INHIBITORS

The present invention relates to a series of novel compounds that are selective inhibitors of the enzyme plasma kallikrein, to pharmaceutical compositions comprising these inhibitors, and the use of such compositions in the treatment of human diseases.

BACKGROUND

The enzyme plasma kallikrein, also known by the classification EC.3.4.21.34, is a member of a family of trypsin-like serine protease that also includes tissue kallikrein, thrombin, trypsin and plasmin. It is found in plasma as an inactive zymogen that is activated by Factor XIIa. The enzyme has a broad spectrum of activity. Plasma kallikrein liberates the vasoactive peptide bradykinin from high molecular weight kininogen by cleavage of Lys-Arg and Arg-Ser bonds. The same peptide can also be liberated from low molecular weight kininogen in the presence of neutrophil elastase. It is also capable of activating prourokinase and plasminogen, and is also thought to participate in the conversion of prorenin to renin. Plasma kallikrein is an essential component of the intrinsic blood coagulation cascade although its role does not involve the release of bradykinin or enzymatic cleavage. High molecular weight kininogen, the preferred substrate for plasma kallikrein, is essential for the activation in this cascade (K. D. Bhoola *et al.*, Pharm. Rev., 1992, 44, 1-80).

The physiological effects of plasma kallikrein are likely to result from the proteolytic cleavage of kininogens to liberate kinins or of other substrates, e.g. precursors of growth factors. Kinins such as bradykinin are potent mediators of inflammation. In addition they influence cellular functions such as blood pressure, local blood flow, glucose transport and cell proliferation. These cellular actions which are modified by release of secondary messengers such as platelet activating factor, leukotrienes, prostaglandins, Substance P, acetylcholine and noradrenaline.

Several groups have disclosed synthetic inhibitors of plasma kallikrein. These include arginine ketomethylene derivatives (WO 92/04371 and D. M. Evans *et al.*, Immunopharmacology, 1996, 32, 115-116), noragmatine and agmatine derivatives (WO 95/07291, WO 94/29335), benzamidine derivatives (J. Stürzbecher *et al.*, Brazilian J. Med. Biol. Res. 1994, 27, 1929-1934), boronic acid derivatives (US 5,187,157) and

aminomethylcyclohexanoyl derivatives (N. Teno *et al.*, Chem. Pharm. Bull., 1993, **41**, 1079-1090). The aminomethylcyclohexanoyl derivatives have been shown to be active in models of collagen-induced arthritis in mice (Y. Fujimora *et al.*, Agents Actions, 1993, **39**, 42-48) and endotoxin-induced disseminated intravascular coagulation (DIC) in rats (S. Okamoto *et al.*, Agents Actions (Supplement), 1992, **38(Part 1)**, 198-205). The boronic acid derivatives are active in models of inflammatory bowel disease (A. Stadnicki *et al.*, Digestive Diseases and Sciences, 1996, **41**, 912-920 and FASEB, 1998, **12**, 325-333).

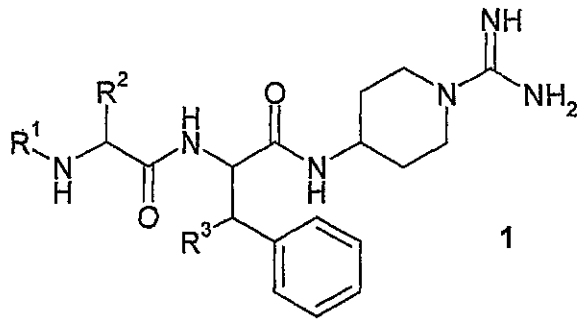
Selectivity with respect to the other members of the trypsin-like serine protease family is an important issue. Inhibitors of tissue kallikrein displaying poor plasma kallikrein activity have previously been reported (M. Szelke *et al.*, Brazilian J. Med. Biol. Res. 1994, **27**, 1935 and D. M. Evans *et al.*, Immunopharmacology, 1996, **32**, 117), but there remains a need for compounds that selectively inhibit plasma kallikrein and not tissue kallikrein.

BRIEF DESCRIPTION OF THE INVENTION

The present invention relates to a series of acylaminopiperidine-1-carboxamidines that are inhibitors of plasma kallikrein. These compounds demonstrate good selectivity for plasma kallikrein, and are potentially useful in the treatment of inflammatory bowel disease, arthritis, inflammation, septic shock, hypotension, cancer, adult respiratory distress syndrome, disseminated intravascular coagulation, cardiopulmonary bypass surgery and bleeding from post-operative surgery. The invention further relates to pharmaceutical compositions of the inhibitors, to the use of the compositions as therapeutic agents, and to methods of treatment using the compositions.

DETAILED DESCRIPTION OF THE INVENTION

In a first aspect, the present invention comprises a series of novel 4-(dipeptidylamino)-piperidine-1-carboxamidines according to general formula **1**.



1

In general formula **1**, R^1 represents a group selected from a hydrogen atom (H), a lower alkyl group, a group according to R^4-CO, a group according to R^4-O_2CCH_2, a group according to R^5-OCO, and a group according to R^5-SO_2. R^2 represents a group selected from a lower alkyl group, a cycloalkyl or (C_5-C_{12})cycloalkylalkyl group, either of which may optionally be substituted with an alkyl or alkoxy group, an aralkyl group which may optionally be substituted with up to three groups chosen from F, Cl, Br, I, OH, lower alkyl, O-(lower alkyl), O-benzyl, NH_2, NO_2, NH-acyl, CN and CF_3, and an aralkyloxymethyl group which may optionally be substituted with up to three groups chosen from F, Cl, Br, OH, lower alkyl and O-(lower alkyl). Alternatively, R^1 and R^2 together may constitute an *ortho*-xylylene group (*o*-$C_6H_4(CH_2)_2$). The aromatic ring of this xylylene group may optionally be substituted with a group selected from F, Cl, Br, OH, lower alkyl and O-(lower alkyl).

R^3 represents a group selected from H, OH and O-(lower alkyl).

R^4 represents a group selected from H, lower alkyl and phenyl.

R^5 represents a group selected from lower alkyl, phenyl and benzyl.

In the context of the present disclosure, the terms "alkyl group" and "lower alkyl group" are used interchangeably to denote linear and branched saturated hydrocarbon groups with between 1 and 8 carbon atoms, such as methyl, ethyl, isopropyl, *tert*-butyl, neopentyl and isooctyl groups.

The term "cycloalkyl group" is used to denote monocyclic or polycyclic saturated hydrocarbon groups with between 3 and 12 carbon atoms, such as cyclopropyl, cyclohexyl, bicyclo[4.4.0]decyl (i.e. decahydronaphthyl) and adamantyl groups.

The term "cycloakylalkyl group" is used to denote alkyl groups that bear a cycloalkyl group as a substituent, such as cyclohexylmethyl and 1-(cyclopentyl)ethyl groups. Where a limit is specified, as in (C_a-C_b)cycloalkylalkyl, this denotes that the cycloalkyl moiety has between a and b carbon atoms.

The term "alkoxy group" is used to denote O-(alkyl) groups.

The term "acyl group" is used to denote formyl (H-CO) and alkyl-CO groups.

The term "aralkyl group" is used to denote alkyl groups that bear an aryl group as a substituent, such as benzyl and 1-naphthylmethyl groups. The term "aryl group" includes phenyl, naphthyl, furyl, thienyl, pyrrolyl and pyridyl groups.

The term "aralkyloxymethyl group" is used to denote aralkyl-OCH_2 groups.

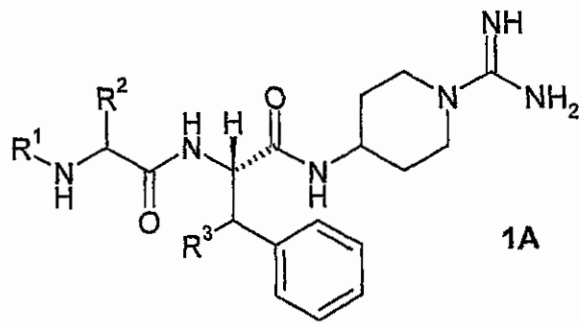
The compounds of the present invention all have a guanidine functional group and so can form addition salts with acids. To the extent that such acids are pharmaceutically acceptable then these salts fall within the scope of the invention. Examples of suitable acids include acetic acid, trifluoroacetic acid, fumaric acid, malic acid, citric acid, benzoic acid, benzenesulphonic acid, hydrochloric acid, sulphuric acid and phosphoric acid. Certain compounds within the invention have an acidic functional group and so can form salts with alkaline and alkaline earth metals. Again, insofar as these are pharmaceutically acceptable they are included in the scope of the invention. Examples of such salts include the sodium, potassium and calcium salts.

The compounds of the present invention all have at least two stereogenic centres (asymmetric carbon atoms) and so can exist as optical isomers, such as enantiomers, diastereomers and epimers. All such isomers are included in the scope of the present invention. Mixtures of such isomers, including (but not limited to) racemic mixtures are also included in the scope of the invention.

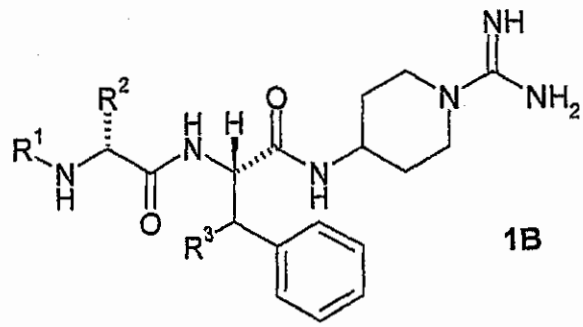
In a preferred embodiment, the present invention comprises compounds according to general formula **1** in which R^1 is selected from H, lower alkyl and R^4-O_2CCH_2.

