



(51) International Patent Classification:

C07D 233/58 (2006.01) A61P 25/28 (2006.01)

A61K 31/4164 (2006.01) A61P 29/00 (2006.01)

C07D 233/64 (2006.01) A61P 27/12 (2006.01)

A61P 9/00 (2006.01) A61P 3/10 (2006.01)

(21) International Application Number:

PCT/IB2018/060332

(22) International Filing Date:

19 December 2018 (19.12.2018)

(25) Filing Language:

Italian

(26) Publication Language:

English

(30) Priority Data:

102017000149628 27 December 2017 (27.12.2017) IT

(71) Applicant: FLAMMA INNOVATION S.R.L. [IT/IT];

Via Bedeschi, 22, 24040 Chignolo d'Isola (BG) (IT).

(72) Inventors: NEGRISOLI, GianPaolo; Via Bedeschi, 22,

24040 Chignolo d'Isola (BG) (IT). GAGLIARDI, Stefa-

nia; Via Bedeschi, 22, 24040 Chignolo d'Isola (BG) (IT).

SILNOVIC, Almin; Via Bedeschi, 22, 24040 Chignolo

d'Isola (BG) (IT). DALLANOCE, Clelia; Via Cola di Rien-

zo, 2, 20144 Milano (IT). DE AMICI, Marco; Via Briga-

ta Pavia, 6, 27100 Pavia (IT). CANEVOTTI, Renato; Via

Bedeschi, 22, 24040 Chignolo d'Isola (BG) (IT).

(74) Agent: PISTOLESI, Roberto; Dragotti & Associati, Srl,

via Nino Bixio, 7, 20129 Milan (IT).

(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH,

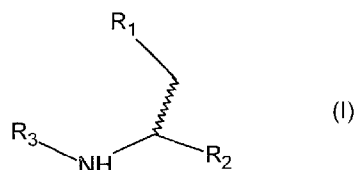
Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

Published:

— with international search report (Art. 21(3))

(54) Title: PEPTIDE DERIVATIVES AND THERAPEUTIC ACTIVITY THEREOF



(57) Abstract: Disclosed are peptide or pseudopeptide derivatives containing a nitrogenous heterocyclic residue with blocking activity against the by-products of lipid oxidative, stress, and in particular of unsaturated aldehydes such as malondialdehyde and 4-hydroxy-trans-2-nonenal (HNE). Formula (I)



PEPTIDE DERIVATIVES AND THERAPEUTIC ACTIVITY THEREOF

The present invention relates to dipeptide or pseudodipeptide derivatives containing an aminoalkanoyl group disubstituted on at least one of the carbon atoms of the chain, with metabolic quenching activity towards carbonyl compounds.

Prior art

5 The influence of oxidative damage on triggering various physiopathological processes, including aging, inflammatory disorders, diabetes, cardiovascular disease and neurodegenerative processes, has long been recognised. The main molecular mechanisms responsible for oxidative damage, such as structural damage to proteins, lipids and nucleic acids caused by radical reactive oxygen species, and an altered cell redox state,
10 are also well known [Halliwell B, Gutteridge JM. Free Radicals in Biology and Medicine (2001) Oxford Science Publications, 3rd Ed.].

It has also been clarified that some products of lipid oxidation characterised by a keto/aldehyde function act as important cytotoxic oxidative mediators, inducing irreversible structural modifications of the biomolecules, leading to alteration of the cell
15 functions [Uchida K. Free Radic. Biol. Med. 2000; 28:1685-96.; Poli G. et al., IUBMB Life. 2000; 50:315-21].

The carbonyl compounds studied include the products of oxidation of polyunsaturated fatty acids, including alpha, beta-unsaturated aldehydes such as 4-hydroxy-trans-2-nonenal (HNE) and acrolein (ACR) [Esterbauer H. et al., Free Radic.
20 Biol. Med. 1991; 11:81-128].

The participation of unsaturated aldehydes in various pathological processes with an oxidative basis has been demonstrated with the use of mono- and polyclonal antibodies [Uchida K. Prog. Lipid Res. 2003; 42:318-43]. In particular, adducts between HNE and acrolein with proteins have been identified in biopsy and autopsy tissue of patients
25 suffering from diabetes, atherosclerosis, muscular dystrophy, rheumatoid arthritis, actinic elastosis, cerebral ischaemia and neurodegenerative diseases such as Alzheimer's disease

and Parkinson's disease [Uchida K. Prog. Lipid Res. 2003; 42:318-43.; Zarkovic N. Mol. Aspects Med. 2003; 24:281-91.; Zarkovic N. Mol. Aspects Med. 2003; 24:293-303.; M. Carini et al. in "Redox Proteomics: from Protein Modifications to Cellular Dysfunction and Diseases" (Ed. I. Dalle-Donne, A. Scaloni, and A. Butterfield); Wiley InterScience Books from John Wiley & Sons (2005)].

The role of HNE as a pathogenetic factor has been demonstrated at molecular level for various disorders such as fibrosis [Chiarpotto E. et al., Biofactors. 2005; 24(1-4):229-36], diabetic nephropathy [Furfaro AL. et al. Biofactors. 2005;24(1-4): 291-8.], atherosclerotic processes [Leonarduzzi G. et al., Mol Nutr Food Res. 2005 Nov;49(11):1044-9] and neurodegenerative disorders [Zarkovic K. Mol Aspects Med. 2003 Aug-Oct;24(4-5):293-303].

It is therefore evident that carbonyl products, especially reactive carbonyl compounds such as HNE, are important targets for the development of a new class of biologically active molecules with carbonyl-quenching activity.

This interest is clearly demonstrated by the considerable number of articles and patents describing compounds with carbonyl-quenching activity, especially dipeptide compounds correlatable with carnosine [Hipkiss A.R., J. Alzheimer's Dis. 2007, 11, 229-240; Guiotto A. et al. Curr. Med. Chem. 2005, 12, 2293-2315; Vistoli G. et al., Chem. Med. Chem. 2009, 4 (6), 967-975].

Peptide compounds which are structurally correlatable with carnosine and their carbonyl-quenching activity are also described, for example, in WO2008/001174, while some aminoalcohol derivatives are described in WO2011/080139.

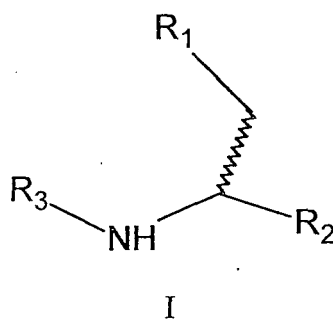
Description of the invention

The present invention relates to peptide or pseudopeptide derivatives containing a nitrogenous heterocyclic residue which have demonstrated an interesting activity of blocking the by-products of oxidative lipid stress, in particular those of unsaturated aldehydes such as malondialdehyde and 4-hydroxy-trans-2-nonenal (HNE), which are known for their contribution to the onset of a considerable number of chronic disorders

such as neurodegenerative disorders, chronic inflammatory disorders, cardiovascular disease, and complications of diabetes and cataract. This novel series of compounds, studied as from the structure of the endogenous L-carnosine dipeptide present in some tissues of the human body, is characterised by the presence of a double substitution on one of the carbons of the beta-alanine chain and the presence of a hydroxymethyl group optionally substituted for the terminal carboxyl group of L-carnosine. The compounds have demonstrated a surprisingly greater activity of the dipeptide L-carnosine and the corresponding aminoalcohols in quenching model carbonyl compounds, and extremely high metabolic stability compared with that of L-carnosine or structurally correlated compounds. These characteristics make them suitable for therapeutic use in all disorders correlatable with the presence of reactive carbonyl compounds.

The invention also relates to said compounds for use in the treatment or prevention of said disorders.

