto-2-(3,4-methylenedioxyphenyl)methyl-propionyl]-(S)-phenylalanine and N-[3-mercapto-2-(3,4-methylenedioxyphenyl)methyl-propionyl]-(S)-tyrosine, described as enkephalinase and ACE-inhibitors in the aforementioned European patent application No. 0419327 and hereinafter referred to as compounds R-1 and R-2 respectively, as well as N-(3-mercapto-2-methylpropanoyl)-L-tyrosine and N-(3-mercapto-2-methylpropanoyl)-L-triptophan, described as ACE-inhibitors in U.S. 4,199,512 and as enkephalinase inhibitors in U.S. 4,401,677 and hereinafter referred to as compounds R-3 and R-4 respectively, were used as comparison compounds.

The ex-vivo ACE/NEP-inhibitory activity, in particular, was determined by evaluating the enzymatic activity in tissue homogenates (lung and kidney for ACE and NEP activity, respectively) from spontaneously hypertensive rats (SHR) treated with the tested compounds by intravenous or oral route.

It is worth noting that the activity shown by the compounds of formula I in the ex vivo tests confirms the mixed activity (dual activity) shown in the in vitro tests, also after oral administration.

Said activity, moreover, resulted to be significantly higher than that of the comparison compounds.

For a practical use in therapy, the compounds of formula I can be formulated in solid or liquid pharmaceutical compositions, suitable to oral or parenteral administration.

Therefore, the pharmaceutical compositions containing a therapeutically effective amount of a

compound of formula I in admixture with a carrier for pharmaceutical use are a further object of the present invention.

Specific examples of pharmaceutical compositions according to the present invention are tablets, coated tablets, capsules, granulates, solutions and suspensions suitable to oral administration, solutions and suspensions suitable to parenteral administration.

The pharmaceutical compositions object of the present invention are prepared according to conventional techniques.

The daily dose of the compound of formula I or of the corresponding pro-drug will depend on several factors such as the seriousness of the disease, the individual response of the patient or the kind of formulation but it is usually comprised between 0.1 mg and 10 mg per kg of body weight divided into a single dose or into more daily doses.

With the aim of illustrating the present invention the following examples are now given.

Unless otherwise specified, the flash chromatographies were carried out by using flash chromatography silica gel from Baker company (code 7024-00).

Example 1

Preparation of N-tert-butoxycarbonyl-4-(5-pyrimidinyl)-L-phenylalanine ethyl ester

A mixture of 5-pyrimidinylboronic acid (850 mg; 2 mmoles), N-tert-butoxycarbonyl-4-trifluoromethylsulphonyl-L-phenylalanine ethyl ester (450 mg; 2.2 mmoles), a solution of sodium carbonate (530 mg) in water (2.59 ml) and a mixture of toluene:ethanol=10:4.5 (20 ml) was degassed with nitrogen for 30 minutes.

Subsequently, palladium(0)tetrakis(triphenylphosphine) (120 mg; 0.06 mmoles) was therein added and the reaction mixture was heated at 90°C and kept under stirring for 3 hours.

The mixture was then kept at room temperature and N-tert-butoxycarbonyl-4-trifluoromethylsulphonyl-L-phenylalanine ethyl ester (112 mg) and palladium(0)tetrakis-(triphenylphosphine) (30 mg) were added again.

The mixture was further heated at the temperature of 90°C and kept under stirring for other 18 hours.

After cooling the reaction mixture at room temperature, ethyl acetate (100 ml) and water (40 ml) were added.

The phases were separated and the organic phase was dried on sodium sulphate and evapo-

rated under vacuum. The obtained residue was purified by flash chromatography (silica gel, eluent hexane:ethyl acetate=7:3, pressure of nitrogen 0.1 atm) thus affording N-tert-butoxycarbonyl-4-(5-pyrimidinyl)-L-phenylalanine ethyl ester (130 mg; 14% yield) as a colourless oil.

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.24 (t, 3H, CH_2-CH_3); 1.40 [s, 9H, $C(CH_3)_3$]; 3.01-3.25 (m, 2H, CH_2-CH); 4.19 (q, 2H, CH_2-CH_3); 4.52-4.65 (m, 1H, CH-COO); 5.06 (d, 1H, NH); 7.25-7.52 (m, 4H, phenylene); 8.91 (s, 2H, N-CH-C-CH-N); 9.19 (s, 1H, N-CH-N).

Example 2

Preparation of N-tert-butoxycarbonyl-4-(2-thiazolyl)-L-phenylalanine methyl ester

N-tert-butoxycarbonyl-4-(trifluoromethylsulphonyl)-L-phenylalanine methyl ester (8 g; 32.3 mmoles) and palladium-bis(triphenylphosphine) chloride (2.3 g) were added to a solution of 2-trimethylstannyl-thiazole (13.8 g; 32.3 mmoles) in a mixture of tetrahydrofuran: toluene=10:1 (50 ml), previously degassed with nitrogen.

The mixture was refluxed for 24 hours and, subsequently, 2-trimethylstannyl-thiazole (2 g) was added again.

After 6 hours at reflux N-tert-butoxycarbonyl-4-(trifluoromethylsulphonyl)-L-phenylalanine methyl ester (2 g) and palladium-bis(triphenylphosphine) chloride (700 mg) were added.

The resultant reaction mixture was kept under stirring at 70°C for 16 hours and, subsequently, cooled at room temperature.

Water (200 ml) was then added to the mixture which was extracted with methylene chloride (4x200 ml).

The collected organic phases were dried on sodium sulphate and evaporated under vacuum.

The obtained residue was purified by flash chromatography (silica gel, eluent methylene chloride:ethyl acetate=9:1, pressure of nitrogen=0.1 atm) thus affording N-tert-butoxycarbonyl-4-(2-thiazolyl)-L-phenylalanine methyl ester (2.3 g; 20% yield).

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.40 [s, 9H, $C(CH_3)_3$]; 3.00-3.21 (m, 2H, CH_2); 3.70 (s, 3H, $COOCH_3$); 4.42-4.65 (m, 1H, CH-COO); 5.02 (bd, 1H, NH); 7.30 (d, 1H, S-CH-CH-N); 7.81 (d, 1H, S-CH-CH-N); 7.15-7.90 (m, 4H, phenylene).

Example 3

Preparation of N-tert-butoxycarbonyl-4-(3-pyridyl)-L-phenylalanine methyl ester

3-Bromopyridine (1.67 g; 10 mmoles) and palladium(0)tetrakis(triphenylphosphine) (370 mg; 0.219 mmoles) were added to a solution of N-tert-butoxycarbonyl-4-(tributylstannyl)-L-phenylalanine methyl ester (4 g; 7.03 mmoles), prepared as described by D.S. Wilbur et al. in Bioconjugate Chem., 1973, 4, 574-580, in anhydrous dimethylformamide (30 ml), previously degassed with nitrogen.