In another preferred embodiment, the present invention comprises compounds according to general formula **1** in which R^2 is selected from (C_6-C_{10})cycloalkylalkyl, benzyl optionally substituted with up to three groups chosen from F, Cl, Br, OH, lower alkyl and O-(lower alkyl), phenethyl optionally substituted with up to three groups chosen from F, Cl, Br, OH, lower alkyl and O-(lower alkyl), and benzyloxymethyl optionally substituted with up to three groups chosen from F, Cl, Br, OH, lower alkyl and O-(lower alkyl). More preferably R^2 is selected from cyclohexylmethyl, decahydronaphth-2-ylmethyl, benzyl, 4-fluorobenzyl, 4-chlorobenzyl, 4-hydroxybenzyl, 4-(lower alkyl)oxybenzyl, α-hydroxybenzyl, α-methoxybenzyl, phenethyl and benzyloxymethyl.

In another preferred embodiment, the present invention comprises compounds according to general formula **1** in which the absolute stereochemistry is as depicted in general formula **1A**. More preferably, the absolute stereochemistry is as depicted in general formula **1B**.



1A



1B

In another preferred embodiment, the present invention comprises a compound selected from:

(2'S,2"*R*)-4-(2'-(2"-amino-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-phenylpropanoyl-amino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-carboxymethylamino-3"-(4'"-ethoxyphenyl)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(3"-(4'"-ethoxyphenyl)-2"-(methyloxycarbonylmethylamino)-propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-amino-3"-cyclohexylpropanoylamino)-3'-phenylpropanoyl-amino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-carboxymethylamino-3"-cyclohexylpropanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(3"-cyclohexyl-2"-(methyloxycarbonylmethylamino)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-amino-3"-phenylpropanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-carboxymethylamino-3"-phenylpropanoylamino)-3'-phenyl-propanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-(methyloxycarbonylmethylamino)-3"-phenylpropanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-amino-3"-decahydronaphth-2'"-ylpropanoylamino)-3'-phenylpropanoyl-amino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-carboxymethylamino-3"-decahydronaphth-2'"-ylpropanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(3"-decahydronaphth-2'"-yl-2"-(methyloxycarbonylmethylamino)propanoyl-amino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*,3'*R*)-4-(2'-(2"-amino-3"-cyclohexylpropanoylamino)-3'-hydroxy-3'-phenyl-propanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*,3'*R*)-4-(2'-(2"-carboxymethylamino-3"-cyclohexylpropanoylamino)-3'-hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

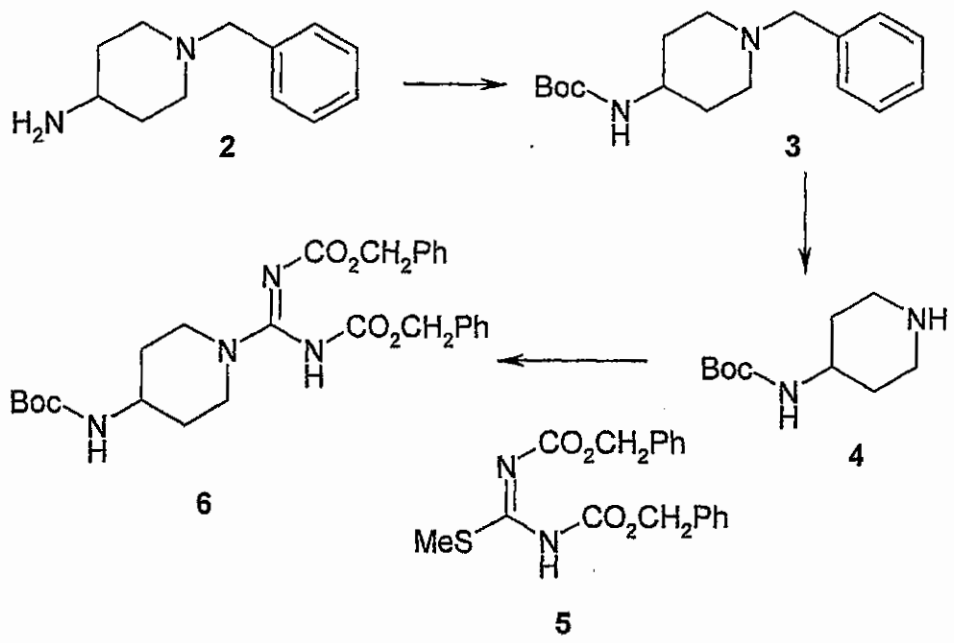
(2'*S*,2"*R*,3'*R*)-4-(2'-(3"-cyclohexyl-2"-(methyloxycarbonylmethylamino)propanoylamino)-3'-hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*,3'*R*)-4-(2'-(2"-amino-3"-(4'"-ethoxyphenyl)propanoylamino)-3'-methoxy-3'-phenyl-propanoylamino)piperidine-1-carboxamidine;

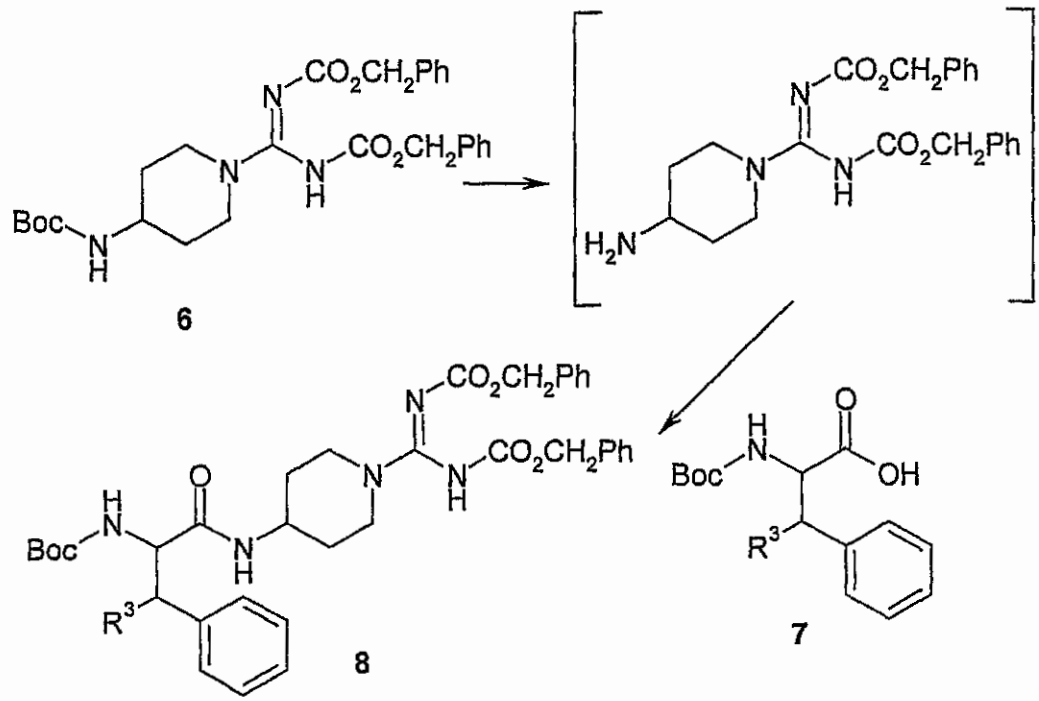
(2'*S*,2"*R*,3'*R*)-4-(2'-(2"-carboxymethylamino-3"-(4'"-ethoxyphenyl)propanoylamino)-3'-methoxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine; and

(2'*S*,2"*R*,3'*R*)-4-(2'-(3"-(4'"-ethoxyphenyl)-2"-(methyloxycarbonylmethylamino)-propanoylamino)-3'-methoxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine.

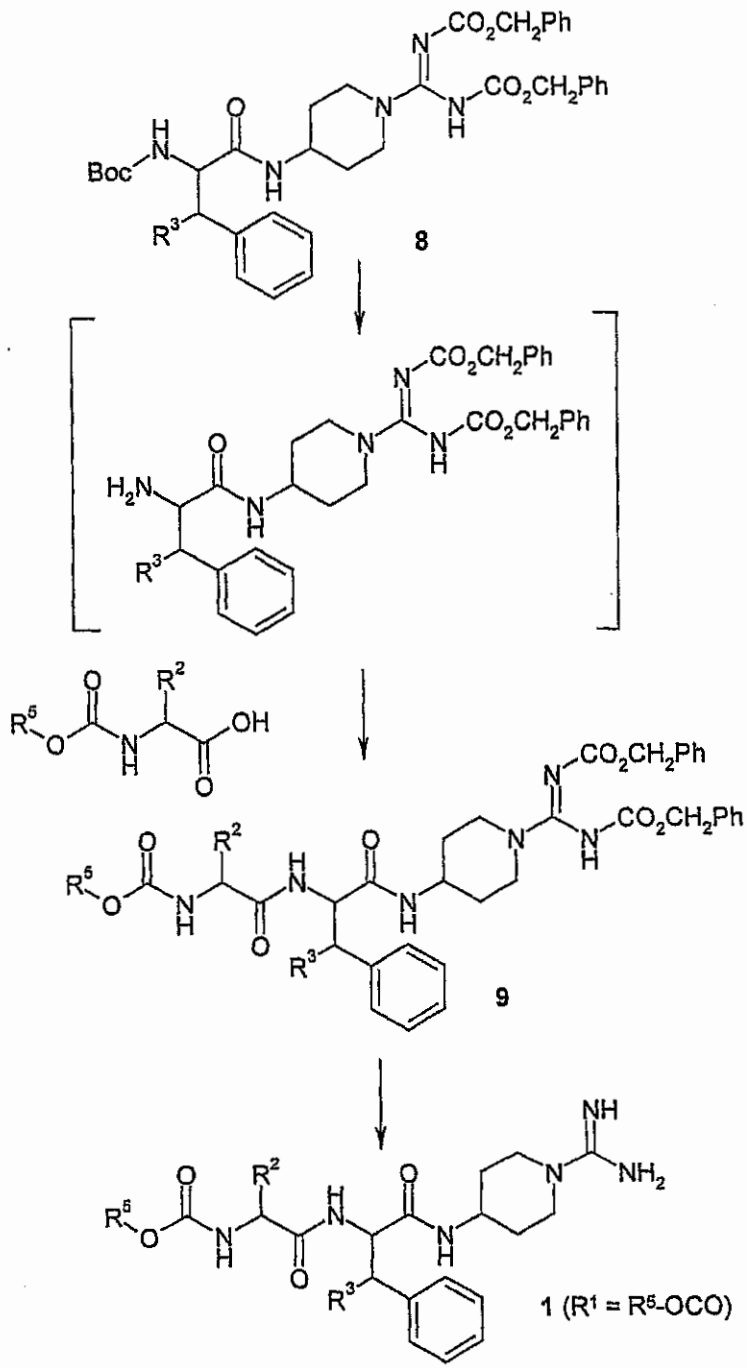
The compounds of the present invention may be prepared by the methods generally known in the art, and particularly those methods used in the field of peptide chemistry. A useful starting material is 4-amino-1-benzylpiperidine (**2**). Protection of the primary amine with a *tert*-butyloxycarbonyl (Boc) group to give **3** and hydrogenolysis provides piperidine derivative **4**. This can be treated with isothiourea derivative **5** to give the carboxamidinopiperidine derivative **6** in which the amine and guanidine functional groups are differentially protected.