The compounds forming the object of the invention have the general formula I



wherein:

R₁ is a 5-6 membered heterocyclic ring optionally fused to a benzene ring, containing one to three nitrogen atoms optionally bearing C₁-C₃ alkyl substituents;

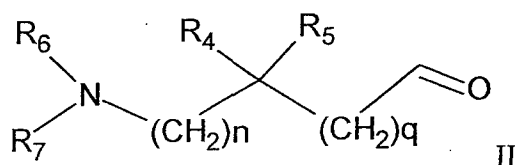
R₂ is:

- a **CH₂-O-R** group wherein **R** represents hydrogen, a straight or branched C₁-C₁₀ alkyl group or a C₃-C₈ cycloalkyl group, a straight or branched C₁-C₈ alkylcarbonyl group, a C₃-C₈ cycloalkylcarbonyl group; or **R** is an **Ar(CH₂)_pC(=O)-** group wherein **Ar** is an aryl selected from phenyl and naphthyl optionally substituted with one or two groups

selected from the group of methyl, methoxy or ethyl, and p is zero or an integer between 1 and 3;

- a COOR_8 group wherein R_8 represents hydrogen, a straight or branched C_1 - C_{10} alkyl group or a C_3 - C_8 cycloalkyl group;

5 R_3 is a group of general formula II



wherein

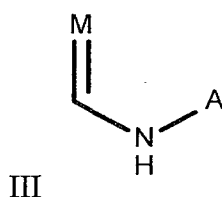
n and q , independently of one another, are equal to zero or represent an integer
10 between 1 and 4;

R_6 and R_7 , which are the same or different, represent:

- hydrogen;
- a straight or branched C_1 - C_{20} alkylcarbonyl group or a C_3 - C_7 cycloalkylcarbonyl group optionally containing one or more double bonds, or an $\text{Ar}(\text{CH}_2)_p\text{C}(=\text{O})$ - group
15 wherein Ar and n are as defined above;

- a straight or branched C_1 - C_{10} alkoxycarbonyl group or a C_3 - C_7 cycloalkoxycarbonyl group optionally containing one or more double bonds, or an $\text{ArO}(\text{CH}_2)_n\text{C}(=\text{O})$ - group wherein Ar and n are as defined above;

- one of R_6 and R_7 is an amino group and the other is hydrogen;
20 - one of R_6 and R_7 is hydrogen and the other is a group of formula III



wherein M represents nitrogen, oxygen or sulphur and A represents hydrogen or an amino group, and the free valence of the carbon atom forms a bond with the nitrogen

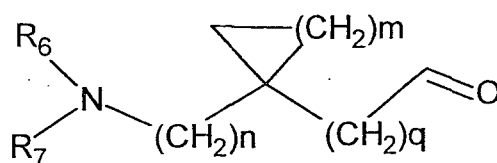
atom of the group of formula II;

R₄ and **R₅**, which are the same or different, represent:

- a straight or branched C₁-C₈ alkyl group, preferably methyl;

- a halogen, preferably fluorine;

5 - can be joined to form, together with the carbon atom to which they are linked, a cycloaliphatic ring with 3 to 6 carbon atoms selected from cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl forming an **R₃** group of general formula IV




IV

wherein

m has a value ranging between 1 and 4;

R₆, **R₇**, **n** and **q** have the meanings described above.

10 In general formula I, wavy bond  indicates that the carbon atom substituted with NH and **R₂** can have the R or S configuration.

In general formulas II and IV, the free valence of the carbon atom of the carbonyl group forms a bond with the nitrogen atom of formula I.

Examples of a 5-6 member heterocyclic ring comprise imidazole, pyrrole, pyrazole, triazole, indole, isoindole, indazole, benzoimidazole and benzotriazole.

20 In one embodiment of the invention:

R₁ is a heterocyclic ring selected from imidazole, pyrrole, pyrazole, triazole, indole, isoindole, indazole, benzoimidazole and benzotriazole, preferably imidazole or imidazole substituted at the nitrogen atom at the 1- position or at the 3- position with methyl, ethyl or propyl;

25 **R₂** and **R₃** are as defined above.

In another embodiment of the invention:

R₁ is imidazole optionally substituted at the nitrogen atom at the 1- position or at the 3- position with methyl, ethyl or propyl;

R₂ is CH₂-OH or a COOR₈ group wherein **R**₈ is selected from hydrogen, methyl and ethyl.

5 **R**₃ is a group of formula II wherein **R**₄ and **R**₅ are both methyl or halogen, **R**₆ and **R**₇ are both hydrogen, or one of **R**₆ and **R**₇ is hydrogen and the other is a straight or branched C₁-C₁₀ alkoxy carbonyl group or a C₃-C₇ cyclic group, and **n** and **q** are as defined above.

In another embodiment of the invention:

10 **R**₁ is imidazole optionally substituted at the nitrogen atom at the 1- position or at the 3- position with methyl, ethyl or propyl;

R₂ is CH₂-OH or a COOR₈ group wherein **R**₈ is selected from hydrogen, methyl and ethyl;

15 **R**₃ is a group of formula IV wherein **R**₄ and **R**₅ are joined to form, together with the carbon atom to which they are bonded, a cycloaliphatic ring with 3 to 6 carbon atoms selected from cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl, **R**₆ and **R**₇ are both hydrogen, or one of **R**₆ and **R**₇ is hydrogen and the other is a straight or branched C₁-C₁₀ alkoxy carbonyl group or a C₃-C₇ cyclic group, and **m**, **n** and **q** are as defined above.

In a preferred embodiment:

20 **R**₁ is imidazole optionally substituted at the nitrogen atom at the 1- position or at the 3- position with methyl;

R₂ is CH₂-OH or a COOR₈ group wherein **R**₈ is hydrogen;

R₃ is a group of formula II wherein **R**₄ and **R**₅ are both methyl or fluorine, **R**₆ and **R**₇ are both hydrogen, and **n** and **q** are as defined above.

25 In another preferred embodiment:

R₁ is imidazole optionally substituted at the nitrogen atom at the 1- position or at the 3- position with methyl;

R₂ is CH₂-OH or a COOR₈ group wherein **R**₈ is hydrogen.

R₃ is a group of formula IV wherein **R**₄ and **R**₅ are joined to form, together with the carbon atom to which they are bonded, a cycloaliphatic ring with 3 to 6 carbon atoms selected from cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl, **R**₆ and **R**₇ are both hydrogen, and **m**, **n** and **q** are as defined above.

5 Examples of compounds according to the invention are:

(S)-2-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-2-methylpropanamide;

(S)-3-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-2,2-dimethylpropanamide;

10 (S)-4-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-2,2-dimethylbutanamide;

(S)-1-(aminomethyl-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)cyclopropanecarboxamide;

15 (S)-1-(aminomethyl-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)cyclobutanecarboxamide;

(S)-1-(aminomethyl-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)cyclopentanecarboxamide;

(S)-1-(aminomethyl-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)cyclohexanecarboxamide;

20 (S)-3-amino-N-(1-hydroxy-3-(1-methyl-1H-imidazol-5-yl)propan-2-yl)-2,2-dimethylpropanamide;

(S)-3-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-3,3-dimethylpropanamide;

25 (S)-3-amino-2,2-difluoro-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-propanamide;

N-(2-amino-2-methylpropanoyl)-L-histidine;

N-(3-amino-2,2-dimethylpropanoyl)-L-histidine

N-(1-aminomethyl)-1-cyclopropylcarbonyl)-L-histidine;

N-(1-aminomethyl)-1-cyclobutylcarbonyl)-L-histidine;

N-(1-aminomethyl)-1-cyclopentylcarbonyl)-L-histidine

N-(1-aminomethyl)-1-cyclohexylcarbonyl)-L-histidine;

N-(4-amino-2,2-dimethylbutanoyl)-L-histidine;

5 N-(3-amino-3,3-dimethylpropanoyl)-L-histidine;

N-(3-amino-2,2-dimethylpropanoyl)-L-histidine

N-(3-amino-2,2-difluoropropanoyl)-L-histidine.

As at least one chiral centre is present in the compounds of formula I, all the optical isomers of the products in question and the mixtures thereof in any proportion, and
10 all the possible diastereoisomers, taken individually or mixed together in any proportion, are deemed to constitute an integral part of the invention. All the pharmacologically acceptable salts of the products of formula I are also part of the invention.