The reaction mixture was kept under stirring for 10 minutes at room temperature and, subsequently, heated at 105°C for 6 hours.

Palladium(0)tetrakis(triphenylphosphine) (0.0035 mmoles) was again added and the mixture was kept under stirring at 105°C for 8 hours and then cooled at room temperature.

Water (100 ml) was therein added and the reaction mixture was extracted with hexane (3x150 ml).

The collected organic phases were washed with a saturated aqueous solution of potassium fluoride, dried on sodium sulphate and evaporated under vacuum.

The obtained residue was collected with ethyl acetate and filtered.

The resultant solution was evaporated under vacuum and the residue was purified by flash chromatography (silica gel, eluent hexane:ethyl acetate=8:2, pressure of nitrogen 0.1 atm) affording N-tert-butoxycarbonyl-4-(3-pyridyl)-L-phenylalanine methyl ester (1.5 g; 60% yield) as a colourless oil.

Mass (C.I.): $(M+H)^+$=357

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.40 [s, 9H, $C(CH_3)_3$]; 3.00-3.20 (m, 2H, CH_2); 3.71 (s, 3H, $COOCH_3$); 4.55 (m, 1H, CH-COO); 5.05 (bd, 1H, NH); 7.19-7.51 (m, 4H, phenylene); 7.30-8.82 (bm, 4H, pyridyl).

By working in an analogous way the following compounds were prepared:

N-tert-butoxycarbonyl-4-(2-pyridyl)-L-phenylalanine methyl ester

Mass (C.I.): $(M+H)^+$=357

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.40 [s, 9H, $C(CH_3)_3$]; 3.03-3.21 (m, 2H, CH_2-CH); 3.70 (s, 3H, $COOCH_3$); 4.54-4.65 (m, 1H, CH-COO); 4.98 (d, 1H, CONH); 7.16-7.21 (m, 1H, N-C-CH-CH); 7.65-7.77 (m, 2H, N-CH-CH-CH); 7.19-7.93 (m, 4H, phenylene); 8.63-8.68 (m, 1H, N-CH);

N-tert-butoxycarbonyl-4-(2-pyrazinyl)-L-phenylalanine methyl ester

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.40 [s, 9H, $C(CH_3)_3$]; 3.02 (m, 2H, CH_2); 3.70 (s, 3H, $COOCH_3$); 4.53-4.70 (m, 1H, CH-COO); 5.03 (bd, 1H, NH); 7.21-7.98 (m, 4H, phenylene); 8.49 and 8.62 [2(bs, 2H, N-CH-CH-N)]; 9.00 (s, 1H, CH-N-CH-CH);

N-tert-butoxycarbonyl-4-(2-thienyl)-L-phenylalanine methyl ester

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.41 [m, 9H, $C(CH_3)_3$]; 2.98-3.18 (m, 2H, CH_2); 3.71 (s, 3H, $COOCH_3$); 4.53-4.64 (m, 1H, CH-COO); 4.98 (bd, 1H, NH); 7.02-7.28 (m, 3H, thienyl); 7.10-7.54 (m, 4H, phenylene).

Example 4

Preparation of 4-(3-pyridyl)-L-phenylalanine methyl ester dihydrochloride

Thionyl chloride (0.85 ml; 4.78 mmoles) was added dropwise to a solution of N-tert-butoxy-carbonyl-4-(3-pyridyl)-L-phenylalanine methyl ester (1.4 g; 3.93 mmoles) in methanol (30 ml), prepared as described in example 3, keeping the temperature at 0°C.

At the end of the addition, the reaction mixture was brought at room temperature and kept under stirring for 8 hours.

The solvent was then evaporated under vacuum affording 4-(3-pyridyl)-L-phenylalanine methyl ester dihydrochloride (820 mg; 71% yield) used as such in the following reactions.

Mass (C.I.):$(M+H)^+$= 257 (free base)

^{1}H-NMR (200 MHz, D_2O): δ (ppm): 3.11-3.32 (m, 2H, CH_2); 3.67 (s, 3H, CH_3); 4.31-4.37 (m, 1H, CH); 7.30-7.63 (m, 4H, phenylene); 7.97 (dd, 1H, CH-N-CH-CH-CH); 8.59 (d, 1H, CH-N-CH-CH-CH); 8.65-8.71 (m, 1H, CH-N-CH-CH); 8.89 (d, 1H, CH-N-CH-CH-CH).

By working in an analogous way the following compounds were prepared:

4-(2-pyridyl)-L-phenylalanine methyl ester dihydrochloride

Mass (C.I.): $(M+H)^+$= 257 (free base)

^{1}H-NMR (200 MHz, D_2O): δ (ppm): 3.12-3.33 (m, 2H, CH_2); 3.64 (s, 3H, CH_3); 4.30-4.37 (m, 1H, CH); 7.36-7.73 (m, 4H, phenylene); 7.78-8.58 (m, 4H, pyridyl);

4-(5-pyrimidinyl)-L-phenylalanine ethyl ester dihydrochloride

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 1.11 (t, 3H, CH_2-CH_3); 3.10-3.35 (m, 2H, CH_2-CH); 4.04-4.20 (m, 2H, CH_2-CH_3); 4.22-4.35 (m, 1H, CH); 7.40-7.84 (m, 4H, phenylene); 9.15 (s, 2H, N-CH-C-CH-N); 9.19 (s, 1H, N-CH-N);

4-(2-pyrazinyl)-L-phenylalanine methyl ester dihydrochloride

Mass (C.I.): $(M+H)^+$= 258 (free base)

^{1}H-NMR (200 MHz, DCl 1N): δ (ppm): 3.45-3.67 (m, 2H, CH_2); 3.98 (s, 3H, CH_3); 4.65-4.72 (m, 1H, CH); 7.68-8.26 (m, 4H, phenylene); 9.02 (d, 1H, CH-N-CH-CH); 9.44 (dd, 1H, CH-N-CH-CH); 9.54 (d, 1H, CH-N-CH-CH);

4-(2-thienyl)-L-phenylalanine methyl ester hydrochloride

Mass (C.I.): $(M+H)^+$= 262 (free base)

^{1}H-NMR (200 MHz, D_2O): δ (ppm): 2.98-3.19 (m, 2H, CH_2); 3.65 (s, 3H, CH_3); 4.21-4.28 (m, 1H, CH); 6.96-7.28 (m, 3H, thienyl); 7.08-7.52 (m, 4H, phenylene);

4-(2-thiazolyl)-L-phenylalanine methyl ester dihydrochloride

^{1}H-NMR (200 MHz, D_2O): δ (ppm): 3.10-3.32 (m, 2H, CH_2-CH); 3.68 (s, 3H, CH_3); 4.30-4.38 (m, 1H, CH); 7.30-7.80 (m, 4H, phenylene); 7.70-7.91 (m, 2H, thiazolyl).