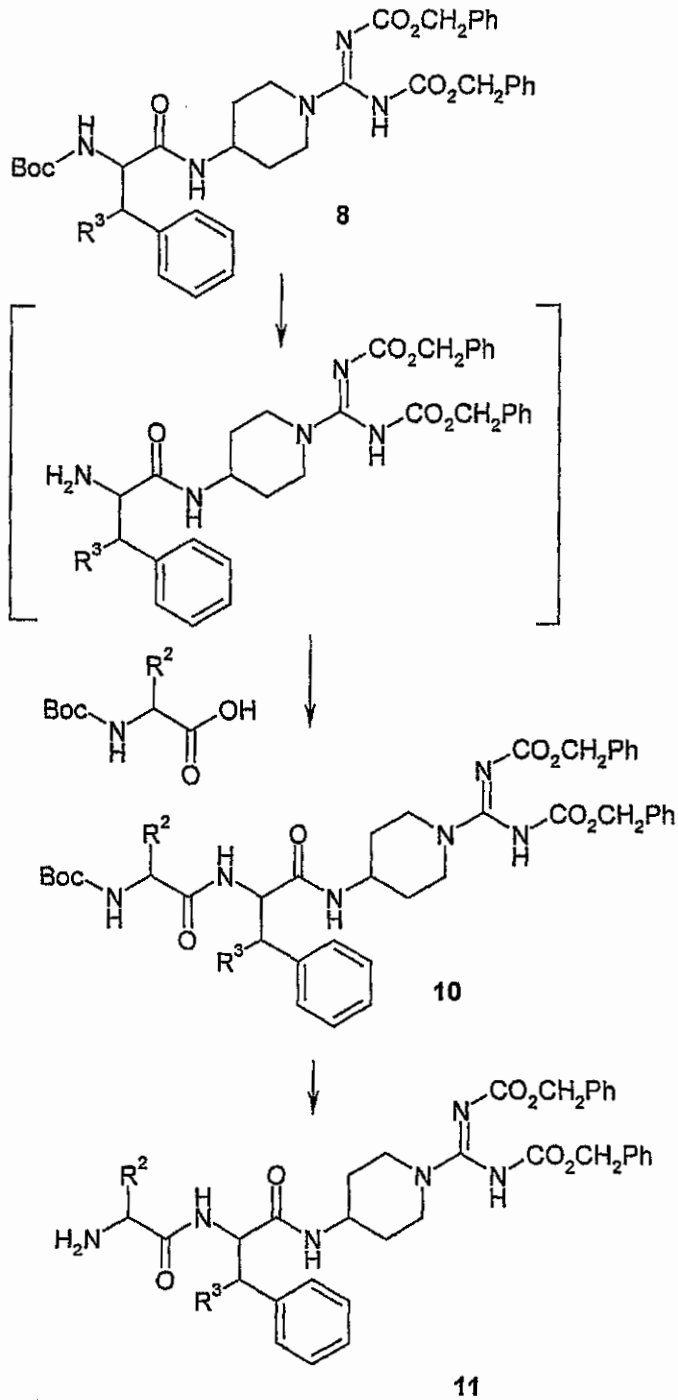
The carboxamidinopiperidine derivative **6** can then be selectively deprotected and coupled to an *N*-protected amino acid **7** to give intermediate **8**.



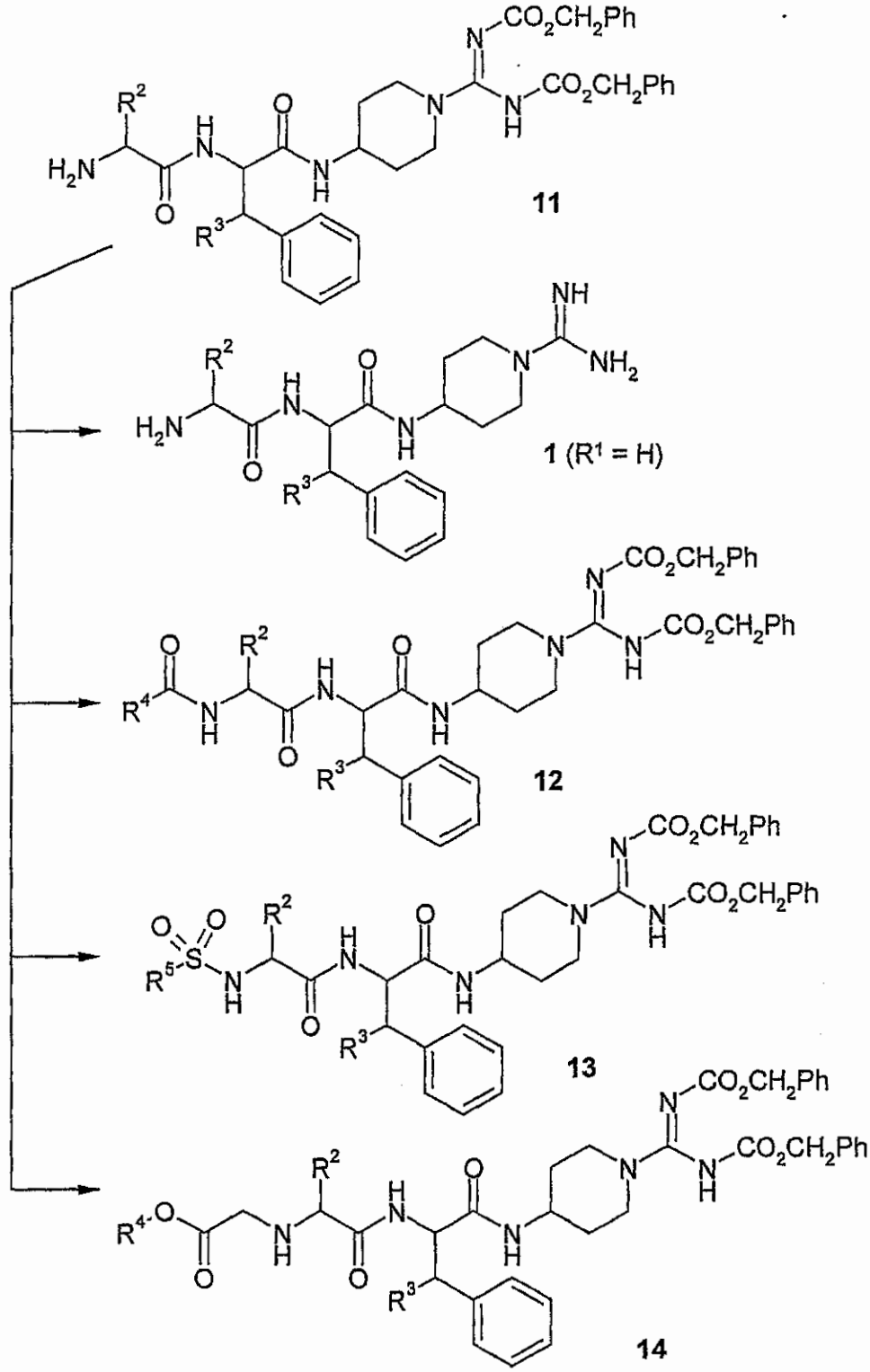
The elaboration of intermediate **8** to give the final product depends to some extent on the nature of R^1. When R^1 is R^5-OCO then the synthesis may conveniently proceed via intermediate **9**.



When R^1 is H, R^4-CO, R^4-O_2CCH_2 or R^5-SO_2 then the synthesis may conveniently proceed via intermediates **10** and **11**.