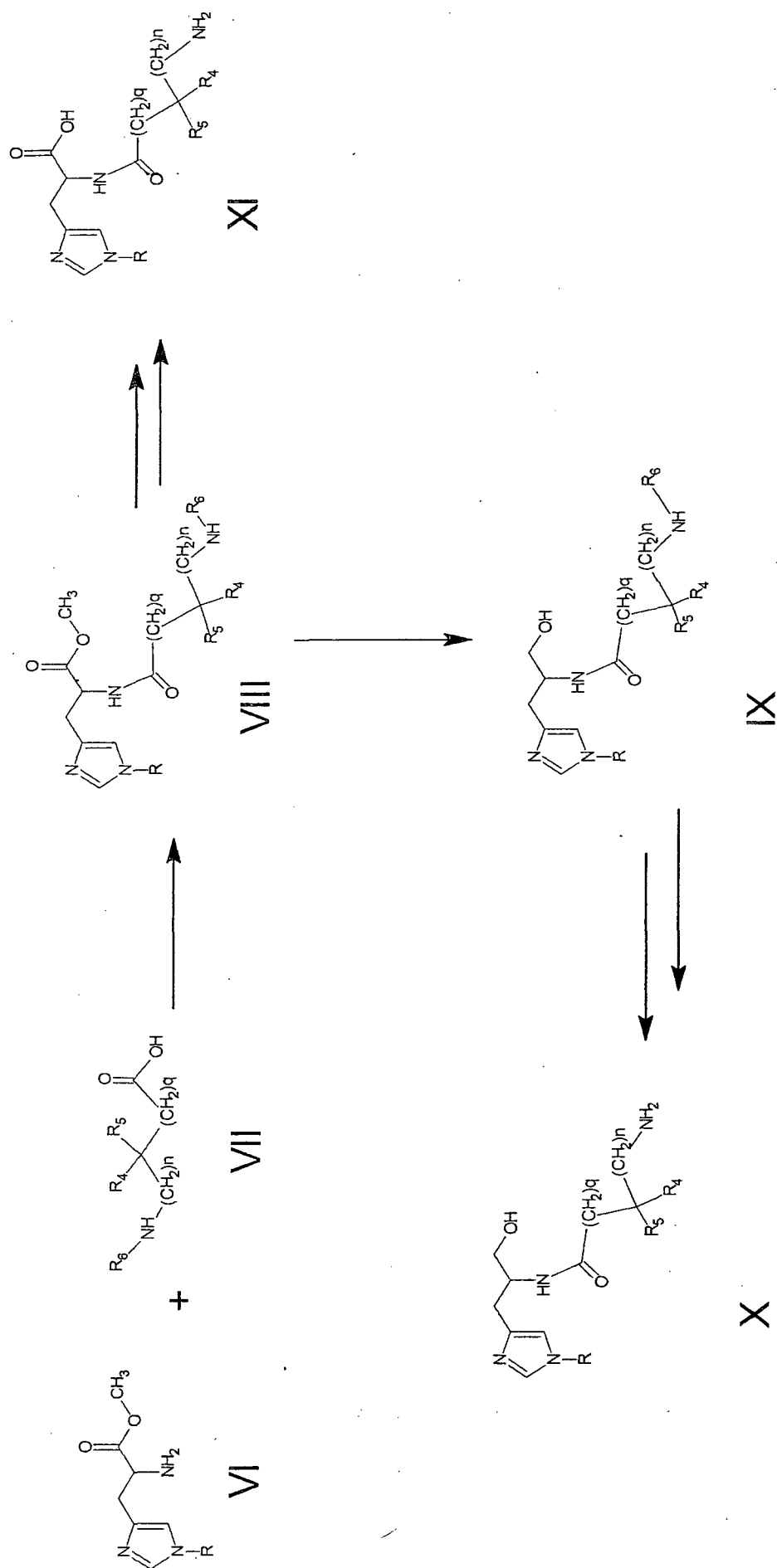
The compounds according to the invention were synthesised by some of the well-known solid-phase or solution-phase peptide synthesis methods reported in the
15 literature, for example in Houben-Weil "Synthesis of peptides and peptidomimetics" vol. E22 a-d or J. Jones "Amino acid and peptide synthesis". The amino acids used in the synthesis, if not available in an already protected form, were suitably functionalised with the necessary protecting groups using well-known methods, such as those reported in T.W. Greene, P.G.M. Wuts "Protective group in organic synthesis" or P.J. Kocienski
20 "Protecting groups". The aminoalcohols used in the synthesis were prepared by reduction of derivatives of the corresponding amino acids using suitable metal or organometal hydrides in inert solvents, and similar procedures were used in the reduction of dipeptide to aminoacylaminoalcohol derivatives.

The end products obtained were purified, when necessary, by one of the methods
25 known to the skilled person, using, for example, crystallisation, chromatographic purification or any other technique required to obtain compounds with the required degree of purity.

By way of example, the compounds according to the invention can be prepared

according to synthesis scheme 1. A suitable aminoester of formula VI wherein R is a C₁-C₃ alkyl is coupled with the corresponding protected amino acid of formula VII wherein R₄, R₅, n and q are as defined above, and R'₆ is a protecting group of the nitrogen atom according to one of the known methods, and the resulting protected dipeptide ester of formula VIII can subsequently be reduced to the corresponding alcohol IX and deprotected to obtain the corresponding aminoalcohol X. Alternatively, the protected dipeptide ester of formula VIII can be hydrolysed and deprotected to obtain the peptides of formula XI.

Synthesis scheme 1



The pharmacological activity of the compounds according to the invention was determined in vitro by evaluating their carbonyl-quenching activity towards 4-hydroxy nonenal (HNE), which is known for its involvement in numerous disorders. Said activity was compared with that of L-carnosine, a compound known as a carbonyl quencher in vitro but having limited metabolic stability caused by serum carnosinase. The metabolic stability of a particularly interesting compound was also evaluated in human serum after 1 and 2 hours.

The results of the pharmacological tests clearly demonstrate that some of the compounds according to the invention possess carbonyl-quenching activity which is considerably greater than that of L-carnosine and significantly greater than that of other compounds that do not bear substituents on the alkyl chain, which are described above. For this purpose it is interesting to compare the compound 2-amino-*N*-(1-hydroxy-3-(1*H*-imidazol-4-yl)propan-2-yl)-2-methylpropanamide (FLM-04-OLO) forming the object of the present invention, which exhibits carbonyl-quenching activity 3.1 times greater than that of L-carnosine (see Table I), with that of the non-disubstituted derivative 3-amino-*N*-[(1*S*)-2-hydroxy-1-(1*H*-imidazol-5-yl-methyl)ethyl]propanamide reported in WO2011/080139, an activity whereof, 1.6 times greater than that of L-carnosine, is reported in the same test.

For the intended uses, the compounds of formula I are conveniently formulated in conventional pharmaceutical, cosmetic or nutritional compositions suitable for oral, parenteral, topical or transdermal administration, which constitute a further object of the invention. Examples of said compositions include capsules, tablets, syrups, injectable solutions or suspensions, ointments, suppositories, controlled-release forms and the like, and water-soluble granulates. Said forms, plus carriers and excipients used in pharmaceutical technology, could optionally also contain other active ingredients which have a complementary activity or are otherwise useful for the treatment/prevention of the disorders in question. The invention is illustrated in detail in the following examples.

General example of procedure 1

In an anhydrous medium, under argon, the acid of structure VII (1 equiv.) is dissolved in anhydrous CH₃CN (5 mL/mmol), and the mixture is cooled to 0°C. HATU

(1.05 equiv.) is added, and DIPEA (2 equiv.) is dripped in. The reaction mixture is left under magnetic stirring at 0°C for 5 min, and a solution of compound VI (1 equiv.) and DIPEA (2 equiv.) in anhydrous CH₃CN (5 mL/mmol) is then dripped in. The resulting mixture is left under stirring at room temperature for 2-4 hours. At the end of the reaction
5 (TLC assay [DCM/MeOH 7:3]) the solvent is evaporated under low pressure. The residue is taken up with EtOAc and washed with a Na₂CO₃ saturated solution and a NH₄Cl saturated solution. The organic phase is dried with anhydrous Na₂SO₄, filtered and concentrated under low pressure. The crude product is optionally purified by column chromatography on silica gel, to obtain the compounds of general structural formula VIII.

10 **General example of procedure 2**

The compound of structure VIII (1 equiv.) is dissolved in THF and MeOH (1:1) (8 mL/mmol), and the mixture is cooled to 0°C with an ice bath. NaBH₄ (10 equiv.) is added slowly under magnetic stirring. The reaction mixture is left under stirring at 0°C for 2 h. The disappearance of the starting compound is tested for by TLC (EtOAc/MeOH 85:15),
15 and the solvent is evaporated under low pressure at the end of the reaction. The crude product is taken up with EtOAc and washed with a NH₄Cl saturated solution. The organic phase dried with anhydrous Na₂SO₄ is filtered and concentrated under low pressure, and the resulting residue is purified by column chromatography on silica gel to obtain the compounds of general structure IX.