Example 5

Preparation of 2-isobutyl-3-phenylcarbonylthio-propionic acid

A mixture of 2-isobutyl-acrylic acid (6.34 g; 49 mmoles) and thiobenzoic acid (5.96 ml; 51 mmoles) was heated at 100°C under stirring for 2 hours.

The reaction mixture was then treated with petroleum ether 40-60°C (100 ml) and cooled at -70°C in a dry ice/acetone bath.

By filtration and washing with petroleum ether at -70°C a residue was collected which, dried at reduced pressure, furnished 2-isobutyl-3-phenylcarbonylthio-propionic acid (11.12 g; 85% yield) as a white solide.

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 0.90-1.00 (m, 6H); 1.40-1.90 (m, 3H); 2.70-2.90 (m, 1H); 3.10-3.40 (m, 2H); 7.35-7.62 (m, 3H); 7.90-8.00 (d, 2H).

By working in an analogous way the following compounds were prepared:

2-(3-methoxyphenyl)methyl-3-phenylcarbonylthio-propionic acid

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.32 (s, 3H); 2.80-3.15 (m, 5H); 3.77 (s, 3H); 6.70-6.80 (m, 3H); 7.14-7.25 (m, 1H).

3-acetylthio-2-(2-fluorophenyl)methyl-propionic acid

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.31 (s, 3H); 2.90-3.20 (m, 5H); 6.95-7.30 (m, 4H).

3-phenylcarbonylthio-2-(2-thienyl)methyl-propionic acid benzylamine salt

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.45-3.25 (m, 5H, S-CH_2-CH-CH_2); 3.90 (s, 2H, CH_2-phenyl); 6.85-7.91 (m, 13H, aryl).

Example 6

Preparation of N-[(2S)-3-phenylcarbonylthio-2-phenylmethylpropionyl]-4-(2-thiazolyl)-L-phenylalanine methyl ester (compound 1)

A solution of hydroxybenzotriazole (0.54 g; 4 mmoles) in tetrahydrofuran (30 ml) and, subsequently, a solution of dicyclohexylcarbodiimide (0.825 g; 4 mmoles) in methylene chloride (15 ml) were added, at 0°C under stirring, to a mixture of (2S)-3-phenylcarbonylthio-2-phenylmethylpropionic acid (1.2 g; 4 mmoles), 4-(2-thiazolyl)-L-phenylalanine methyl ester dihydrochloride (1.34 g; 4 mmoles), prepared as described in example 4, triethylamine (1.11 ml; 8 mmoles) in tetrahydrofuran (20 ml) and methylene chloride (30 ml).

The reaction mixture was kept under stirring for 20 hours, then dicyclohexyl urea was filtered off and the solvent was evaporated at reduced pressure.

The residue was collected with ethyl acetate and the solution was washed with an aqueous solution of sodium chloride at 20%, sodium bicarbonate at 5% and sodium chloride at 20% again.

After separation of the phases and evaporation of the organic phase, the resultant white solid was purified by flash chromatography (silica gel, eluent ethyl acetate:hexane=40:60, pressure of nitrogen 0.1 atm) thus affording N-[(2S)-3-phenylcarbonylthio-2-phenylmethylpropionyl]-4-(2-thiazolyl)-L-phenylalanine methyl ester (1.5 g).

m.p. 98-100°C

Mass (C.I.): $(M+H)^+$= 545

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.63-3.35 (m, 7H, CH_2-CH-CH_2, CH_2-C_6H_4-thiazolyl); 3.68 (s, 3H, $COOCH_3$); 4.75-4.85 (m, 1H, CH-COO); 5.78 (d, 1H, NH); 7.10-8.00 (m, 16H, aryl).

By working in an analogous way, starting from compounds known or prepared as described in examples 4 and 5, the following compounds were obtained:

N-[(2S)-3-phenylcarbonylthio-2-phenylmethylpropionyl]-4-(2-furyl)-L-phenylalanine methyl ester (compound 2)

m.p. 132-134°C

Mass (C.I.) $(M+H)^+$= 528

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.60-3.35 (m, 7H, CH_2-CH-CH_2, CH_2-C_6H_4-furyl); 3.58 (s, 3H, $COOCH_3$); 4.71-4.81 (m, 1H, CH-COO); 5.73 (d, 1H, NH); 6.40-8.00 (m, 17H, aryl);

N-[(2S)-3-phenylcarbonylthio-2-phenylmethylpropionyl]-4-(5-pyrimidinyl)-L-phenylalanine ethyl ester (compound 3)

m.p. 117-119°C

Mass (C.I.) $(M+H)^+$= 554

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.18 (t, 3H, CH_3-CH_2); 2.65-3.35 (m, 7H, CH_2-CH-CH_2, CH_2-C_6H_4-pyrimidinyl); 3.95-4.20 (m, 2H, $COOCH_2$); 4.70-4.80 (m, 1H, CH-COO); 5.78 (d, 1H, NH); 7.05-8.00 (m, 14H, phenyl, phenylene); 8.68 (s, 2H, CH-N-CH-N-CH); 9.11 (s, 1H, N-CH-N);

N-[(2S)-3-phenylcarbonylthio-2-phenylmethylpropionyl]-4-(2-pyrazinyl)-L-phenylalanine methyl ester (compound 4)

m.p. 145-147°C

Mass (C.I.) $(M+H)^+$= 540

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.65-3.35 (m, 7H, CH_2-CH-CH_2, CH_2-C_6H_4-pyrazinyl); 3.61 (s, 3H, $COOCH_3$); 4.75-4.85 (m, 1H, CH-COO); 5.78 (d, 1H, NH); 7.10-8.00 (m, 14H, phenyl, phenylene); 8.48-8.75 (m, 3H, CH-N-CH-CH-N);

N-[(2S)-3-phenylcarbonylthio-2-phenylmethylpropionyl]-4-(3-pyridyl)-L-phenylalanine methyl ester (compound 5)

m.p. 132-134°C

Mass (C.I.) $(M+H)^+$= 539

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.66-2.79 (m, 1H, CH_2-CH-CH_2); 2.88-3.35 (m, 6H, CH_2-CH-CH_2, CH_2-C_6H_4-pyridyl); 3.61 (s, 3H, $COOCH_3$); 4.75-4.85 (m, 1H, CH-COO); 5.77 (d, 1H, NH); 7.07-7.99 (m, 16H, CH-CH-CH-N, phenyl, phenylene); 8.51-8.64 (m, 2H, CH-N-CH);