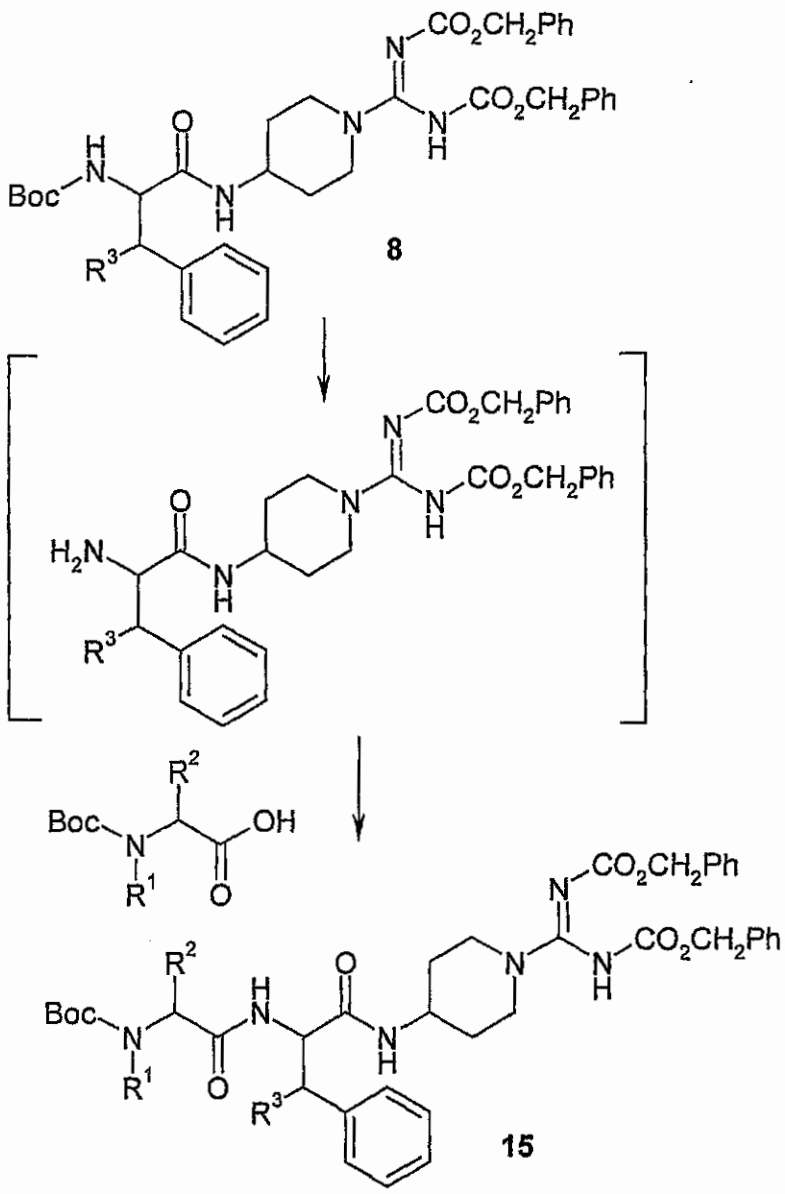
Deprotection of the guanidine functional group gives compounds according to general formula **1** in which R^1 is H. Derivatisation of the primary amine prior to deprotection of the guanidine gives access to other embodiments of R^1.



Intermediates **12**, **13** and **14** may then be deprotected to give the corresponding compounds according to general formula **1**.

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When R^1 is alkyl, or when R^1 and R^2 together form a xylylene group, the synthesis may conveniently proceed via intermediate **15**.



Two deprotection steps then give the corresponding compounds according to general formula **1**.

The compounds of the present invention are potent and selective inhibitors of plasma kallikrein. They are therefore useful in the treatment of disease conditions for which over activity of plasma kallikrein is a causative factor. Generally, for use in such treatment the compounds will be formulated for administration to the patient. The pharmaceutical

formulation may be a solid or liquid, such as a tablet, capsule, solution or suspension. Methods of preparing such formulations are well known in the pharmaceutical art.

The compositions will be administered to the patient under the supervision of the attending physician.

EXAMPLES

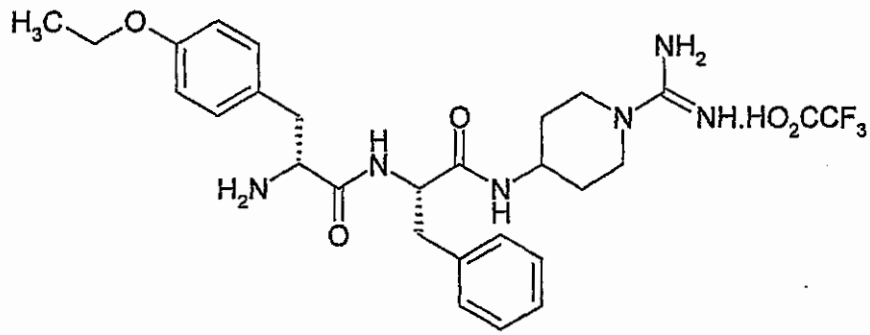
The following abbreviations have been used:

AcOH	acetic acid
Boc-DCha-OH	*N*-(*tert*-butyloxycarbonyl)-3-cyclohexyl-*D*-alanine
Boc-DTyr(Et)-OH	*N*-(*tert*-butyloxycarbonyl)-*O*-ethyl-*D*-tyrosine
Boc-Phe-ONSu	*N*-(*tert*-butyloxycarbonyl)-phenylalanine succinimidyl ester
DMF	dimethylformamide
H-thPse-OH	*threo*-3-phenylserine
mplc	medium pressure liquid chromatography
TFA	trifluoroacetic acid

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EXAMPLE 1

(2'*S*,2"*R*)-4-(2'-(2"-Amino-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-phenylpropanoyl-amino)piperidine-1-carboxamidine trifluoroacetate



1A. 1-Benzyl-4-(*tert*-butyloxycarbonylamino)piperidine

4-Amino-1-benzylpiperidine (3.2g, 16.8mmol) was dissolved in CH_2Cl_2 (100ml). Di-*tert*-butyl dicarbonate (3.7g, 17.0mmol) and *N*,*N*-diisopropylethylamine (1.9g, 19mmol) were

added. The mixture was stirred for 18h at room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (150ml). This solution was washed with 0.3M $KHSO_4$ (2 x 30ml), sat. $NaHCO_3$ (2 x 30ml), water (2 x 30ml) and brine (1 x 30ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil which was purified by flash chromatography on silica gel (eluant: 70% chloroform, 30% cyclohexane) to give a yellow solid identified as 1-benzyl-4-(*tert*-butyloxycarbonylamino)piperidine (4.9g, 18.9 mmol, 100%).

1B. 4-(*tert*-Butyloxycarbonylamino)piperidine

1-Benzyl-4-(*tert*-butyloxycarbonylamino)piperidine (4.9g, 18.9mmol) was dissolved in ethanol (100ml). This solution was hydrogenated over 10% palladium on charcoal at 60 psi. After 18h at room temperature the mixture was filtered through Celite and the residue washed with ethanol (100ml). The combined filtrates were evaporated *in vacuo* to give a white solid identified as 4-(*tert*-butyloxycarbonylamino)piperidine (2.3g, 7.1mmol, 51%).

1C. *N,N'*-Di(benzyloxycarbonyl)-4-(*tert*-butyloxycarbonylamino)piperidine-1-carboxamidine

4-(*tert*-Butyloxycarbonylamino)piperidine (1.5g, 7.5mmol) was dissolved in ethanol (100ml). *N,N'*-Bis(benzyloxycarbonyl)-*S*-methylisothiourea (3.1g, 8.7mmol) and mercuric oxide (1.9g, 8.8mmol) were added. The mixture was stirred at 40°C for 4h then the solid was filtered off and washed with ethanol (50 ml). The combined filtrates were evaporated *in vacuo* to give a colourless oil which was purified by flash chromatography on silica gel (eluant: 90% pet ether 60-80, 10% ethyl acetate) to give a colourless oil identified as *N,N'*-di(benzyloxycarbonyl)-4-(*tert*-butyloxycarbonylamino)piperidine-1-carboxamidine (3.3g, 6.6mmol, 87%).

1D. (2'*S*)-*N,N'*-Di(benzyloxycarbonyl)-4-(2'-(*tert*-butyloxycarbonylamino)-3'-phenyl-propanoylamino)piperidine-1-carboxamidine

N,N'-Di(benzyloxycarbonyl)-4-(*tert*-butyloxycarbonylamino)piperidine-1-carboxamidine (3.1g,6.1mmol) was dissolved in 4M HCl/dioxan (70ml). After 30min at room temperature the solvent was evaporated *in vacuo* and the residue was dissolved in CH_2Cl_2 (60ml). This solution was cooled to 0°C, Boc-Phe-ONSu (2.2g, 6.1mmol) was added and the pH adjusted to 9 with *N*-methylmorpholine. The mixture was stirred at room temperature for 4 h, the solvent was evaporated *in vacuo* and the residue dissolved in ethyl acetate (200ml). This solution was washed with 0.3M $KHSO_4$ (2 x 30ml), sat. $NaHCO_3$ (2 x 30ml), water(2 x

30ml) and brine (1 x 30ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a white solid which was purified by flash chromatography on silica gel (eluant: 70% chloroform, 30% cyclohexane) to give a white solid identified as (2'*S*)-*N*,*N*'-di(benzyloxycarbonyl)-4-(2'-(*tert*-butyloxycarbonylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine (3.56g, 5.4 mmol, 89%).

1E. (2'*S*,2"*R*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(2"-(*tert*-butyloxycarbonylamino)-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine

(2'*S*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(*tert*-butyloxycarbonylamino)-3'-phenylpropanoyl-amino)piperidine-1-carboxamidine (2.5g, 3.85mmol) was dissolved in 4M HCl/dioxan (70ml). After 30min at room temperature the solvent was evaporated *in vacuo* and the residue dissolved in CH_2Cl_2/DMF (9:1, 50ml). This solution was cooled to 0°C and Boc-DTyr(Et)-OH (1.2g, 3.84mmol) was added followed by 1-hydroxybenzotriazole hydrate (680mg, 5.0mmol) and water-soluble carbodiimide (1.0g, 5.0mmol). After 15min the pH adjusted to 8 with *N*-methylmorpholine The mixture was stirred at room temperature for 18 h, after which time the solvent was evaporated *in vacuo* and the residue dissolved in chloroform (200ml). This solution was washed with 0.3M $KHSO_4$ (2 x 30ml), sat. $NaHCO_3$ (2 x 30ml), water (2 x 30ml) and brine (1 x 30ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a white solid which was purified by flash chromatography on silica gel (eluant: 85% chloroform, 15% hexane) to give a white solid identified as (2'*S*,2"*R*)-*N*,*N*'-di(benzyloxycarbonyl)-4-(2'-(2"-(*tert*-butyloxycarbonylamino)-3"-(4"'-ethoxyphenyl)-propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine (2.47g, 3.2mmol, 65%).