20 **General example of procedure 3**

Trifluoroacetic acid (10 equiv.) is slowly dripped into a solution of the N-protected aminoalcohol of structure IX in dichloromethane (10 mL/mmol), precooled to 0°C, under magnetic stirring. The reaction mixture is left under stirring at room temperature for 1.5 h. When the reaction is complete (TLC assay [EtOAc/MeOH 8:2]),
25 the solvent is removed under low pressure. The residue is diluted in 2N HCl and loaded into a Dowex50WX2[®] ion-exchange resin column. The purification is carried out by washing with deionised water and eluting the aminoalcohol of structure X with a 10% aqueous solution of NH₃.

General example of procedure 4

A solution of intermediate VIII in DCM (10 mL/mmol) is cooled to 0°C with an ice bath. TFA (10 equiv.) is dripped in, and the resulting mixture is left under stirring at room temperature for 2 h. On the disappearance of the starting product (TLC assay [EtOAc/MeOH 8:2]), the solvent and the excess TFA are removed under low pressure. The residue is dissolved in THF and the mixture is cooled to 0°C. An aqueous solution of 2N LiOH is slowly dripped in under magnetic stirring until a basic pH is reached, and the reaction mixture is left under stirring at room temperature until the reaction is complete (TLC assay [EtOAc/MeOH/NH₃ 10% aqueous solution]). The reaction mixture is concentrated under low pressure, acidified with 2N HCl and loaded into a Dowex50WX2® ion-exchange resin column. After washing with deionised water, compound XI is released from the resin with a 10% aqueous solution of NH₃.

The following compounds were obtained with the general procedure described above.

Example 1: (S)-2-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-2-methylpropanamide (code FLM-05 OLO)

(S)-methyl-2-(2-((tert-butoxycarbonyl)amino)-2-methylpropanamido)-3-(1H-imidazol-4-yl)propanoate (Procedure 1)

¹H NMR (300 MHz, CDCl₃) δ 7.52 (s, 1H), 6.76 (s, 1H), 5.24 (br s, 1H), 4.70 (dd, J = 11.2, 4.8 Hz, 1H), 3.70 (s, 3H), 3.33 – 3.18 (m, 1H), 3.11 (dd, J = 15.0, 5.2 Hz, 1H), 1.49 (s, 6H), 1.42 (s, 9H).

(S)-tert-butyl(1-((1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)carbamate (Procedure 2 Yield 46%).

¹H NMR (300 MHz, CD₃OD) δ 7.61 (s, 1H), 6.87 (s, 1H), 4.20 – 4.00 (m, 1H), 3.58 – 3.45 (m, 2H), 2.88 (dd, J = 14.9, 6.0 Hz, 1H), 2.77 (dd, J = 14.9, 7.9 Hz, 1H), 1.42 (s, 9H), 1.36 (s, 3H), 1.32 (s, 3H).

(S)-2-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-2-methylpropanamide (Procedure 3. Yield 79%).

¹H NMR (300 MHz, CD₃OD) δ 7.57 (s, 1H), 6.85 (s, 1H), 4.15 – 3.99 (m, 1H),

3.55 (d, $J = 5.1$ Hz, 2H), 2.89 (dd, $J = 14.8, 6.0$ Hz, 1H), 2.78 (dd, $J = 14.8, 8.0$ Hz, 1H), 1.26 (s, 3H), 1.23 (s, 3H).

Example 2: (S)-3-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-2,2-dimethylpropanamide (code FLM 04 OLO)

5 (S)-methyl-2-(3-((tert-butoxycarbonyl)amino)-2,2-dimethylpropanamido)-3-(1H-imidazol-4-yl)propanoate (procedure 1)

^1H NMR (300 MHz, CDCl_3) δ 7.59 (s, 1H), 7.54 (br s, 1H), 6.80 (s, 1H), 5.68 (br s, 1H), 4.74 (dd, $J = 11.9, 4.9$ Hz, 1H), 3.70 (s, 3H), 3.35 – 3.03 (m, 4H), 1.42 (s, 9H), 1.22 (s, 3H), 1.20 (s, 3H).

10 (S)-tert-butyl(3-((1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)amino)-2,2-dimethyl-3-oxopropyl) carbamate (Procedure 2, yield 80%).

^1H NMR (300 MHz, CD_3OD) δ 7.61 (s, 1H), 6.86 (s, 1H), 4.19 – 4.10 (m, 1H), 3.55 (d, $J = 5.5$ Hz, 2H), 3.23 – 3.06 (m, 2H), 2.88 (dd, $J = 14.9, 5.9$ Hz, 1H), 2.77 (dd, $J = 14.9, 8.2$ Hz, 1H), 1.43 (s, 9H), 1.10 (s, 3H), 1.07 (s, 3H).

15 (S)-3-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-2,2-dimethylpropanamide (Procedure 3, yield 92%).

^1H NMR (300 MHz, CD_3OD) δ 7.56 (s, 1H), 6.84 (s, 1H), 4.22 – 4.12 (m, 1H), 3.55 (d, $J = 5.5$ Hz, 2H), 2.88 (dd, $J = 14.8, 5.7$ Hz, 1H), 2.77 (dd, $J = 14.8, 8.4$ Hz, 1H), 2.65 (s, 2H), 1.11 (s, 3H), 1.09 (s, 3H).

20 **Example 3: (S)-4-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-2,2-dimethylbutanamide** (code FLM 13 OLO)

(S)-methyl 2-(4-(tert-butoxycarbonylamino)-2,2-dimethylbutanamido)-3-(1H-imidazol-4-yl)propanoate (procedure 1)

25 ^1H NMR (300 MHz, acetone) δ 7.99 (d, $J = 6.4$ Hz, 1H), 7.70 (s, 1H), 6.98 (s, 1H), 6.05 (br s, 1H), 4.71 – 4.65 (m, 1H), 3.62 (s, 3H), 3.20 – 2.86 (m, 4H), 1.72 (dd, $J = 9.1, 5.6$ Hz, 2H), 1.39 (s, 9H), 1.18 (s, 6H).

(S)-tert-butyl 4-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-ylamino)-3,3-dimethyl-4-oxobutylcarbamate (procedure 2, yield 68%)

¹H NMR (300 MHz, CD₃OD) δ 7.73 (s, 1H), 6.91 (s, 1H), 4.27 – 4.07 (m, 1H), 3.63 – 3.49 (m, 2H), 2.96 – 2.86 (m, 3H), 2.81 (dd, *J* = 14.9, 8.6 Hz, 1H), 1.69 – 1.61 (m, 2H), 1.42 (s, 9H), 1.12 (s, 3H), 1.11 (s, 3H).

(S)-4-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-2,2-dimethylbutanamide (67 mg, 0.27 mmol) (procedure 3, yield 83%.

¹H NMR (300 MHz, D₂O) δ 7.66 (s, 1H), 6.92 (s, 1H), 4.33 – 4.18 (m, 1H), 3.71 – 3.56 (m, 2H), 2.89 (dd, *J* = 14.9, 4.7 Hz, 1H), 2.79 – 2.67 (m, 1H), 2.45 – 2.27 (m, 2H), 1.69 – 1.50 (m, 2H), 1.09 (s, 3H), 1.07 (s, 3H).

Example 4: (S)-1-(aminomethyl)-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)cyclopropanecarboxamide (code FLM-9 OLO)

(S)-methyl 2-(1-(((tert-butoxycarbonyl)amino)methyl)cyclopropanecarboxamido)-3-(1H-imidazol-4-yl)propanoate (procedure 1)

¹H NMR (300 MHz, acetone-d₆) δ 8.08 (br s, 1H), 7.64 (s, 1H), 6.99 (s, 1H), 6.68 (br s, 1H), 4.59 (dd, *J* = 12.7, 6.8 Hz, 1H), 3.61 (s, 3H), 3.44 (dd, *J* = 15.0, 7.0 Hz, 1H), 3.29 (dd, *J* = 15.0, 5.5 Hz, 1H), 3.07 – 2.95 (m, 2H), 1.43 (s, 9H), 1.10 – 0.99 (m, 2H), 0.85 – 0.75 (m, 2H).

(S)-tert-butyl (((1-((1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)carbamoyl)cyclopropyl)methyl) carbamate (procedure 2, yield 93%).