N-[(2S)-3-phenylcarbonylthio-2-phenylmethylpropionyl]-4-(2-pyridyl)-L-phenylalanine methyl ester (compound 6)

m.p. 123-125°C

Mass (C.I.) $(M+H)^+$= 539

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.63-2.77 (m, 1H, CH_2-C<u>H</u>-CH_2); 2.85-3.35 (m, 6H, CH_2-CH-CH_2, CH_2-C_6H_4-pyridyl); 3.56 (s, 3H, $COOCH_3$); 4.75-4.85 (m, 1H, CH-COO); 5.75 (d, 1H, NH); 7.09-7.99 (m, 16H, C<u>H</u>-C<u>H</u>-C<u>H</u>-N, phenyl, phenylene); 8.61-8.65 (m, 1H, N-C<u>H</u>-CH);

<u>N-[(2S)-3-phenylcarbonylthio-2-phenylmethylpropionyl]-4-(2-thienyl)-L-phenylalanine methyl ester</u> (compound 7)

Mass (C.I.) $(M+H)^+$= 544

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.64-3.36 (m, 7H, CH_2-CH-CH_2, CONH-CH-CH_2); 3.58 (s, 3H, $COOCH_3$); 4.74-4.83 (m, 1H, CH-COO); 5.74 (d, 1H, NH); 6.97-7.99 (m, 17H, aryl);

<u>N-[(2S)-3-phenylcarbonylthio-2-phenylmethylpropionyl]-4-(3-thienyl)-L-phenylalanine methyl ester</u> (compound 8)

Mass (C.I.) $(M+H)^+$= 544

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.63-3.35 (m, 7H, CH_2-CH-CH_2, NH-CH-CH_2); 3.58 (s, 3H, $COOCH_3$); 4.73-4.85 (m, 1H, CH-COO); 5.70-5.76 (bd, 1H, NH); 7.00-7.62 (m, 15H, phenyl, phenylene, C<u>H</u>-CH-S); 7.93-8.00 (m, 2H, CH-S-CH);

<u>N-[(2S)-3-phenylcarbonylthio-2-phenylmethylpropionyl]-4-(3-furyl)-L-phenylalanine methyl ester</u> (compound 9)

m.p. 115-117°C

Mass (C.I.) $(M+H)^+$= 528

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.62-3.36 (m, 7H, CH_2-CH-CH_2, NH-CH-CH_2); 3.58 (s, 3H, $COOCH_3$); 4.71-4.82 (m, 1H, CH-COO); 5.72 (bd, 1H, NH); 6.50-8.00 (m, 17H, aryl);

<u>N-[3-phenylcarbonylthio-2-(3-pyridylmethyl)propionyl]-4-(2-thiazolyl)-L-phenylalanine methyl ester</u> - stereoisomer A (compound 10)

Mass (C.I.) $(M+H)^+$= 546

TLC (ethyl acetate:petroleum ether=95:5), Rf = 0.33

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.60-3.38 (m, 7H, CH_2-CH-CH_2, NH-CH-CH_2); 3.60 (s, 3H, $COOCH_3$); 4.79-4.90 (m, 1H, CH-COO); 6.21 (bd, 1H, NH); 7.29-7.81 (m, 2H,

thiazolyl); 6.75-8.48 (m, 13H, phenyl, phenylene, pyridyl);

N-[3-phenylcarbonylthio-2-(3-pyridylmethyl)propionyl]-4-(2-thiazolyl)-L-phenylalanine methyl ester - stereoisomer B (compound 11)

Mass (C.I.) $(M+H)^+ = 546$

TLC (ethyl acetate:petroleum ether=95:5), Rf = 0.24

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.61-3.25 (m, 7H, CH_2-CH-CH_2, NH-CH-CH_2); 3.60 (s, 3H, $COOCH_3$); 4.80-4.91 (m, 1H, CH-COO); 6.09 (bd, 1H, NH); 7.27-7.80 (m, 2H, thiazoly); 7.10-8.40 (m, 13H, phenyl, phenylene, pyridyl);

N-[2-isobutyl-3-phenylcarbonylthio-propionyl]-4-(2-thiazolyl)-L-phenylalanine methyl ester (compound 12)

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 0.80-0.95 (m, 6H); 1.30-1.80 (m, 3H); 2.40-2.60 (m, 1H); 3.00-3.30 (m, 4H); 3.70 (d, 3H); 4.90-5.05 (m, 1H); 6.00-6.15 (bt, 1H); 7.10-8.00 (m, 11H);

N-[3-acetylthio-2-(3-methoxyphenyl)methyl-propionyl]-4-(2-thiazolyl)-L-phenylalanine methyl ester (compound 13)

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.30 (d, 3H); 2.45-3.20 (m, 7H); 3.63 (d, 3H); 3.75 (d, 3H); 4.70-4.93 (m, 1H); 5.70-5.90 (dd, 1H); 6.60-6.85 (m, 4H); 7.17-7.32 (m, 3H); 7.68-7.90 (m, 3H);

N-[3-acetylthio-2-(3-fluorophenyl)methyl-propionyl]-4-(2-thiazolyl)-L-phenylalanine methyl ester (compound 14)

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.29 (s, 3H); 2.55-3.20 (m, 7H); 3.65 (2s, 3H); 4.70-4.90 (m, 1H); 5.80-6.00 (2d, 1H); 6.70-7.32 (m, 3H);

N-[3-phenylcarbonylthio-2-(2-thienyl)methyl-propionyl]-4-(2-thiazolyl)-L-phenylalanine methyl ester (compound 15)

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.68-3.37 (m, 7H); 3.60-3.61 (2s, 3H, $COOCH_3$); 4.31-4.45 (m, 1H, C<u>H</u>-$COOCH_3$); 6.01-6.10 (2d, 1H, NH); 6.80-8.00 (m, 14H);

Example 7

Preparation of N-[(2S)-3-mercapto-2-phenylmethylpropionyl]-4-(2-thiazolyl)-L-phenylalanine (compound 16)

N-[(2S)-3-phenylcarbonylthio-2-phenylmethylpropionyl]-4-(2-thiazolyl)-L-phenylalanine

methyl ester (1.4 g; 2.57 mmoles), prepared as described in example 6, was suspended in ethanol (30 ml), degassed by nitrogen bubbling to eliminate the oxygen.

An aqueous degassed solution of sodium hydroxide 1N (7.7 ml) and, at the end of the addition, further degassed ethanol (20 ml) were added dropwise at 5°C to the suspension.