1F. (2'*S*,2"*R*)-4-(2'-(2"-Amino-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-phenyl-propanoylamino)piperidine-1-carboxamidine trifluoroacetate

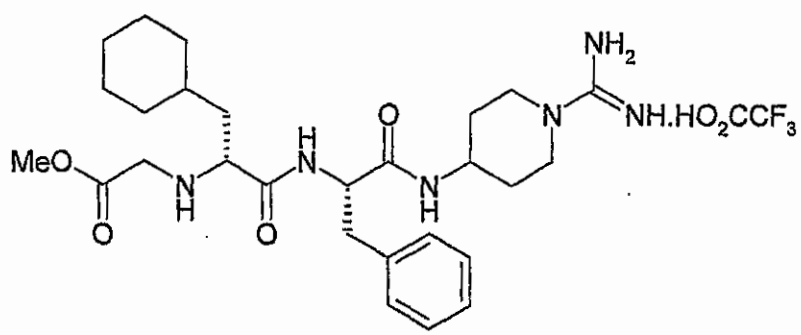
(2'*S*,2"*R*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(2"-(*tert*-butyloxycarbonylamino)-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine (2.1g, 2.7mmol) was dissolved in 4M HCl/dioxan (50ml). After 30min at room temperature the solvent was evaporated *in vacuo* and the residue dissolved in AcOH/water (95:5, 50ml). This solution was hydrogenated over 10% palladium on charcoal. After 2h at room temperature the mixture was filtered through Celite and the residue washed with AcOH/water (9:1, 30ml). The combined filtrates were evaporated *in vacuo* and the

residue purified by mplc on Vydac C_{18} (15-25μ) using MeCN/$H_2$0/TFA to give a white solid identified as H-DTyr(Et)-Phe-4-amino-1-amidinopiperidine trifluoroacetate (1.12g).

$[M+H]^+$ = 480.6

EXAMPLE 2

(2'*S*,2"*R*)-4-(2'-(3"-Cyclohexyl-2"-(methyloxycarbonylmethylamino)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine trifluoroacetate



2A. (2'*S*,2"*R*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(2"-(*tert*-butyloxycarbonylamino)-3"-cyclohexylpropanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine

(2'*S*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(*tert*-butyloxycarbonylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine (from Example 1D, 1.9g, 2.99mmol) was dissolved in 4M HCl/dioxan (70ml). After 30min at room temperature the solvent was evaporated *in vacuo* and the residue dissolved in CH_2Cl_2/DMF (9:1, 50ml). This solution was cooled to 0°C and Boc-DCha-OH (900mg, 3.3mmol) was added followed by 1-hydroxybenzotriazole hydrate (820mg, 6.1mmol) and water-soluble carbodiimide (730mg, 3.6mmol). After 15min the pH was adjusted to 8 with *N*-methylmorpholine. The mixture was stirred at room temperature for 18 h, after which time the solvent was evaporated *in vacuo* and the residue dissolved in chloroform (200ml). This solution was washed with 0.3M $KHSO_4$ (2 x 30ml), sat. $NaHCO_3$ (2 x 30ml), water (2 x 30ml) and brine (1 x 30ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a white solid which was purified by flash chromatography on silica gel (eluant: 90% chloroform, 10% hexane) to give a white solid identified as (2'*S*,2"*R*)-*N*,*N*'-di(benzyloxycarbonyl)-4-(2'-(2"-(*tert*-butyloxycarbonylamino)-3"-cyclohexylpropanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine (1.73g, 2.14 mmol, 72%).

2B. (2'*S*,2"*R*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(3"-cyclohexyl-2"-(methyloxycarbonylmethylamino)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine

(2'*S*,2"*R*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(2"-(*tert*-butyloxycarbonylamino)-3"-cyclohexyl-propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine (1.73g, 2.14mmol) was dissolved in 4M HCl/dioxan (50ml). After 30min at room temperature the solvent was evaporated *in vacuo* and the residue dissolved in acetonitrile (100ml). Methyl bromoacetate (400mg, 2.6mmol) and *N*,*N*-diisopropylethylamine (440mg, 4.4mmol) were added. The reaction mixture was stirred at 60°C for 5h after which time the solvent was evaporated *in vacuo* and the residue dissolved in ethyl acetate (200ml). This solution was washed with 0.3M $KHSO_4$ (2 x 30ml), sat. $NaHCO_3$ (2 x 30ml), water (2 x 30ml) and brine (1 x 30ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil which was purified by flash chromatography on silica gel (eluant: 90% chloroform, 10% hexane) to give a white solid identified as (2'*S*,2"*R*)-*N*,*N*'-di(benzyloxycarbonyl)-4-(2'-(3"-cyclohexyl-2"-(methyloxycarbonylmethylamino)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine (1.65g, 2.11 mmol, 98%).

2C. (2'*S*,2"*R*)-4-(2'-(3"-Cyclohexyl-2"-(methyloxycarbonylmethylamino)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine trifluoroacetate

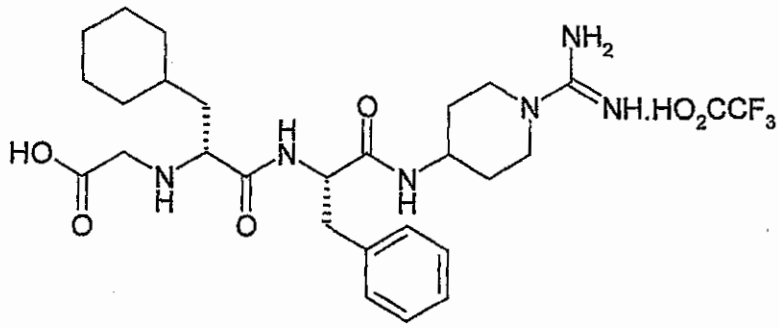
(2'*S*,2"*R*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(3"-cyclohexyl-2"-(methyloxycarbonylmethylamino)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine (1.62g, 2.11mmol) was dissolved in AcOH/water (95:5, 50ml). This solution was hydrogenated over 10% palladium on charcoal. After 2h at room temperature the mixture was filtered through Celite and the residue washed with AcOH/water (9:1, 30ml). The combined filtrates were evaporated *in vacuo* and the residue purified by mplc on VydacC_{18} (15-25μ) using MeCN/$H_2$0/TFA to give a white solid identified as (2'*S*,2"*R*)-4-(2'-(3"-cyclohexyl-2"-(methyloxycarbonylmethylamino)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine trifluoroacetate (570mg).

$[M+H]^+ = 515$

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EXAMPLE 3

(2'*S*,2"*R*)-4-(2'-(2"-(Carboxymethylamino)-3"-cyclohexylpropanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine trifluoroacetate



3A. (2'*S*,2"*R*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(2"-(carboxymethylamino)-3"-cyclohexylpropanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine

(2'*S*,2"*R*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(3"-cyclohexyl-2"-(methyloxycarbonylmethylamino)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine (from Example 2B, 1.6g, 2.1mmol) was dissolved in tetrahydrofuran (50ml). 1M Lithium hydroxide (3ml, 3mmol) was added. After 18h the solvent was evaporated *in vacuo* and the residue dissolved in ethyl acetate (150ml). This solution was washed with 1M citric acid (1 x 30ml), water (2 x 30ml) and brine (1 x 30ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a white solid identified as (2'*S*,2"*R*)-*N*,*N*'-di(benzyloxycarbonyl)-4-(2'-(2"-(carboxymethylamino)-3"-cyclohexylpropanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine (1.62g, 2.11mmol, 100%).

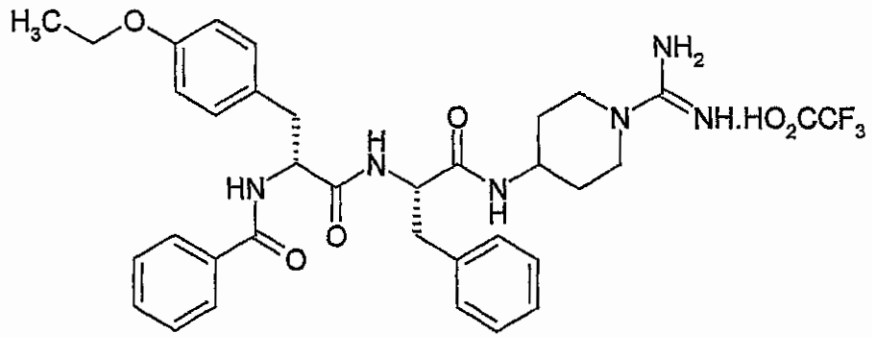
3B. (2'*S*,2"*R*)-4-(2'-(2"-(Carboxymethylamino)-3"-cyclohexylpropanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine trifluoroacetate

(2'*S*,2"*R*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(2"-(carboxymethylamino)-3"-cyclohexylpropanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine (1.62g, 2.11mmol) was dissolved in AcOH/water (95:5, 50ml). This solution was hydrogenated over 10% palladium on charcoal. After 2h at room temperature the mixture was filtered through Celite and the residue washed with AcOH/water (9:1, 30ml). The combined filtrates were evaporated *in vacuo* and the residue purified by mplc on Vydac C_{18} (15-25μ) using MeCN/$H_2$0/TFA to give a white solid identified as (2'*S*,2"*R*)-4-(2'-(2"-(carboxymethylamino)-3"-cyclohexylpropanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine trifluoroacetate (570mg).

$[M+H]^+ = 501$

EXAMPLE 4

(2'*S*,2"*R*)-4-(2'-(2"-Benzoylamino-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine trifluoroacetate



4A. (2'*S*,2"*R*)-4-(2'-(2"-Benzoylamino-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-phenylpropanoylamino)-*N*,*N*'-di(benzyloxycarbonyl)piperidine-1-carboxamidine

(2'*S*,2"*R*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(2"-(*tert*-butyloxycarbonylamino)-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine(from Example 1F, 100mg, 0.12mmol) was dissolved in 4M HCl/dioxan (20ml). After 30min at room temperature the solvent was evaporated *in vacuo* and the residue dissolved in CH_2Cl_2 (20ml). This solution was cooled to 0°C and benzoyl chloride (19.7mg, 0.141mmol) and triethylamine (36mg, 0.36mmol) were added. The mixture was stirred at room temperature for 18 h, the solvent was evaporated *in vacuo* and the residue dissolved in ethyl acetate (70ml). This solution was washed with 0.3M $KHSO_4$ (2 x 20ml), sat. $NaHCO_3$ (2 x 20ml), water (2 x 20ml) and brine (1 x 20ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a white solid which was purified by flash chromatography on silica gel (eluant: 85% chloroform, 15% cyclohexane) to give a white solid identified as (2'*S*,2"*R*)-4-(2'-(2"-benzoylamino-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-phenylpropanoylamino)-*N*,*N*'-di(benzyloxycarbonyl)piperidine-1-carboxamidine (65mg, 0.072 mmol, 60%).