¹H NMR (300 MHz, CD₃OD) δ 7.61 (s, 1H), 6.87 (s, 1H), 4.17 – 4.08 (m, 1H), 3.53 (d, *J* = 5.4 Hz, 2H), 3.27 (s, 2H), 2.90 (dd, *J* = 14.8, 6.0 Hz, 1H), 2.75 (dd, *J* = 14.8, 8.1 Hz, 1H), 1.44 (s, 9H), 1.09 – 1.01 (m, 2H), 0.75 – 0.72 (m, 2H).

(S)-1-(aminomethyl)-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)cyclopropanecarboxamide (procedure 3, yield 86%).

¹H NMR (300 MHz, CD₃OD) δ 7.57 (s, 1H), 6.84 (s, 1H), 4.19 – 4.08 (m, 1H), 3.55 (d, *J* = 5.3 Hz, 2H), 2.92 – 2.71 (m, 4H), 1.09 – 0.97 (m, 2H), 0.74 – 0.63 (m, 2H).

Example 5: (S)-1-(aminomethyl)-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-

2-yl)cyclobutanecarboxamide (code FLM-10 OLO)

(S)-methyl 2-(1-(((tert-butoxycarbonyl)amino)methyl)cyclobutanecarboxamido)-3-(1H-imidazol-4-yl)propanoate (procedure 1)

¹H NMR (300 MHz, CD₃OD) δ 7.61 (s, 1H), 6.89 (s, 1H), 4.67 (dd, *J* = 8.3, 5.3 Hz, 1H), 3.71 (s, 3H), 3.48 – 3.33 (m, 2H), 3.16 (dd, *J* = 14.8, 5.2 Hz, 1H), 3.06 (dd, *J* = 14.8, 8.4 Hz, 1H), 2.32 – 2.13 (m, 2H), 2.04 – 1.85 (m, 3H), 1.84 – 1.67 (m, 1H), 1.43 (s, 9H).

(S)-tert-butyl ((1-((1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)carbamoyl)cyclobutyl)methyl) carbamate (procedure 2, yield 73%).

¹H NMR (300 MHz, CD₃OD) δ 7.60 (s, 1H), 6.87 (s, 1H), 4.20 – 4.12 (m, 1H), 3.61 – 3.51 (m, 2H), 3.45 – 3.31 (m, 2H), 2.87 (dd, *J* = 14.8, 6.0 Hz, 1H), 2.78 (dd, *J* = 14.8, 8.2 Hz, 1H), 2.31 – 2.09 (m, 2H), 2.01 – 1.80 (m, 3H), 1.80 – 1.60 (m, 1H), 1.41 (s, 9H).

(S)-1-(aminomethyl)-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)cyclobutanecarboxamide (procedure 3, yield 96%).

¹H NMR (300 MHz, CD₃OD) δ 7.57 (s, 1H), 6.86 (s, 1H), 4.26 – 4.13 (m, 1H), 3.66 – 3.48 (m, 2H), 2.97 – 2.83 (m, 3H), 2.76 (dd, *J* = 14.8, 8.7 Hz, 1H), 2.37 – 2.17 (m, 2H), 2.00 – 1.67 (m, 4H).

Example 6: (S)-1-(aminomethyl)-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)cyclopentanecarboxamide (code FLM-11 OLO)

(S)-methyl 2-(1-(((tert-butoxycarbonyl)amino)methyl)cyclopentanecarboxamido)-3-(1H-imidazol-4-yl)propanoate (procedure 1)

¹H NMR (300 MHz, CD₃OD) δ 7.59 (s, 1H), 6.87 (s, 1H), 4.64 (dd, *J* = 8.3, 5.3 Hz, 1H), 3.71 (s, 3H), 3.24 (d, *J* = 6.1 Hz, 2H), 3.15 (dd, *J* = 14.7, 5.1 Hz, 1H), 3.06 (dd, *J* = 14.8, 8.4 Hz, 1H), 1.97 – 1.78 (m, 2H), 1.73 – 1.51 (m, 6H), 1.43 (s, 9H).

(S)-tert-butyl ((1-((1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)carbamoyl)cyclopentyl)methyl) carbamate (procedure 2, yield 92%).

¹H NMR (300 MHz, acetone-d₆) δ 7.69 (s, 1H), 7.17 (d, *J* = 7.4 Hz, 1H), 6.94 (s, 1H), 6.44 (br s, 1H), 6.06 (br s, 2H), 4.16 – 4.03 (m, 1H), 3.58 – 3.41 (m, 2H), 3.31 (dd, *J* = 13.8, 6.7 Hz, 1H), 3.22 (dd, *J* = 13.7, 6.1 Hz, 1H), 2.93 – 2.78 (m, 2H), 2.02 – 1.82 (m,

2H), 1.71 – 1.50 (m, 6H), 1.40 (s, 9H).

(S)-1-(aminomethyl)-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)cyclopentanecarboxamide (procedure 3, yield 78%).

¹H NMR (300 MHz, CD₃OD) δ 7.56 (s, 1H), 6.84 (s, 1H), 4.25 – 4.14 (m, 1H),
5 3.62 – 3.49 (m, 2H), 2.88 (dd, *J* = 14.8, 5.6 Hz, 1H), 2.77 (dd, *J* = 14.9, 8.7 Hz, 1H), 2.71 (s, 2H), 2.03 – 1.86 (m, 2H), 1.68 – 1.44 (m, 6H).

Example 7: (S)-1-(aminomethyl)-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)cyclohexanecarboxamide (code FLM-14 OLO)

(S)-methyl 2-(1-((tert-butoxycarbonylamino)methyl)cyclohexanecarboxamido)-3-(1H-imidazol-4-yl)propanoate (procedure 3)

¹H NMR (300 MHz, CD₃OD) δ 7.62 (s, 1H), 6.89 (s, 1H), 4.69 (dd, *J* = 8.5, 5.2 Hz, 1H), 3.73 (s, 3H), 3.23 – 3.04 (m, 4H), 2.03 – 1.89 (m, 2H), 1.61 – 1.48 (m, 3H), 1.43 (s, 9H), 1.37 – 1.16 (m, 5H).

(S)-tert-butyl (1-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-ylcarbamoyl)-cyclohexyl)methyl carbamate (procedure 2, yield 88%).

¹H NMR (300 MHz, CD₃OD) δ 7.59 (s, 1H), 6.87 (s, 1H), 4.28 – 4.15 (m, 1H), 3.63 – 3.50 (m, 2H), 3.23 – 3.12 (m, 1H), 3.10 – 2.99 (m, 1H), 2.93 – 2.74 (m, 2H), 2.03 – 1.86 (m, 2H), 1.59 – 1.46 (m, 3H), 1.42 (s, 9H), 1.36 – 1.08 (m, 5H).

(S)-1-(aminomethyl)-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)cyclohexanecarboxamide (procedure 3, yield 92%).

¹H NMR (300 MHz, CD₃OD) δ 7.56 (s, 1H), 6.86 (s, 1H), 4.36 – 4.19 (m, 1H), 3.65 – 3.50 (m, 2H), 2.88 (dd, *J* = 14.9, 5.4 Hz, 1H), 2.78 (dd, *J* = 14.9, 9.0 Hz, 1H), 2.58 (s, 2H), 2.06 – 1.94 (m, 2H), 1.60 – 1.44 (m, 3H), 1.36 – 1.11 (m, 5H).

Example 8: (S)-3-amino-N-(1-hydroxy-3-(1-methyl-1H-imidazol-5-yl)propan-2-yl)-2,2-dimethylpropanamide (FLM-12 OLO)

(S)-methyl 2-(3-((tert-butoxycarbonyl)amino)-2,2-dimethylpropanamido)-3-(1-methyl-1H-imidazol-5-yl)propanoate (procedure 1)

¹H NMR (300 MHz, CD₃OD) δ 7.54 (s, 1H), 6.75 (s, 1H), 4.68 (dd, *J* = 9.7, 5.4

Hz, 1H), 3.74 (s, 3H), 3.66 (s, 3H), 3.23 (dd, $J = 15.5, 5.4$ Hz, 1H), 3.15 (s, 2H), 3.08 (dd, $J = 15.5, 9.8$ Hz, 1H), 1.43 (s, 9H), 1.11 (s, 3H), 1.08 (s, 3H).

(S)-tert-butyl (3-((1-hydroxy-3-(1-methyl-1H-imidazol-5-yl)propan-2-yl)amino)-2,2-dimethyl-3-oxopropyl)carbamate (procedure 2, yield 91%).