The reaction mixture was kept under stirring for 4 hours at room temperature, then cooled at 0°C and acidified with hydrochloric acid 5% (10 ml).

The reaction mixture was evaporated to dryness and the residue was collected with acetonitrile and water and, subsequently, was filtered thus affording N-[(2S)-3-mercapto-2-phenylmethylpropionyl]-4-(2-thiazolyl)-L-phenylalanine (1 g).

m.p. 180-182°C

Mass (C.I.) $(M+H)^+ = 427$

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 1.80-1.88 (m, 1H, SH); 2.22-2.84 (m, 5H, CH_2-CH-CH_2); 2.86-3.18 (m, 2H, CH_2-CH-COO); 4.46-4.57 (m, 1H, CH-COO); 7.10-7.25 (m, 5H, phenyl); 7.32-7.84 (m, 4H, phenylene); 7.74 (d, 1H, N-CH=CH-S); 7.89 (d, 1H, N-CH=CH-S); 8.35 (d, 1H, NH); 12.76 (s, 1H, COOH);

By working in an analogous way the following compounds were prepared:

N-[(2R)-3-mercapto-2-phenylmethylpropionyl]-4-(2-tiazolyl)-L-phenylalanine (compound 17)

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.15-2.23 (m, 1H, SH); 2.31-2.74 (m, 5H, CH_2-CH-CH_2); 2.78-3.07 (m, 2H, CH_2-CH-COO); 4.42-4.53 (m, 1H, CH-COO); 7.02-7.80 (m, 9H, phenyl and phenylene); 7.75 (d, 1H, N-CH=CH-S); 7.90 (d, 1H, N-CH=CH-S); 8.36 (d, 1H, NH);

N-[(2S)-3-mercapto-2-phenylmethylpropionyl]-4-(2-furyl)-L-phenylalanine (compound 18)

m.p. 153-155°C

Mass (C.I.) $(M+H)^+ = 410$

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.40 (t, 1H, SH); 2.45-3.25 (m, 7H, CH_2-CH-CH_2, CH_2-CH-COO); 4.80-4.90 (m, 1H, CH-COO); 5.86 (d, 1H, NH); 6.42-7.42 (m, 3H, furyl); 7.07-7.57 (m, 9H, phenyl, phenylene);

N-[(2S)-3-mercapto-2-phenylmethylpropionyl]-4-(5-pyrimidinyl)-L-phenylalanine (compound 19)

m.p. 193-195°C

Mass (C.I.) $(M+H)^+$= 422

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 1.81 (bm, 1H, SH); 2.21-3.20 (m, 7H, CH_2-CH-CH_2, CH_2-C_6H_4-pyrimidinyl); 4.46-4.57 (m, 1H, CH-COO); 7.06-7.29 (m, 5H, phenyl); 7.36-7.72 (m, 4H, phenylene); 8.33 (d, 1H, NHCO); 9.08 (s, 2H, N-CH-C-CH-N); 9.15 (s, 1H, N-CH-N);

N-[(2S)-3-mercapto-2-phenylmethylpropionyl]-4-(2-pyrazinyl)-L-phenylalanine (compound 20)

m.p. 176-178°C

Mass (C.I.) $(M+H)^+$= 422

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 1.84 (bt, 1H, SH); 2.21-3.21 (m, 7H, CH_2-CH-CH_2, CH_2-CH-COO); 4.48-4.59 (m, 1H, CH-COO); 7.10-7.26 (m, 5H, phenyl); 7.37-8.05 (m, 4H, phenylene); 8.37 (d, 1H, NHCO); 8.58 (d, 1H, CH-N-CH-CH-N); 8.68-8.70 (m, 1H, CH-N-CH-CH-N); 9.21 (d, 1H, C-CH-N); 12.78 (b, 1H, COOH);

N-[(2S)-3-mercapto-2-phenylmethylpropionyl]-4-(3-pyridyl)-L-phenylalanine (compound 21)

m.p. 146-148°C

Mass (C.I.) $(M+H)^+$= 421

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 1.69-1.89 (b, 1H, SH); 2.22-3.18 (m, 7H, CH_2-CH-CH_2, CH_2-phenylene); 4.45-4.56 (m, 1H, CH-COO); 7.09-7.26 (m, 5H, phenyl); 7.42-7.48 (m, 1H, CH-N-CH-CH-CH); 7.62-7.33 (m, 4H, phenylene); 7.98-8.04 (m, 1H, CH-N-CH-CH-CH); 8.30 (d, 1H, CONH); 8.53 (dd, 1H, CH-N-CH-CH-CH); 8.83 (d, 1H, CH-N-CH-CH-CH);

N-[(2S)-3-mercapto-2-phenylmethylpropionyl]-4-(2-pyridyl)-L-phenylalanine (compound 22)

m.p. 157-159°C

Mass (C.I.) $(M+H)^+$= 421

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 1.87 (b, 1H, SH); 2.23-3.19 (m, 7H, S-CH_2-CH-CH_2, CONH-CH-CH_2); 4.44-4.45 (m, 1H, CH-COO); 7.09-7.93 (m, 8H, N-CH-CH-CH-CH, phenyl); 7.30-7.99 (m, 4H, phenylene); 8.29 (d, 1H, CONH); 8.61-8.65 (m, 1H, C-N-CH);

N-[(2S)-3-mercapto-2-phenylmethylpropionyl]-4-(2-thienyl)-L-phenylalanine (compound 23)

m.p. 152-154°C

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.34-1.43 (m, 1H, SH); 2.44-3.26 (m, 7H, S-CH_2-CH-CH_2, CONH-CH-$C\underline{H}_2$); 4.80-4.89 (m, 1H, NH-C<u>H</u>-COO); 5.81 (d, 1H, NH); 7.02-7.52 (m, 12H, aryl);

<u>N-[(2S)-3-mercapto-2-phenylmethylpropionyl]-4-(3-thienyl)-L-phenylalanine</u> (compound 24)

m.p. 169-171°C

Mass (C.I.) $(M+H)^+$ = 426

TLC (ethyl acetate:hexane:acetic acid=50:50:5), Rf = 0.44

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.84 (bs, 1H, SH); 2.23-3.13 (m, 7H, S-CH_2-CH-CH_2, NH-CH-$C\underline{H}_2$); 4.42-4.53 (m, 1H, NH-C<u>H</u>-COO); 7.12-7.80 (m, 12H, aryl); 8.30 (d, 1H, JHH=8.2 Hz, NH);