4B. (2'*S*,2"*R*)-4-(2'-(2"-Benzoylamino-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine trifluoroacetate

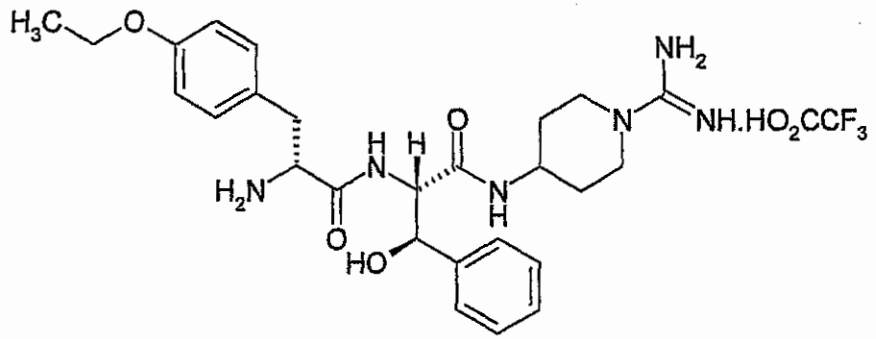
(2'*S*,2"*R*)-4-(2'-(2"-Benzoylamino-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-phenylpropanoylamino)-*N*,*N*'-di(benzyloxycarbonyl)piperidine-1-carboxamidine (65mg,

0.072mmol) was dissolved in AcOH/water (95:5, 25ml). This solution was hydrogenated over 10% palladium on charcoal. After 1 hour at room temperature the mixture was filtered through Celite and the residue washed with AcOH/water (9:1, 20ml). The combined filtrates were evaporated *in vacuo* and the residue purified by mplc on VydacC_{18} (15-25μ) using MeCN/$H_2$0/TFA to give a white solid identified as (2'*S*,2"*R*)-4-(2'-(2"-benzoylamino-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine trifluoroacetate (44mg).

$[M+H]^+ = 585$

EXAMPLE 5

(2'*S*,2"*R*,3'*R*)-4-(2'-(2"-Amino-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine trifluoroacetate



5A. (2*S*,3*R*)-2-(*tert*-Butyloxycarbonylamino)-3-hydroxy-3-phenylpropanoic acid

H-thPse-OH (J. Biol. Chem., 1953, **204**, 323) (1.4g, 7.73mmol) was dissolved in dioxan (75ml). Sodium hydroxide 820mg, 20.5mmol) in water (75ml) was added followed by di-*tert*-butyl dicarbonate (2.1g, 9.6mmol). The mixture was stirred for 18h at room temperature then the dioxan was removed *in vacuo* and the residue was washed with diethyl ether (1 x 100ml), acidified to pH 4 with 1M HCl and extracted with $CHCl_3$ (3 x 100ml). The combined extracts were washed with water (1 x 50ml) and brine (1 x 50ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a white solid identified as (2*S*,3*R*)-2-(*tert*-butyloxycarbonylamino)-3-hydroxy-3-phenylpropanoic acid (1.6g, 5.7 mmol, 74%).

5B (2'*S*,3'*R*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(*tert*-butyloxycarbonylamino)-3'-hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine

N,*N*'-Di(benzyloxycarbonyl)-4-(*tert*-butyloxycarbonylamino)piperidine-1-carboxamidine (from Example 1C, 2.3g, 4.5mmol) was dissolved in 4M HCl/dioxan (70ml). After 30min at

room temperature the solvent was evaporated *in vacuo* and the residue dissolved in CH_2Cl_2/DMF (9:1, 50ml). This solution was cooled to 0°C and (2*S*,3*R*)-2-(tert-butyloxycarbonylamino)-3-hydroxy-3-phenylpropanoic acid (1.6g, 5.6mmol) was added followed by 1-hydroxybenzotriazole hydrate (1.1g, 8.1mmol) and water-soluble carbodiimide (1.4g, 75.0mmol). After 15 min the pH adjusted to 8 with *N*-methylmorpholine. The mixture was stirred at room temperature for 18 h, after which time the solvent was evaporated *in vacuo* and the residue dissolved in ethyl acetate (200ml). This solution was washed with 0.3M $KHSO_4$ (2 x 30ml), sat. $NaHCO_3$ (2 x 30ml), water (2 x 30ml) and brine (1 x 30ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a white solid which was purified by flash chromatography on silica gel (eluant: 50% ethyl acetate, 50% pet. ether) to give a white solid identified as (2'*S*,3'*R*)-*N*,*N*'-di(benzyloxycarbonyl)-4-(2'-(*tert*-butyloxycarbonylamino)-3'-hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine (1.1g, 1.6 mmol, 36%).

5C. (2'*S*,2"*R*,3'*R*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(2"-(*tert*-butyloxycarbonylamino)-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-hydroxy-3'-phenylpropanoyl-amino)piperidine-1-carboxamidine

(2'*S*,3'*R*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(*tert*-butyloxycarbonylamino)-3'-hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine (1.1g, 1.6mmol) was dissolved in 4M HCl/dioxan (70ml). After 30min at room temperature the solvent was evaporated *in vacuo* and the residue dissolved in CH_2Cl_2/DMF (9:1, 50ml). This solution was cooled to 0°C and Boc-DTyr(Et)-OH (620mg, 2.0mmol) was added followed by 1-hydroxybenzotriazole hydrate (270mg, 2.0mmol) and water-soluble carbodiimide (420mg, 2.1mmol). After 15min the pH was adjusted to 8 with *N*-methylmorpholine The mixture was stirred at room temperature for 18 h, after which time the solvent was evaporated *in vacuo* and the residue dissolved in ethyl acetate (200ml). This solution was washed with 0.3M $KHSO_4$ (2 x 30ml), sat. $NaHCO_3$ (2 x 30ml), water (2 x 30ml) and brine (1 x 30ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a white solid which was purified by flash chromatography on silica gel (eluant: 60% ethyl acetate, 40% pet. ether) to give a white solid identified as (2'*S*,2"*R*,3'*R*)-*N*,*N*'-di(benzyloxycarbonyl)-4-(2'-(2"-(*tert*-butyloxycarbonylamino)-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine (1.2g, 1.3 mmol, 80%).

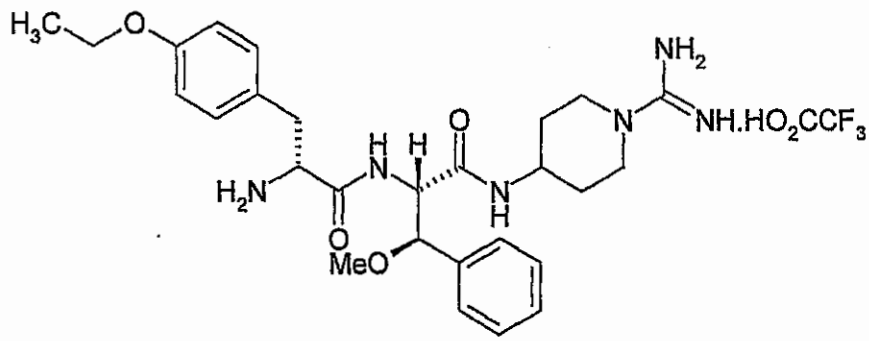
5D. (2'*S*,2"*R*,3'*R*)-4-(2'-(2"-Amino-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine trifluoroacetate

(2'*S*,2"*R*,3'*R*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(2"-(*tert*-butyloxycarbonylamino)-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine (1.2g, 1.3mmol) was dissolved in 4M HCl/dioxan (50ml). After 30min at room temperature the solvent was evaporated *in vacuo* and the residue dissolved in AcOH/water (95:5, 50ml). This solution was hydrogenated over 10% palladium on charcoal. After 2h at room temperature the mixture was filtered through Celite and the residue washed with AcOH/water (9:1, 30ml). The combined filtrates were evaporated *in vacuo* and the residue purified by mplc on Vydac C_{18} (15-25μ) using MeCN/H_20/TFA to give a white solid identified as (2'*S*,2"*R*,3'*R*)-4-(2'-(2"-amino-3"-(4"'-ethoxyphenyl)-propanoylamino)-3'-hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine trifluoroacetate (540mg).

$[M+H]^+$ = 497.0

EXAMPLE 6

(2'*S*,2"*R*,3'*R*)-4-(2'-(2"-Amino-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-methoxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine trifluoroacetate



6A. (2'*SR*,3'*RS*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(*tert*-butyloxycarbonylamino)-3'-hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine

(2'*SR*,3'*RS*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(*tert*-butyloxycarbonylamino)-3'-hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine was prepared by the same method as described in Example 5 but starting with racemic H-thPse-OH.

6B. (2'*SR*,3'*RS*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(*tert*-butyloxycarbonylamino)-3'-methoxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine

(2'*SR*,3'*RS*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(*tert*-butyloxycarbonylamino)-3'-hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine (180mg, 0.27mmol), was dissolved in CH_2Cl_2 (30ml). Iodomethane (190mg, 1.3mmol) and silver oxide (132mg, 0.8mmol) were added. After 18h at 60°C the mixture was filtered and the filtrate was evaporated *in vacuo* to give a brown oil which was purified by flash chromatography on silica gel (eluant: 50% ethyl acetate, 50% pet. ether) to give a white solid identified as (2'*SR*,3'*RS*)-*N*,*N*'-di(benzyloxycarbonyl)-4-(2'-(*tert*-butyloxycarbonylamino)-3'-methoxy-3'-phenylpropanoyl-amino)piperidine-1-carboxamidine (112mg, 0.16mmol, 61%).