5 ^1H NMR (300 MHz, CD_3OD) δ 7.55 (s, 1H), 6.75 (s, 1H), 4.22 – 4.10 (m, 1H), 3.69 (s, 3H), 3.58 (d, $J = 5.4$ Hz, 2H), 3.21 – 3.05 (m, 2H), 2.91 (dd, $J = 15.4, 5.7$ Hz, 1H), 2.75 (dd, $J = 15.3, 9.2$ Hz, 1H), 1.43 (s, 9H), 1.09 (s, 3H), 1.05 (s, 3H).

(S)-3-amino-N-(1-hydroxy-3-(1-methyl-1H-imidazol-5-yl)propan-2-yl)-2,2-dimethylpropanamide (procedure 3, yield 73%).

10 ^1H NMR (300 MHz, CD_3OD) δ 7.51 (s, 1H), 6.74 (s, 1H), 4.25 – 4.15 (m, 1H), 3.68 (s, 3H), 3.59 (d, $J = 5.4$ Hz, 2H), 2.90 (dd, $J = 15.3, 5.6$ Hz, 1H), 2.76 (dd, $J = 15.3, 9.4$ Hz, 1H), 2.64 (s, 2H), 1.11 (s, 3H), 1.07 (s, 3H).

Example 9: (S)-3-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-3,3-dimethylpropanamide (FLM-02 OLO)

15 (S)-methyl-2-(3-((tert-butoxycarbonyl)amino)-3,3-dimethylpropanamido)-3-(1H-imidazol-4-yl)propanoate (procedure 1)

^1H NMR (300 MHz, CDCl_3) δ 7.60 (s, 1H), 7.15 (d, $J = 7.3$ Hz, 1H), 6.83 (s, 1H), 5.39 (s, 1H), 4.88 – 4.74 (m, 1H), 3.70 (s, 3H), 3.12 (d, $J = 5.3$ Hz, 2H), 2.56 (q, $J = 13.7$ Hz, 2H), 1.41 (s, 9H), 1.38 (s, 3H), 1.37 (s, 3H).

20 (S)-tert-butyl(3-((1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)amino)-3,3-dimethyl-3-oxopropyl) carbamate (procedure 2, yield 81%).

^1H NMR (300 MHz, CD_3OD) δ 7.64 (s, 1H), 6.89 (s, 1H), 4.20 – 4.05 (m, 1H), 3.53 (d, $J = 5.2$ Hz, 2H), 2.89 (dd, $J = 14.9, 6.0$ Hz, 1H), 2.74 (dd, $J = 14.9, 8.2$ Hz, 1H), 2.45 (s, 2H), 1.42 (s, 9H), 1.29 (s, 3H), 1.26 (s, 3H).

25 (S)-3-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-3,3-dimethylpropanamide (procedure 3, yield 90%).

^1H NMR (300 MHz, CD_3OD) δ 7.56 (s, 1H), 6.85 (s, 1H), 4.22 – 4.10 (m, 1H), 3.61 – 3.48 (m, 2H), 2.88 (dd, $J = 14.9, 5.7$ Hz, 1H), 2.73 (dd, $J = 14.9, 8.5$ Hz, 2H), 2.22

(s, 2H), 1.11 (s, 3H), 1.09 (s, 3H).

Example 10: *(S)*-3-amino-2,2-difluoro-*N*-(1-hydroxy-3-(1*H*-imidazol-4-yl)propan-2-yl)propanamide (FLM-07-OLO)

The compound was prepared with a slightly different procedure by coupling
5 between (*S*)-2-amino-3-(1*H*-imidazol-4-yl)propyl acetate and Fmoc-3-amino-2,2-difluoro-propionyl chloride.

The resulting compound (*S*)-2-(3-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-2,2-difluoropropanamido)-3-(1*H*-imidazol-4-yl)propyl acetate was then deprotected to give the final aminoalcohol.

10 (*S*)-2-(3-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-2,2-difluoropropanamido)-3-(1*H*-imidazol-4-yl)propyl acetate (Yield 70%).

¹H NMR (300 MHz, CD₃OD) δ 7.79 (d, *J* = 7.5 Hz, 2H), 7.64 (d, *J* = 7.5 Hz, 2H), 7.58 (s, 1H), 7.38 (t, *J* = 7.3 Hz, 2H), 7.30 (t, *J* = 7.3 Hz, 2H), 6.87 (s, 1H), 4.44 – 4.32 (m, 3H), 4.25 – 4.15 (m, 2H), 4.07 – 3.99 (m, 1H), 3.70 (t, *J* = 14.5 Hz, 2H), 2.93 – 2.77
15 (m, 2H), 2.01 (s, 3H).

(*S*)-3-amino-2,2-difluoro-*N*-(1-hydroxy-3-(1*H*-imidazol-4-yl)propan-2-yl)propanamide

(Yield 99%)

¹H NMR (300 MHz, D₂O) δ 7.73 (s, 1H), 6.97 (s, 1H), 4.38 – 4.22 (m, 1H), 3.79
20 (dd, *J* = 11.7, 4.6 Hz, 1H), 3.66 (dd, *J* = 11.8, 7.4 Hz, 1H), 3.05 (t, *J* = 15.3 Hz, 2H), 2.95 (dd, *J* = 14.9, 4.9 Hz, 1H), 2.77 (dd, *J* = 14.9, 10.0 Hz, 1H).

Example 11: *N*-(2-amino-2-methylpropanoyl)-*L*-histidine (FLM-05-ACI)

(procedure 4. Yield 80%)

¹H NMR (300 MHz, D₂O) δ 7.75 (s, 1H), 6.93 (s, 1H), 4.45 (dd, *J* = 9.0, 4.9 Hz, 1H),
25 3.16 (dd, *J* = 15.0, 4.8 Hz, 1H), 2.98 (dd, *J* = 15.0, 9.0 Hz, 1H), 1.49 (s, 3H), 1.37 (s, 3H).

Example 12: *N*-(3-amino-2,2-dimethylpropanoyl)-*L*-histidine(FLM-04-ACI)

(procedure 4. Yield 65%)

¹H NMR (300 MHz, D₂O) δ 7.73 (s, 1H), 6.95 (s, 1H), 4.46 (dd, *J* = 8.7, 4.8 Hz,

1H), 3.18 (dd, $J = 15.0, 4.9$ Hz, 1H), 3.11 – 2.95 (m, 3H), 1.29 (s, 3H), 1.16 (s, 3H).

Example 13: N-(1-aminomethyl)-1-cyclopropylcarbonyl)-L-histidine.
(FLM-09-ACI)

(procedure 4. Yield 99%)

5 ^1H NMR (300 MHz, D_2O) δ 7.64 (s, 1H), 6.89 (s, 1H), 4.42 (dd, $J = 8.7, 4.8$ Hz, 1H), 3.16 – 2.82 (m, 4H), 1.22 – 1.07 (m, 1H), 1.05 – 0.83 (m, 3H).

Example 14: N-(1-aminomethyl)-1-cyclobutylcarbonyl)-L-histidine
(FLM-10-ACI)

(procedure 4. Yield 93%)

10 ^1H NMR (300 MHz, d_2o) δ 7.77 (s, 1H), 7.01 (s, 1H), 4.52 (dd, $J = 9.1, 4.7$ Hz, 1H), 3.42 – 3.29 (m, 2H), 3.24 (dd, $J = 15.0, 4.6$ Hz, 1H), 3.04 (dd, $J = 15.0, 9.1$ Hz, 1H), 2.43 – 2.19 (m, 2H), 2.18 – 1.93 (m, 3H), 1.92 – 1.78 (m, 1H).

Example 15: N-(1-aminomethyl)-1-cyclopentylcarbonyl)-L-histidine
(FLM-11-ACI)

15 (procedure 4. Yield 99%)

^1H NMR (300 MHz, CD_3OD) δ 7.56 (s, 1H), 6.83 (s, 1H), 4.45 (dd, $J = 8.1, 4.3$ Hz, 1H), 3.23 (dd, $J = 14.9, 4.3$ Hz, 1H), 3.10 – 2.93 (m, 3H), 2.18 – 1.95 (m, 2H), 1.75 – 1.47 (m, 6H).