<u>N-[(2S)-3-mercapto-2-phenylmethylpropionyl]-4-(3-furyl)-L-phenylalanine</u> (compound 25)

m.p. 140-142°C

Mass (C.I.) $(M+H)^+$ = 410

TLC (ethyl acetate:hexane:acetic acid=50:50:5), Rf = 0.42

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.79-1.90 (m, 1H, SH); 2.22-3.11 (m, 7H, S-CH_2-CH-CH_2, NH-CH-$C\underline{H}_2$); 4.41-4.53 (m, 1H, NH-C<u>H</u>-COO); 7.11-7.50 (m, 9H, phenyl, phenylene); 6.91-8.12 (m, 3H, furyl); 8.30 (d, 1H, JHH=8.2 Hz, NH);

<u>N-[3-mercapto-2-(3-pyridylmethyl)propionyl]-4-(2-thiazolyl)-L-phenylalanine</u> - stereoisomer A (compound 26)

m.p. 193-196°C

Mass (C.I.) $(M+H)^+$ = 428

TLC (methylene chloride:methanol:acetic acid=85:15:1.5), Rf = 0.53

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.37-3.05 (m, 7H, S-CH_2-CH-CH_2, NH-CH-$C\underline{H}_2$); 4.36-4.47 (m, 1H, NH-C<u>H</u>-COO); 7.76-7.90 (m, 2H, thiazolyl); 7.10-8.33 (m, 9H, NH, pyridyl, phenylene);

<u>N-[3-mercapto-2-(3-pyridylmethyl)propionyl]-4-(2-thiazolyl)-L-phenylalanine</u> - stereoisomer B (compound 27)

Mass (C.I.) $(M+H)^+$ = 428

TLC (methylene chloride:methanol:acetic acid=85:15:1.5), Rf = 0.47

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.28-3.20 (m, 7H, S-CH_2-CH-CH_2, NH-CH-

$C\underline{H}_2$); 4.20-4.35 (m, 1H, NH-C<u>H</u>-COO); 7.70-7.90 (m, 2H, thiazolyl); 7.17-8.40 (m, 9H, NH, pyridyl, phenylene);

N-(2-isobutyl-3-mercapto-propionyl)-4-(2-thiazolyl)-L-phenylalanine (compound 28)

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 0.50-1.44 (m, 9H); 1.68-2.25 (m, 4H); 2.79-3.22 (m, 2H); 4.50-4.63 (m, 1H); 7.34-7.85 (m, 4H); 7.73-7.90 (m, 2H); 8.27-8.39 (2d, 1H);

Mass (C.I.) $(M+H)^+$= 393

N-[3-mercapto-2-(3-methoxyphenyl)methyl-propionyl]-4-(2-thiazolyl)-L-phenylalanine (compound 29)

Mass (C.I.) $(M+H)^+$=457

^{1}H-NMR (200 MHz, DMSO-d_6+D_2O): δ (ppm): 2.20-3.21 (m, 7H); 3.70 (d, 3H); 4.48 (m, 1H); 6.55-6.86 (m, 3H); 7.00-7.40 (m, 3H); 7.65-7.95 (m, 4H); 8.27-8.45 (bt, CONH);

N-[3-mercapto-2-(2-fluorophenyl)methyl-propionyl]-4-(2-thiazolyl)-L-phenylalanine (compound 30)

Mass (C.I.) $(M+H)^+$=445

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.20-3.18 (m, 8H); 4.35-4.55 (m, 1H); 6.85-7.35 (m, 6H); 7.65-7.90 (m, 4H); 8.35 (d, 1H);

N-[3-mercapto-2-(2-thienyl)methyl-propionyl]-4-(2-thiazolyl)-L-phenylalanine (compound 31)

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 1.82-3.19 (m, 8H, CH_2-CH_2-CH_2; NH-CH-$C\underline{H}_2$); 4.44-4.59 (m, 1H, CH-COO); 6.62-7.91 (m, 9H, aryl); 8.42 (d, 1H, NH);

N-[3-mercapto-2-(2-furyl)methyl-propionyl]-4-(2-thiazolyl)-L-phenylalanine (compound 32)

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 1.79-3.18 (m, 8H, S-CH_2-CH_2-CH_2; NH-CH-$C\underline{H}_2$); 4.43-4.58 (m, 4H, CH-NH); 5.79-7.47 (m, 3H, furyl); 7.30-7.85 (m, 4H, phenylene); 7.73-7.91 (m, 2H, thiazolyl); 8.40-8.46 (2d, 1H, NH);

N-[3-mercapto-2-(3-methyl-5-isoxazolyl)methyl-propionyl]-4-(2-thiazolyl)-L-phenylalanine (compound 33)

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.00-2.12 (2S, 3H, $C\underline{H}_3$-isoxazolyl); 2.28-3.19 (m, 8H, S-CH_2-CH_2-CH_2; $C\underline{H}_2$-phenylene); 4.43-4.59 (m, 1H, C<u>H</u>-NH); 5.75-6.03 (2s, 1H, isoxazolyl); 7.29-7.86 (m, 4H, phenylene); 7.74-7.90 (m, 2H, thiazolyl); 8.46-8.53 (2d, 1H, NH);

Example 8

In vitro evaluation of the pharmacologic activity

a) NEP-inhibitory activity

The NEP-inhibitory activity was evaluated in rat kidney cortex membranes prepared according to the procedure described by T. Maeda et al. in Biochim. Biophys. Acta 1983, 731(1), 115-120.

Kidneys were removed from male Sprague-Dawley rats weighing approximately 300 g and kept at 4°C.

Kidney cortex was carefully dissected, finely minced and suspended in a homogenization buffer (10 mM sodium phosphate pH 7.4 containing 1 mM $MgCl_2$, 30 mM NaCl, 0.02% NaN_3) 1:15 weight/volume.

The tissue was then cold homogenized for 30 seconds using an Ultra-Turrax homogenizer.

Approximately 10 ml of homogenate were layered over 10 ml of sucrose (41% weight/volume) and centrifuged at 31200 rpm for 30 minutes at 4°C in a fixed angle rotor.

The membranes were collected from the buffer/sucrose interface, washed twice with 50 mM TRIS/HCl buffer (pH 7.4) and resuspended into the same buffer for storage in small aliquots at -80°C until use.

The NEP-inhibitory activity was evaluated by using the method described by C. Llorens et al., in Eur. J. Pharmacol., 69, (1981), 113-116, as herinafter reported.

Aliquots of the membrane suspension prepared as above described (concentration 5 µg/ml of proteins) were preincubated in the presence of an aminopeptidase inhibitor (Bestatin - 1 mM) for 10 minutes at 30°C.

[^{3}H][Leu^5]-enkephaline (15 nM) and buffer TRIS/HCl pH 7.4 (50 mM) were added in order to obtain a final volume of 100 µl.

Incubation (20 minutes at 30°C) was stopped by adding HCl 0.1M (100 µl).