6C. (2'*SR*,2"*R*,3'*RS*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(2"-(*tert*-butyloxycarbonylamino)-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-methoxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine

(2'*SR*,3'*RS*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(*tert*-butyloxycarbonylamino)-3'-methoxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine (112mg, 0.16mmol) was dissolved in 4M HCl/dioxan (20ml). After 30min at room temperature the solvent was evaporated *in vacuo* and the residue dissolved in CH_2Cl_2 (30ml). This solution was cooled to 0°C and Boc-DTyr(Et)-OH (50mg, 0.16mmol) was added followed by PyBrop (76mg, 0.16mmol). The pH adjusted to 9 with *N*,*N*-diisopropylethylamine. The mixture was stirred at room temperature for 18 h, after which time the solvent was evaporated *in vacuo* and the residue dissolved in ethyl acetate (70ml). This solution was washed with 0.3M $KHSO_4$ (1 x 20ml), sat. $NaHCO_3$ (1 x 20ml), water (1 x 20ml) and brine (1 x 20ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a white solid which was purified by flash chromatography on silica gel (eluant: 65% chloroform, 15% hexane) to give a white solid identified as (2'*SR*,2"*R*,3'*RS*)-*N*,*N*'-di(benzyloxycarbonyl)-4-(2'-(2"-(*tert*-butyloxycarbonylamino)-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-methoxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine (108mg, 0.12 mmol, 75%).

6D. (2'*S*,2"*R*,3'*R*)-4-(2'-(2"-Amino-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-methoxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine trifluoroacetate

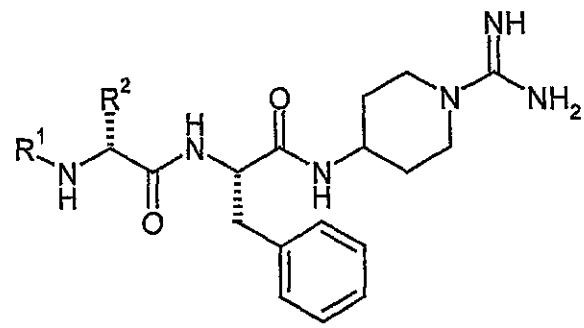
(2'*SR*,2"*R*,3'*RS*)-*N*,*N*'-Di(benzyloxycarbonyl)-4-(2'-(2"-(*tert*-butyloxycarbonylamino)-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-methoxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine (108mg, 0.12mmol) was dissolved in 4M HCl/dioxan (20ml). After 30min at room temperature the solvent was evaporated *in vacuo* and the residue dissolved in

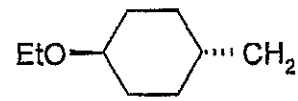
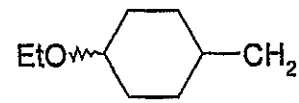
AcOH/water (95:5, 20ml). This solution was hydrogenated over 10% palladium on charcoal. After 2h at room temperature the mixture was filtered through Celite and the residue washed with AcOH/water (9:1, 30ml). The combined filtrates were evaporated *in vacuo* and the residue purified by mplc on Vydac C_{18} (15-25μ) using MeCN/$H_2$0/TFA to give a white solid identified as (2'*S*,2"*R*,3'*R*)-4-(2'-(2"-amino-3"-(4"'-ethoxyphenyl)-propanoylamino)-3'-methoxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine trifluoroacetate (18mg).

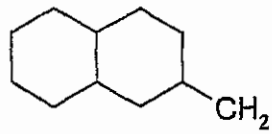
$[M+H]^+ = 511.3$

The following compounds were prepared using analogous methods.

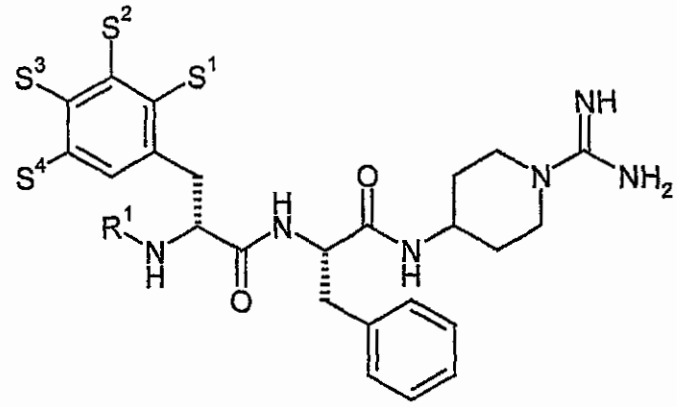
Examples 7 - 17



Ex.	R^1	R^2	m/e
7	H	$(CH_3)_2CHCH_2$	403.3
8	H	c-C_6H_{11}	429.3
9	H	c-$C_6H_{11}CH_2$	443.4
10	H	c-$C_6H_{11}CH_2CH_2$	457.4
11	H		487.4
12	H		487.4

Ex.	R^1	R^2	m/e
13	H		497.4
14	HO_2CCH_2		555
15	MeO_2CCH_2		569
16	H	$PhCH_2CH_2$	451.2
17	H	$PhCH_2OCH_2$	467.2

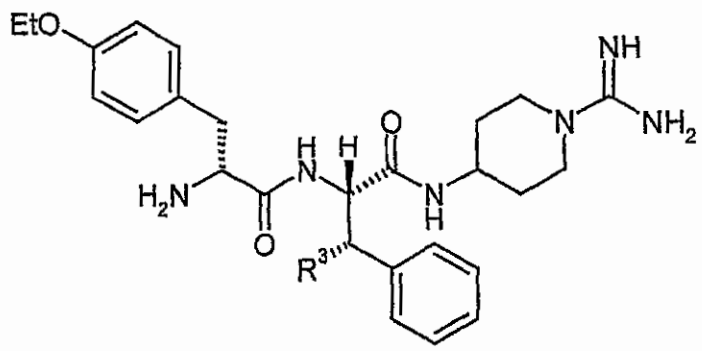
Examples 18 - 52



Ex.	R^1	S^1	S^2	S^3	S^4	m/e
18	H	H	H	H	H	437.3
19	H	H	H	$CH_3CH_2CH_2$	H	479
20	H	H	H	NO_2	H	482.3
21	H	H	H	NH_2	H	452.3
22	H	H	H	I	H	563.1
23	H	H	H	F	H	455.2
24	H	H	H	CN	H	462.3
25	H	H	H	Cl	H	471.6
26	H	H	H	CF_3	H	505.3
27	H	H	H	$NHCOCH_3$	H	494.3
28	H	H	F	H	H	455
29	H	H	Cl	Cl	H	505.1
30	H	Cl	H	Cl	H	505.1
31	H	H	H	OH	H	453.3

Ex.	R^1	S^1	S^2	S^3	S^4	m/e
32	H	H	H	OCH_2Ph	H	543.5
33	H	H	H	$OC(CH_3)_3$	H	509.3
34	H	H	H	$OCH_2CH_2CH_3$	H	495.4
35	H	H	H	OCH_3	H	467.3
36	H	H	H	$OCH(CH_3)_2$	H	495.2
37	H	H	H	OnC_6H_{13}	H	537.3
38	H	H	I	OCH_2CH_3	I	733.1
39	CH_3	H	H	OCH_2CH_3	H	493.3
40	CH_3SO_2	H	H	OCH_2CH_3	H	559.3
41	$CH_3CH_2SO_2$	H	H	OCH_2CH_3	H	573.3
42	$PhSO_2$	H	H	OCH_2CH_3	H	621
43	CH_3CO	H	H	OCH_2CH_3	H	523.3
44	$CH_3CH_2CH_2CO$	H	H	OCH_2CH_3	H	551
45	CH_3CH_2CO	H	H	OCH_2CH_3	H	537
46	$PhCH_2OCO$	H	H	OCH_2CH_3	H	615
47	MeO_2CCH_2	H	H	OCH_2CH_3	H	553
48	HO_2CCH_2	H	H	OCH_2CH_3	H	539
49	H	-CH=CH-CH=CH-		H	H	487.4
50	H	H	-CH=CH-CH=CH-		H	487.3
51	$-CH_2-$		H	H	H	449.3
52	$-CH_2-$		H	OCH_2CH_3	H	493.3

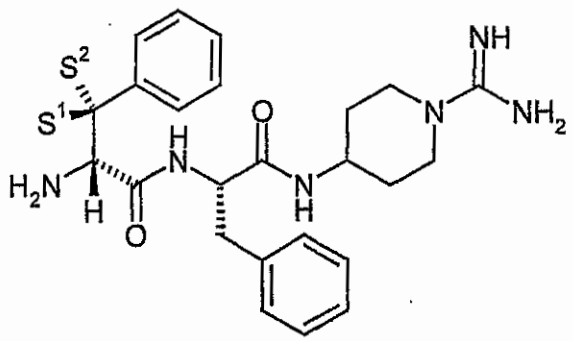
Examples 53 - 54



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Ex.	R^3	m/e
53	OH	497.4
54	OMe	511.3

Examples 55 - 58



Ex.	S^1	S^2	m/e
55	H	OH	453.3
56	H	OMe	467.3
57	OH	H	453.3
58	OMe	H	467.3

Example 59

Determination of the inhibition constant K_i for plasma kallikrein

Inhibition of plasma kallikrein activity *in vitro* was determined using standard published methods (see e.g. Johansen *et al.*, Int. J. Tiss. Reac. 1986, **8**, 185; Shori *et al.*, Biochem. Pharmacol., 1992, **43**, 1209; Stürzebecher *et al.*, Biol. Chem. Hoppe-Seyler, 1992, **373**, 1025). Human plasma kallikrein (Callbiochem) was incubated at 37°C with three different concentrations of the chromogenic substrate S-2302 (Chromogenix AB) and various concentrations of the test compound. Residual enzyme activity (initial rate of reaction) was determined by measuring the change in optical absorbance at 405nm and the inhibitory constant K_i for the test compound as determined from a Dixon plot (Dixon, Biochem. J., 1953, **55**, 170). Typical results are presented in the Table.