Example 16: N-(1-aminomethyl)-1-cyclohexylcarbonyl)-L-histidine (FLM-14-
20 **ACI)**

(procedure 4. Yield 100%)

^1H NMR (300 MHz, CD_3OD) δ 7.56 (s, 1H), 6.84 (s, 1H), 4.48 (dd, $J = 8.6, 4.3$ Hz, 1H), 3.26 (dd, $J = 15.0, 4.5$ Hz, 1H), 3.05 (dd, $J = 15.0, 8.7$ Hz, 1H), 2.98 – 2.79 (m, 2H), 2.10 (d, $J = 12.2$ Hz, 2H), 1.64 – 1.45 (m, 3H), 1.45 – 1.12 (m, 5H).

25 **Example 17: N-(4-amino-2,2-dimethylbutanoyl)-L-histidine(FLM-13-ACI)**

(procedure 4. Yield 100%)

^1H NMR (300 MHz, D_2O) δ 7.59 (s, 1H), 6.86 (s, 1H), 4.40 (dd, $J = 9.1, 4.7$ Hz, 1H), 3.08 (dd, $J = 14.9, 4.7$ Hz, 1H), 2.90 (dd, $J = 14.9, 9.1$ Hz, 1H), 2.58 – 2.38 (m, 2H),

1.67 – 1.56 (m, 2H), 1.07 (s, 3H), 1.04 (s, 3H).

Example 18: N-(3-amino-3,3-dimethylpropanoyl)-L-histidine (FLM-02-ACI)

(procedure 4. Yield 100%)

¹H NMR (300 MHz, D₂O) δ 7.65 (s, 1H), 6.93 (s, 1H), 4.49 (dd, J = 9.8, 4.4 Hz, 1H), 3.15 (dd, J = 15.0, 4.4 Hz, 1H), 2.92 (dd, J = 14.9, 9.9 Hz, 1H), 2.37 (s, J = 14.1 Hz, 2H), 1.13 (s, 3H), 1.08 (s, 3H).

Example 19: N-(3-amino-2,2-dimethylpropanoyl)-L-histidine (FLM-12-ACI)

(procedure 4. Yield 90%)

¹H NMR (300 MHz, D₂O) δ 7.69 (s, 1H), 6.86 (s, 1H), 4.53 (dd, J = 10.0, 4.8 Hz, 1H), 3.70 (s, 3H), 3.26 (dd, J = 15.5, 4.7 Hz, 1H), 3.13 – 2.96 (m, 3H), 1.32 (s, 3H), 1.17 (s, 3H).

Example 20: N-(3-amino-2,2-difluoropropanoyl)-L-histidine (FLM-07-ACI)

(procedure 4. Yield 95%)

¹H NMR (300 MHz, D₂O) δ 8.16 (s, 1H), 7.11 (s, 1H), 4.53 (dd, J = 9.1, 4.8 Hz, 1H), 3.34 – 3.23 (m, 3H), 3.07 (dd, J = 15.2, 9.2 Hz, 1H).

¹H NMR (300 MHz, D₂O) δ 8.16 (s, 1H), 7.11 (s, 1H), 4.53 (dd, J = 9.1, 4.8 Hz, 1H), 3.34 – 3.23 (m, 3H), 3.07 (dd, J = 15.2, 9.2 Hz, 1H).

Pharmacological Tests

The carbonyl-quenching activity of a selected series of compounds according to the invention has been demonstrated *in vitro* by incubating HNE (50 μ M) with the molecule under study (1 mM), in phosphate buffer (10 mM), pH 7.4, at 37°C. The activity was evaluated one and three hours after incubation, determining the residual HNE content by reverse-phase chromatography as previously described by Aldini G. et al. [Biochem Biophys Res Commun. 2002 Nov 15;298(5):699-706]. The carbonyl-quenching activity is evaluated on the basis of the percentage of HNE reacted, compared with the HNE content in the absence of the molecule to be tested.

The stability in human serum of the compound most active in the preceding test was also evaluated by incubation at 37°C and subsequent sampling at 0 min, 15 min, 30

min, 1 h and 2 h. The samples taken from each matrix, suitably treated, were analysed by HPLC-MS, obtaining recovery after 1 h and 2 h.

The results are summarised in the tables below:

Table I

5

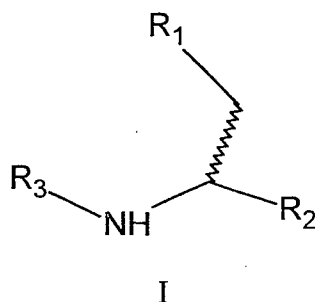
COMPOUND	ACTIVITY (CARNOSINE UNIT)		HNE CONSUMPTION (%)	
	<i>1 h</i>	<i>3 h</i>	<i>1 h</i>	<i>3 h</i>
FLM-02-ACI	0.02	0.14	0.40±1.73	6.05±1.66
FLM-04-ACI	1.76	1.51	30.06±4.68	64.44±4.37
FLM-05-ACI	0.33	0.37	5.58±7.50	15.84±10.65
FLM-07-ACI	0.46	0.69	7.88±5.41	29.42±6.42
FLM-09-ACI	0.81	0.89	13.70±0.83	36.11±2.87
FLM-10-ACI	1.06	1.11	17.89±1.97	45.04±2.94
FLM-11-ACI	1.41	1.31	23.82±1.55	53.54±1.10
FLM-12-ACI	1.58	1.20	27.07±9.77	51.35±9.22
FLM-13-ACI	0.69	0.48	11.77±6.79	20.43±7.94
FLM-14-ACI	1.71	1.36	29.10±2.97	58.03±2.37
FLM-02-OLO	0.25	0.24	4.09±2.61	10.50±2.24
FLM-04-OLO	3.13	2.00	52.14±5.56	86.47±2.73
FLM-05-OLO	0.38	0.45	6.40±1.72	19.44±2.36
FLM-07-OLO	1	0.82	17.07±1.76	34.93±0.58
FLM-09-OLO	1.55	1.45	26.21±1.04	59.09±1.49
FLM-10-OLO	2.82	2.08	47.68±3.02	84.63±2.70
FLM-11-OLO	2.82	2.06	47.56±0.69	83.90±0.33
FLM-12-OLO	2.98	1.64	50.77±3.84	70.08±2.98
FLM-13-OLO	0.62	0.54	10.54±10.04	23.05±12.28
FLM-14-OLO	2.84	1.96	48.36±6.22	83.50±5.85
BLANK	0.12	0.04	2.00±0.66	1.88±0.69
CARNOSINE	1.00	1.00	17.05±1.24	42.66±0.89

Table II

COMPOUND	Stability in plasma (% residual amount)	
	1h	2h
FLM-04-OL	101.78±16.20	100.34±9.46

CLAIMS

1. Compounds of general formula I



wherein:

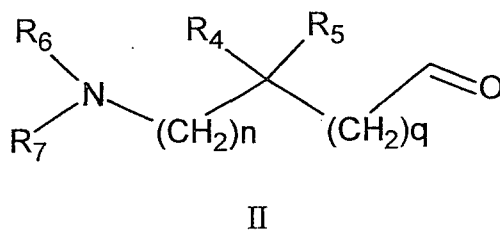
R1 is a 5-6-membered heterocyclic ring optionally fused to a benzene ring, containing one to three nitrogen atoms optionally bearing C₁-C₃ alkyl substituents;

R2 is selected from:

- a **CH₂ O-R** group wherein **R** is hydrogen, a straight or branched C₁-C₁₀ alkyl group or a C₃-C₈ cycloalkyl group, a straight or branched C₁-C₈ alkylcarbonyl group, a C₃-C₈ cycloalkylcarbonyl group; or **R** is an Ar(CH₂)_pC(=O)- group wherein Ar is aryl selected from phenyl and naphthyl optionally substituted with one or two groups selected from the group consisting of methyl, methoxy or ethyl and p is zero or an integer ranging from 1 to 3;

- a **COOR₈** group wherein R₈ is hydrogen, a straight or branched C₁-C₁₀ alkyl group or a C₃-C₈ cycloalkyl group;

R3 is a group of general formula II



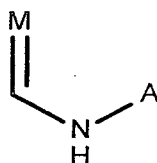
wherein

n and **q**, independently one from the other, are zero or are an integer ranging from

1 to 4;

R₆ and **R₇**, which can be the same or different, are:

- hydrogen;
- a straight or branched C₁-C₂₀ alkylcarbonyl or C₃-C₇ cycloalkylcarbonyl group optionally containing one or more double bonds, or an Ar(CH₂)_pC(=O)- group wherein
- 5 Ar and p are as defined above;
- a straight or branched C₁-C₁₀ alkoxy carbonyl or C₃-C₇ cycloalkoxy carbonyl group optionally containing one or more double bonds or an ArO(CH₂)_nC(=O)- group wherein Ar and p are as defined above;
- one of **R₆** and **R₇** is an amino group and the other is hydrogen;
- 10 - one or **R₆** and **R₇** is hydrogen and the other is a group of formula III