The formation of the metabolite [^{3}H]Tyr-Gly-Gly was quantified by chromatography on polystyrene columns (Porapak Q).

The percentage of inhibition of the metabolite formation in the membrane preparations treated with the compounds of formula I and with the comparative compounds with respect to the untreated membrane preparations was expressed as IC_{50} (nM) value.

b) ACE-inhibitory activity

The ACE-inhibitory activity was evaluated according to the method reported in the literature by B. Holmquist et al., in Analytical Biochemistry 95, 540-548 (1979).

50 μM of ACE (250 mU/ml purified by lung rabbit, EC 3.4.15.1 SIGMA) were preincubated with 50 μl of the compound of formula I or of the reference compound or related vehicle in thermostated cuvettes at 37°C.

The reaction was started by adding furylacryloylphenylalanylglycylglycine 0.8 mM (FAPGG-SIGMA).

Contemporaneously, by using a Beckman DU-50 spectrophotometer provided with a program for calculating delta A/minutes and regression coefficients of the enzyme kinetics curves, the absorbance at 340 nm was recorded in continuo for 5 minutes.

The percentage of the enzyme inhibition in the preparations treated with the compounds of formula I or with the comparative compounds with respect to the untreated preparations was expressed as IC_{50} (nM) value.

The IC_{50} (nM) values related to the ACE-inhibitory activity and NEP-inhibitory activity of the compounds 16, 18-25, 27-33 and of the comparison compounds Thiorphan and Captopril are reported in the following table 1.

Table 1

ACE-inhibitory and NEP-inhibitory activity, expressed as IC_{50} (nM) value, of compounds 16, 18-25, 27-33 and of Thiorphan and Captopril.

Compound	ACE-inhibitory activity IC_{50} (nM)	NEP-inhibitory activity IC_{50} (nM)
16	3.2	1.8
18	1.8	1.8
19	1.9	1.8
20	1.5	2.5
21	1.7	2.6
22	1.8	2.0
23	1.6	0.6
24	2.5	1.3
25	2.4	1
27	9.1	17.3
28	5.8	1.8
29	4.6	9.0
30	10.7	11.2
31	8.6	1.2
32	8.6	4.0
33	7.9	4.5
Thiorphan	99	18
Captopril	4.6	not active

The data reported in table 1 show that the compounds of formula I, object of the present invention, are endowed with a significant mixed ACE/NEP inhibitory activity.

Said activity is comparable to that of Captopril, to what it concerns the ACE inhibitory activity, and greater than that of Thiorphan, to what it concerns the NEP inhibitory activity.

Example 9

"Ex vivo" evaluation of the pharmacologic activity

a) NEP-inhibitory activity

The ex vivo NEP-inhibitory activity was evaluated according to the procedure reported in the literature by M. Orlowsky et al., in Biochemistry 1981, 20, 4942-4950.

The inhibitory activity of the compounds of formula I was evaluated in kidneys of spontaneously hypertensive rats (SHR), 5 minutes after i.v. injection (0.6 and 21 μmoles/kg) and 30 minutes, 60 minutes and 4 hours after oral administration (30 μmoles/kg) of the tested compounds.

After the removal of the kidneys from SHR, the renal tissue was homogenized and incubated for 15 minutes at 37°C in the presence of Glutaryl-Ala-Ala-Phe-2-naphthylamide (GAAP), as a substrate, and aminopeptidase M at pH 7.6.

The reaction was stopped by adding an aqueous solution at 10% of trichloroacetic acid.

The released 2-naphthylamine was determined by adding fast garnet dye (2 ml).

Enzyme reaction rates were determined by measuring the increase in the optical density at 524 nm (OD_{524}) with respect to a standard obtained with 2-naphthylamine complexed with fast garnet.

The NEP-inhibitory activity of the tested compounds was expressed as percentage of NEP-inhibition in SHR kidneys.

b) ACE-inhibitory activity

The ex vivo ACE-inhibitory activity was evaluated by using a radiometric assay method, as reported in the literature by J. W. Ryan et al. in Biochem. J. (1977), 167, 501-504.

The inhibitory activity of the compounds of formula I was evaluated in lungs of spontaneously hypertensive rats (SHR), 5 minutes after i.v. injection (0.6 and 21 μmoles/kg) and 30 minutes, 60 minutes and 4 hours after oral administration (30 μmoles/kg) of the tested compounds.

After the removal of the lungs from SHR, the lung tissue was homogenized and incubated for 2 hours at 37°C in the presence of [^{3}H]Hyp-Gly-Gly, as a substrate.

The reaction was stopped by adding hydrochloric acid.

The released radiolabelled hyppuric acid was extracted with ethyl acetate and counted by liquid scintillation spectrometry, according to conventional methods.

The ACE-inhibitory activity of the tested compounds was expressed as percentage of ACE-inhibition in SHR lungs.

As an example, the percentages of basal enzymatic activity obtained in the ex vivo tests after intravenous or oral administration of compound 16 and of comparison compounds R-1, R-2, R-3 and R-4 are reported in the following table 2.

Table 2

Percentages of ex vivo ACE-inhibition and NEP-inhibition of compound 16 and of compounds R-1, R-2, R-3 and R-4.

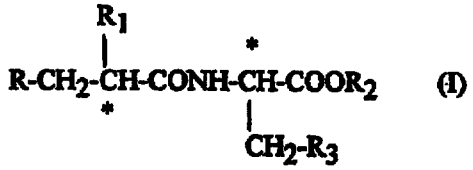
Compound	Treatment	ACE-inhibition (lungs)		NEP-inhibition (kidneys)	
		5 minutes	*60 minutes*	*5 minutes*	*60 minutes*
16	i.v. (0.6 µmoles/kg)	15%		34%	
16	i.v. (21 µmoles/kg)	72%		49%	
16	oral (30 µmoles/kg)		36%		39%
		30 minutes	*4 hours*	*30 minutes*	*4 hours*
16	oral (30 µmoles/kg)	60%	45%	55%	40%
R-1	oral (30 µmoles/kg)	25%	20%	5%	not active
R-2	oral (30 µmoles/kg)	30%	25%	30%	not active
R-3	oral (30 µmoles/kg)	25%	20%	10%	5%
R-4	oral (30 µmoles/kg)	25%	10%	not active	not active

The data reported in table 2 confirm that the compounds of formula I, object of the present invention, are endowed with a significant ACE/NEP-inhibitory activity after intravenous administration as well as after oral administration.

The ex-vivo ACE/NEP-inhibitory activity of the compounds of formula I, moreover, results to be significantly higher than that of the comparison compounds.