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Compound of Example No	K_i (nM)	Compound of Example No	K_i (nM)
1	4.5	34	7.0
2	66.0	39	15.0
3	3.0	40	9.0
4	1.4	42	4.5
5	6.6	43	7.3
6	25.0	44	4.1
14	2.6	45	3.0
15	19.0	52	18.0
32	5.5		

Example 60

Determination of enzyme selectivity

Selected compounds were further screened for inhibitory activity against other trypsin-like proteases following the method of Example 59 and using the appropriate enzyme and chromogenic substrate (Chromogenix AB). Representative results are presented in the Table. The selectivity is given by:

Selectivity = (K_i for test enzyme) / (K_i for plasma kallikrein)

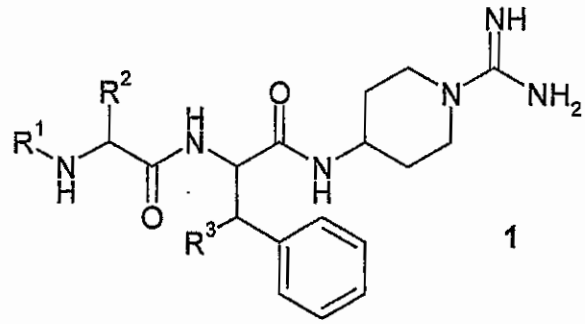
Enzyme	Substrate	Compound of Example No	K_i (nM)	Selectivity
Human Tissue Kallikrein	S-2266	1	45,000	10,000
		3	6,000	2,000
		4	18,500	13,000
		5	>70,000	>10,000
		39	14,000	930
		40	1,950	210
		42	6,800	1,500
		43	69,000	9,400
		45	49,000	16,000
Thrombin	S-2238	3	310	100
		40	16,500	1,800

Enzyme	Substrate	Compound of Example No	K_i (nM)	Selectivity
Plasmin	S-2390	**3**	3,200	1,000
		40	1,440	160
Trypsin	S-2222	**3**	825	270
		40	3,500	380

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CLAIMS

1 A compound according to general formula **1**, or a pharmaceutically acceptable salt thereof,



1

wherein

R^1 is selected from H, lower alkyl, R^4-CO, R^4-O_2CCH_2, R^5-OCO and R^5-SO_2;

R^2 is selected from lower alkyl, cycloalkyl optionally substituted with an alkyl or alkyloxy group, (C_5-C_{12})cycloalkylalkyl optionally substituted with an alkyl or alkyloxy group, aralkyl optionally substituted with up to three groups chosen from F, Cl, Br, I, OH, lower alkyl, O-(lower alkyl), O-benzyl, NH_2, NO_2, NH-acyl, CN and CF_3, and aralkyloxymethyl optionally substituted with up to three groups chosen from F, Cl, Br, OH, lower alkyl and O-(lower alkyl); or

R^1 and R^2 together are an *o*-xylylene group optionally substituted on the aromatic ring with a group selected from F, Cl, Br, OH, lower alkyl and O-(lower alkyl);

R^3 is selected from H, OH and O-lower alkyl;

R^4 is selected from H, lower alkyl and phenyl; and

R^5 is selected from lower alkyl, phenyl and benzyl.

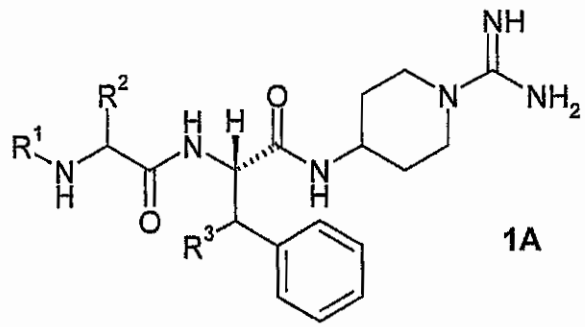
2 A compound according to Claim 1 wherein R^1 is selected from H, lower alkyl, and R^4-O_2CCH_2.

3 A compound according to either of Claims 1 and 2 wherein R^2 is selected from (C_6-C_{10})cycloalkylmethyl, benzyl optionally substituted with up to three groups chosen from F, Cl, Br, OH, lower alkyl and O-(lower alkyl), phenethyl optionally substituted with up to three groups chosen from F, Cl, Br, OH, lower alkyl and O-(lower alkyl)and benzyloxymethyl optionally substituted with up to three groups chosen from F, Cl, Br, OH, lower alkyl and O-(lower alkyl).

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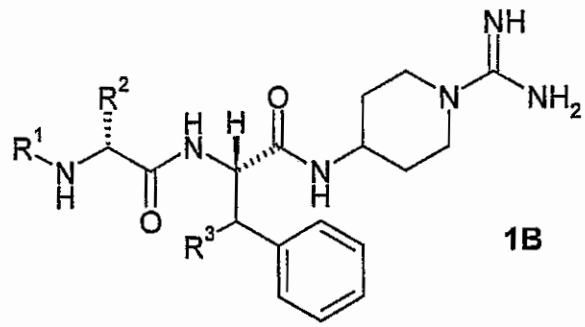
4 A compound according to any of Claims 1 to 3 wherein R^2 is selected from cyclohexylmethyl, decahydronaphth-2-ylmethyl, benzyl, 4-fluorobenzyl, 4-chlorobenzyl, 4-hydroxybenzyl, 4-(lower alkyl)oxybenzyl, α-hydroxybenzyl, α-methoxybenzyl, phenethyl and benzyloxymethyl.

5 A compound according to any of Claims 1 to 4 wherein the absolute stereochemistry is as depicted in general formula **1A**.



1A

6 A compound according to any of Claims 1 to 5 wherein the absolute stereochemistry is as depicted in general formula **1B**.



1B

7 A compound according to any of Claims 1 to 6 selected from

(2'*S*,2"*R*)-4-(2'-(2"-amino-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-phenyl-propanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-carboxymethylamino-3"-(4"'-ethoxyphenyl)propanoyl-amino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(3"-(4"'-ethoxyphenyl)-2"-(methyloxycarbonylmethylamino)-propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-amino-3"-cyclohexylpropanoylamino)-3'-phenylpropanoyl-amino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-carboxymethylamino-3"-cyclohexylpropanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(3"-cyclohexyl-2"-(methyloxycarbonylmethylamino)propanoyl-amino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-amino-3"-phenylpropanoylamino)-3'-phenylpropanoyl-amino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-carboxymethylamino-3"-phenylpropanoylamino)-3'-phenyl-propanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-(methyloxycarbonylmethylamino)-3"-phenylpropanoyl-amino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-amino-3"-decahydronaphth-2"'-ylpropanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(2"-carboxymethylamino-3"-decahydronaphth-2"'-yl-propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*)-4-(2'-(3"-decahydronaphth-2"'-yl-2"-(methyloxycarbonylmethyl-amino)propanoylamino)-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*,3'*R*)-4-(2'-(2"-amino-3"-cyclohexylpropanoylamino)-3'-hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*,3'*R*)-4-(2'-(2"-carboxymethylamino-3"-cyclohexylpropanoylamino)-3'-

hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*,3'*R*)-4-(2'-(3"-cyclohexyl-2"-(methyloxycarbonylmethylamino)-propanoylamino)-3'-hydroxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

(2'*S*,2"*R*,3'*R*)-4-(2'-(2"-amino-3"-(4"'-ethoxyphenyl)propanoylamino)-3'-methoxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine;

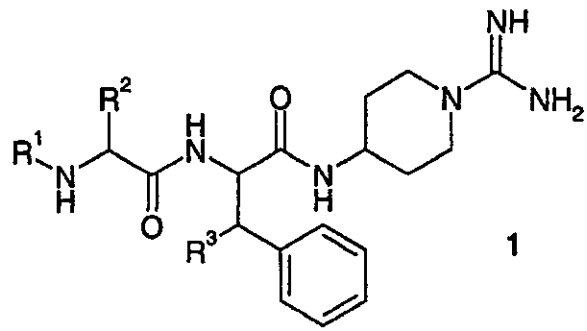
(2'*S*,2"*R*,3'*R*)-4-(2'-(2"-carboxymethylamino-3"-(4"'-ethoxyphenyl)propanoyl-amino)-3'-methoxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine; and

(2'*S*,2"*R*,3'*R*)-4-(2'-(3"-(4"'-ethoxyphenyl)-2"-(methyloxycarbonylmethylamino)-propanoylamino)-3'-methoxy-3'-phenylpropanoylamino)piperidine-1-carboxamidine

or a pharmaceutically acceptable salt thereof.

8 A pharmaceutical composition comprising a compound according to any of Claims 1 to 7 or a pharmaceutically acceptable salt thereof.

9 Use of a compound of general formula 1, or a pharmaceutically acceptable salt thereof,



1

wherein

R^1 is selected from H, lower alkyl, R^4-CO, R^4-O_2CCH_2, R^5-OCO and R^5-SO_2;

R^2 is selected from lower alkyl, cycloalkyl optionally substituted with an alkyl or alkyloxy group, (C_5-C_{12})cycloalkylalkyl optionally substituted with an alkyl or alkyloxy group, aralkyl optionally substituted with up to three groups chosen

from F, Cl, Br, I, OH, lower alkyl, O-(lower alkyl), O-benzyl, NH_2, NO_2, NH-acyl, CN and CF_3, and aralkyloxymethyl optionally substituted with up to three groups chosen from F, Cl, Br, OH, lower alkyl and O-(lower alkyl); or
R^1 and R^2 together are an *o*-xylylene group optionally substituted on the aromatic ring with a group selected from F, Cl, Br, OH, lower alkyl and O-(lower alkyl);
R^3 is selected from H, OH and O-lower alkyl;
R^4 is selected from H, lower alkyl and phenyl; and
R^5 is selected from lower alkyl, phenyl and benzyl; for the manufacture of a pharmaceutical for the treatment of a disease condition for which over activity of plasma kallikrein is a causative factor.

10 A use according to claim 9 for the treatment of a disease selected among inflammatory bowel disease, arthritis, inflammation, septic shock, hypotension, cancer, adult respiratory distress syndrome, disseminated intravascular coagulation, cardiopulmonary bypass surgery and bleeding from post-operative surgery.

11 The use, in the manufacture of a medicament for treatment of disease in a human or animal, of a compound according to any of claims 1 to 7.

12 A pharmaceutical composition according to claim 8 for the treatment of a disease condition for which over activity of plasma kallikrein is a causative factor.