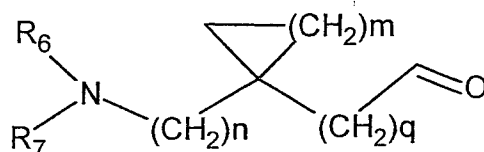


III

- wherein **M** is nitrogen, oxygen or sulphur and **A** is hydrogen or an amino group,
- 15 and the free valence of the carbon atom forms a bond with the nitrogen atom of the group of formula II;

R₄ and **R₅**, which can be the same or different, are:

- a straight or branched C₁-C₈ alkyl group;
- a halogen;
- 20 - can be linked to form, together with the carbon atom to which they are bonded, a C₃-C₆ cycloaliphatic ring selected from cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl, forming an **R₃** group of general formula IV




IV

wherein

m has a value ranging from 1 to 4;

R₆, **R₇**, **n** and **q** have the meanings described above;

wherein:

5 in general formula I, the wavy bond  indicates that the carbon atom substituted with NH and **R₂** can have the R or S configuration.

in general formulas II and IV the free valence at the carbon atom of the carbonyl group forms a bond with the nitrogen atom of formula I;

the optical isomers and diastereomers thereof, either in isolated or mixture form,
10 and the pharmacologically acceptable salts thereof.

2. Compounds according to claim 1 wherein:

R₁ is a heterocyclic ring selected from imidazole, pyrrole, pyrazole, triazole, indole, isoindole, indazole, benzimidazole and benzotriazole, preferably imidazole or imidazole substituted at the nitrogen atom at the 1- position or at the 3- position with
15 methyl, ethyl or propyl;

R₂ and **R₃** are as defined above.

3. Compounds according to claim 1 or 2 wherein:

R₁ is imidazole optionally substituted at the nitrogen atom at the 1- position or at the 3- position with methyl, ethyl or propyl;

20 **R₂** is CH₂-OH or a COOR₈ group wherein **R₈** is selected from hydrogen, methyl and ethyl;

R₃ is a group of formula II wherein **R₄** and **R₅** are both methyl or halogen, **R₆** and **R₇** are both hydrogen or one of **R₆** and **R₇** is hydrogen and the other is a straight or branched C₁-C₁₀ alkoxy carbonyl or C₃-C₇ cyclic group, and **n** and **q** are as defined above.

25 4. Compounds according to claim 1 or 2 wherein:

R₁ is imidazole optionally substituted at the nitrogen atom at the 1- position or at the 3- position with methyl, ethyl or propyl;

R₂ is CH₂-OH or a COOR₈ group wherein **R₈** is selected from hydrogen, methyl

and ethyl;

R₃ is a group of formula IV wherein **R₄** and **R₅** are joined to form, together with the carbon atom to which they are bonded, a C3-C6 cycloaliphatic ring selected from cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl; **R₆** and **R₇** are both hydrogen or one of **R₆** and **R₇** is hydrogen and the other is a straight or branched C₁-C₁₀ alkoxy carbonyl or
5 a C₃-C₇ cyclic group, and **m**, **n** and **q** are as defined above.

5. Compounds according to claim 1, 2 or 3 wherein:

R₁ is imidazole optionally substituted at the nitrogen atom at the 1- position or at the 3- position with methyl;

10 **R₂** is CH₂-OH or a COOR₈ group wherein **R₈** is hydrogen;

R₃ is a group of formula II wherein **R₄** and **R₅** are both methyl or fluorine, **R₆** and **R₇** are both hydrogen, and **n** and **q** are as defined above.

6. Compounds according to claim 1, 2 or 4, wherein:

15 **R₁** is imidazole optionally substituted at the nitrogen atom at the 1- position or at the 3- position with methyl;

R₂ is CH₂-OH or a COOR₈ group wherein **R₈** is hydrogen;

R₃ is a group of formula IV wherein **R₄** and **R₅** are joined to form, together with the carbon atom to which they are bonded, a C3-C6 cycloaliphatic ring selected from cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl, **R₆** and **R₇** are both hydrogen, and **m**,
20 **n** and **q** are as defined above.

7. A compound according to claim 1 selected from: (S)-2-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-2-methylpropanamide;

(S)-3-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-2,2-dimethylpropanamide;

25 (S)-4-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-2,2-dimethylbutanamide;

(S)-1-(aminomethyl)-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)cyclopropanecarboxamide;

(S)-1-(aminomethyl)-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)cyclobutanecarboxamide;

(S)-1-(aminomethyl)-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)cyclopentanecarboxamide;

5 (S)-1-(aminomethyl)-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)cyclohexanecarboxamide;

(S)-3-amino-N-(1-hydroxy-3-(1-methyl-1H-imidazol-5-yl)propan-2-yl)-2,2-dimethylpropanamide;

(S)-3-amino-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)-3,3-dimethylpropanamide;

10 (S)-3-amino-2,2-difluoro-N-(1-hydroxy-3-(1H-imidazol-4-yl)propan-2-yl)propanamide;

N-(2-amino-2-methylpropanoyl)-L-histidine;

N-(3-amino-2,2-dimethylpropanoyl)-L-histidine

15 N-(1-aminomethyl)-1-cyclopropylcarbonyl)-L-histidine;

N-(1-aminomethyl)-1-cyclobutylcarbonyl)-L-histidine;

N-(1-aminomethyl)-1-cyclopentylcarbonyl)-L-histidine

N-(1-aminomethyl)-1-cyclohexylcarbonyl)-L-histidine;

N-(4-amino-2,2-dimethylbutanoyl)-L-histidine;

20 N-(3-amino-3,3-dimethylpropanoyl)-L-histidine;

N-(3-amino-2,2-dimethylpropanoyl)-L-histidine

N-(3-amino-2,2-difluoropropanoyl)-L-histidine.

8. A compound according to claims 1 to 7 for use as a medicament.

9. A compound according to claim 8 for use in the treatment or prevention of
25 neurodegenerative, chronic inflammatory and cardiovascular disorders and the complications of diabetes and cataract.

10. A pharmaceutical composition containing an effective amount of one or more of the compounds of claims 1-7 in combination with suitable excipients.

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2018/060332

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D233/58 A61K31/4164 C07D233/64 A61P9/00 A61P25/28
A61P29/00 A61P27/12 A61P3/10

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D A61K A61P

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

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

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/080139 A1 (FLAMMA SPA [IT]; UNIV DEGLI STUDI MILANO [IT]; NEGRISOLI GIANPAOLO [IT]) 7 July 2011 (2011-07-07) cited in the application	1-3,5, 7-10
A	claims; examples	4,6
X	WO 2008/001174 A1 (FLAMMA SPA [IT]; NEGRISOLI GIANPAOLO [IT]; CANEVOTTI RENATO [IT]) 3 January 2008 (2008-01-03) cited in the application	1-3,5, 7-10
A	claims; examples	4,6
	----- -/-	

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

6 February 2019

Date of mailing of the international search report

19/02/2019

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Fazzi, Raffaella

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2018/060332

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>GUIOTTO ANDREA ET AL: "Carnosine and carnosine-related antioxidants: A review", CURRENT MEDICINAL CHEMISTRY : THE NEW INTERNATIONAL JOURNAL FOR TIMELY IN-DEPTH REVIEWS IN MEDICINAL CHEM, BENTHAM, NL, vol. 12, no. 20, September 2005 (2005-09), pages 2293-2315, XP008106853, ISSN: 0929-8673, DOI: 10.2174/0929867054864796 the whole document -----</p>	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2018/060332

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
WO 2011080139	A1	07-07-2011	CN 102791696 A	21-11-2012
			EP 2519507 A1	07-11-2012
			ES 2439322 T3	22-01-2014
			IT 1397212 B1	04-01-2013
			JP 5787899 B2	30-09-2015
			JP 2013515753 A	09-05-2013
			US 2012316212 A1	13-12-2012
			WO 2011080139 A1	07-07-2011

WO 2008001174	A1	03-01-2008	EP 2041157 A1	01-04-2009
			JP 2009541295 A	26-11-2009
			US 2009247601 A1	01-10-2009
			WO 2008001174 A1	03-01-2008