Claims

1) A compound of formula



(I)

wherein

R is a mercapto group or a R_4COS group convertible in the organism to mercapto group;

R_1 is a straight or branched C_2-C_4 alkyl group or an aryl or arylalkyl group having from 1 to 6 carbon atoms in the alkyl moiety wherein the aryl is a phenyl or a 5 or 6 membered aromatic heterocycle with one or two heteroatoms selected from the group consising of nitrogen, oxygen and sulphur, optionally substituted with one or more substituents, the same or different, selected from the group consisting of halogen atoms, hydroxy groups, alkoxy, alkyl, alkylthio, alkylsulphonyl or alkoxycarbonyl groups having from 1 to 6 carbon atoms in the alkyl moiety, C_1-C_3 alkyl groups containing one or more fluorine atoms, carboxy groups, nitro groups, amino or aminocarbonyl groups, acylamino groups, aminosulphonyl groups, mono- or di-alkylamino or mono- or di-alkylaminocarbonyl groups having from 1 to 6 carbon atoms in the alkyl moiety;

R_2 is a hydrogen atom, a straight or branched C_1-C_4 alkyl or a benzyl group;

R_3 is a phenyl group substituted by a 5 or 6 membered aromatic heterocycle with one or two heteroatoms selected from the group consisting of nitrogen, oxygen and sulphur, being the phenyl and the heterocyclic groups optionally substituted with one or more substituents, the same or different, as indicated for R_1;

R_4 is a straight or branched C_1-C_4 alkyl group or a phenyl group;

the carbon atoms marked with an asterisk are stereogenic centers;

and pharmaceutically acceptable salts thereof.

2) A compound of formula I according to claim 1 wherein R_3 is a phenyl group substituted in position 4 with a heterocyclic group.

3) A compound of formula I according to claim 2 wherein R_1 represents an arylalkyl group optionally substituted with one or more substituents, the same or different, selected from the groups consisting of halogen atoms, hydroxy, alkyl or alkoxy groups.

4) A compound of formula I according to claim 3 wherein R_1 represents a phenylalkyl group optionally substituted with one or more substituents, the same or different, selected among halogen atoms, hydroxy, alkyl or alkoxy groups.

5) A compound of formula I according to claim 1 in the form of a salt with an alkali metal selected from the group consisting of sodium, lithium and potassium.

6) A process for the preparation of a compound of formula I according to claim 1 comprising the reaction between a compound of formula

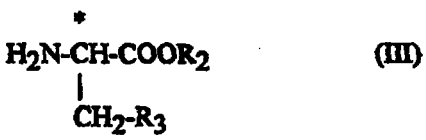


(II)

wherein

R and R_1 have the above reported meanings;

and a phenylalanine derivative of formula



(III)

wherein

R_2 and R_3 have the above reported meanings.

7) A pharmaceutical composition containing a therapeutically effective amount of a compound of formula I according to claim 1 in admixture with a carrier for pharmaceutical use.

8) A pharmaceutical composition according to claim 6 for the treatment of cardiovascular diseases.

9) A method for the treatment of cardiovascular diseases comprising the administration of a therapeutically effective amount of a compound according to claim 1.

10) A compound selected from the group consisting of:

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(2-thiazolyl)-L-phenylalanine methyl ester;

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(2-pyridyl)-L-phenylalanine methyl ester;

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(3-pyridyl)-L-phenylalanine benzyl ester;

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N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(2-furyl)-L-phenylalanine methyl ester;

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(5-pyrimidinyl)-L-phenylalanine ethyl ester;

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(2-pyrazinyl)-L-phenylalanine methyl ester;

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(2-thienyl)-L-phenylalanine methyl ester;

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(3-thienyl)-L-phenylalanine methyl ester;

N-(3-phenylcarbonylthio-2-phenylmethylpropionyl)-4-(3-furyl)-L-phenylalanine methyl ester;

N-[3-phenylcarbonylthio-2-(3-pyridylmethyl)propionyl]-4-(2-thiazolyl)-L-phenylalanine methyl ester;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(2-thiazolyl)-L-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(2-pyridyl)-L-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(3-pyridyl)-L-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(2-furyl)-L-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(5-pyrimidinyl)-L-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(2-pyrazinyl)-L-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(2-thienyl)-L-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(3-thienyl)-L-phenylalanine;

N-(3-mercapto-2-phenylmethylpropionyl)-4-(3-furyl)-L-phenylalanine;

N-[3-mercapto-2-(3-pyridylmethyl)propionyl]-4-(2-thiazolyl)-L-phenylalanine;

N-[3-acetylthio-2-(3-methoxyphenyl)methyl-propionyl]-4-(2-thiazolyl)-L-phenylalanine methyl ester;

N-[3-acetylthio-2-(2-fluorophenyl)methyl-propionyl]-4-(2-thiazolyl)-L-phenylalanine methyl ester;

N-[3-phenylcarbonylthio-2-(2-thienyl)methyl-propionyl]-4-(2-thiazolyl)-L-phenylalanine methyl ester;

N-[2-(2-furyl)methyl-3-phenylcarbonylthio-propionyl]-4-(2-thiazolyl)-L-phenylalanine methyl ester;

N-[2-(3-methyl-5-isoxazolyl)methyl-3-phenylcarbonylthio-propionyl]-4-(2-thiazolyl)-L-phenylalanine methyl ester;

N-[3-mercapto-2-(3-methoxyphenyl)methyl-propionyl]-4-(2-thiazolyl)-L-phenylalanine;

N-[3-mercapto-2-(2-thienyl)methyl-propionyl]-4-(2-thiazolyl)-L-phenylalanine;

N-[3-mercapto-2-(3-methyl-5-isoxazolyl)methyl-propionyl]-4-(2-thiazolyl)-L-phenylalanine;

N-[2-(2-fluorophenyl)methyl-3-mercapto-propionyl]-4-(2-thiazolyl)-L-phenylalanine;

N-[2-(2-furyl)methyl-3-mercapto-propionyl]-4-(2-thiazolyl)-L-phenylalanine;

and the stereoisomers (2S) or (2R) thereof.

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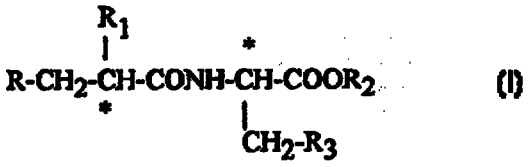
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(57) Abstract

Compounds of formula (I), wherein R, R_1, R_2, and R_3 have the meanings reported in the description, processes for their preparation and pharmaceutical compositions which contain them as active ingredients are described. The compounds of formula (I) are endowed with a mixed ACE-inhibitory and NEP-inhibitory activity and are useful in the treatment of cardiovascular diseases.



(I)