3-FLUORO-PYRROLIDINES AS ANTIDIABETIC AGENTS

The present invention relates to novel compounds that are inhibitors of dipeptidyl peptidase IV or prodrugs thereof. The compounds are useful in the treatment of, *inter alia*, type 2 diabetes and impaired glucose tolerance.

BACKGROUND

The enzyme dipeptidyl peptidase IV, herein abbreviated DP-IV (and elsewhere as DAP-IV or DPP-IV) and also known by the classification EC.3.4.14.5, is a serine protease that cleaves the N-terminal dipeptide from peptides that begin with the sequence H-Xaa-Pro (where Xaa is any amino acid, although preferably a lipophilic one, and Pro is proline). It will also accept as substrates peptides that begin with the sequence H-Xaa-Ala (where Ala is alanine). DP-IV was first identified as a membrane-bound protein. More recently a soluble form has been identified.

Initial interest in DP-IV focussed on its role in the activation of T lymphocytes. DP-IV is identical to the T cell protein CD26. It was proposed that inhibitors of DP-IV would be capable of modulating T cell responsiveness, and so could be developed as novel immunomodulators. It was further suggested that CD26 was a necessary co-receptor for HIV, and thus that DP-IV inhibitors could be useful in the treatment of AIDS.

Attention was given to the role of DP-IV outside the immune system. It was recognised that DP-IV has a key role in the degradation of several peptide hormones, including growth hormone releasing hormone (GHRH) and glucagon-like peptide-1 and –2 (GLP-1 and GLP-2). Since GLP-1 is known to have a potentiating effect on the action of insulin in the control of post-prandial blood glucose levels it is clear that DP-IV inhibitors might also be usefully employed in the treatment of type II diabetes and impaired glucose tolerance. At least two DP-IV inhibitors are currently undergoing clinical trials to explore this possibility.

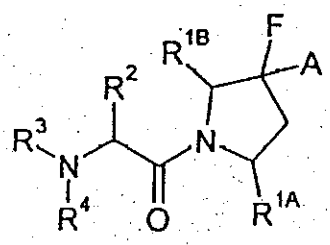
Several groups have disclosed inhibitors of DP-IV. While some leads have been found from random screening programs, the majority of the work in this field has been directed towards the investigation of substrate analogs. Inhibitors of DP-IV that are substrate analogs are disclosed in, for example, US 5,462,928, US 5,543,396, WO95/15309

(equivalent to US 5,939,560 and EP 0731789), WO98/19998 (equivalent to US 6,011,155), WO99/46272 and WO99/61431. The most potent inhibitors are aminoacyl pyrrolidine boronic acids, but these are unstable and tend to cyclise, while the more stable pyrrolidine and thiazolidine derivatives have a lower affinity for the enzyme and so would require large doses in a clinical situation. Pyrrolidine nitriles appear to offer a good compromise since they have both a high affinity for the enzyme and a reasonably long half-life in solution as the free base. There remains, however, a need for inhibitors of DP-IV with improved properties.

SUMMARY OF THE INVENTION

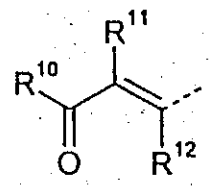
Disclosed herein is a series of inhibitors of DP-IV with improved affinity for the enzyme and prodrugs thereof. The compounds can be used for the treatment of a number of human diseases, including impaired glucose tolerance and type II diabetes.

Also disclosed herein are the use of the compounds in the preparation of pharmaceutical compositions, such compositions *per* se, and the use of such compositions in human therapy. In one embodiment, the compounds are described by general formula 1.



1

In this general formula A is F or H; one of R^{1A} and R^{1B} is selected from H and CN and the other is H; R^2 is selected from H, $C_1 - C_8$ alkyl, optionally substituted phenyl, optionally substituted benzyl and R^5; R^3 is selected from H, R^6OCO, $H_2NCH(R^7)CO$, $H_2NCH(R^8)CONHCH(R^9)CO$, and a group according to general formula 2;



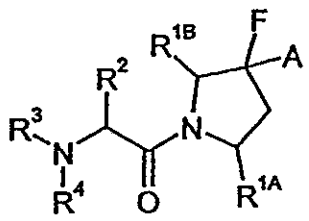
2

R^4 is selected from H, $C_1 - C_8$ alkyl, adamantyl, adamantylmethyl, adamantylethyl and $Het\text{-}NH(CH_2)_a$; or R^2 and R^4 together constitute a chain of three or four methylene groups

so as to form, together with the atoms to which they are attached, a pyrrolidine or piperidine ring, which ring may further be fused with a benzenoid ring; R^5 is selected from CH_2R^{13}, $CH_2CH_2R^{13}$ and $C(R^{14})(R^{15})$-X^1-R^{16}; R^6 is selected from C_1 – C_6 alkyl, optionally substituted phenyl, optionally substituted benzyl and $R^{17}CO_2C(R^{18})(R^{19})$; R^7, R^8 and R^9 are each independently selected from the side chains of the proteinaceous amino acids; R^{10} is selected from C_1 – C_8 alkyl, phenyl and O-(C_1 – C_8 alkyl); R^{11} is selected from H and C_1 – C_8 alkyl; R^{12} is selected from H, C_1 – C_8 alkyl and phenyl; R^{13} is selected from CO-$N(R^{20})(R^{21})$, $N(R^{22})$-$C(=X^2)R^{23}$ and $N(R^{22})(R^{24})$; R^{14} and R^{15} are independently selected from H and methyl, or together are –$(CH_2)_z$–; R^{16} is selected from C_1 – C_8 alkyl, optionally substituted phenyl, optionally substituted benzyl and –$(CH_2)_b$-R^{13}; R^{17} is selected from H and C_1 – C_8 alkyl; R^{18} and R^{19} are independently selected from H and C_1 – C_8 alkyl, or together are –$(CH_2)_y$–; R^{20} and R^{21} are independently selected from H, C_1 – C_8 alkyl, optionally substituted phenyl, optionally substituted phenylalkyl, Het and –$(CH_2)_c$Het; or R^{20} and R^{21} together constitute a chain of four or five methylene groups so as to form, together with the nitrogen atom to which they are attached, a pyrrolidine or piperidine ring, which ring may further be fused with a benzenoid ring; R^{22} is selected from H and methyl; R^{23} is selected from R^{25}, O-R^{25} and $N(R^{26})(R^{27})$; R^{24} is selected from optionally substituted phenyl, Het and –CH_2-Het; R^{25} is selected from C_1 – C_8 alkyl, optionally substituted phenyl, optionally substituted phenylalkyl, Het and –$(CH_2)_c$Het; R^{26} and R^{27} are independently selected from H, C_1 – C_8 alkyl, optionally substituted phenyl, optionally substituted phenylalkyl, Het and –$(CH_2)_c$Het, or R^{26} and R^{27} together constitute a chain of four or five methylene groups so as to form, together with the nitrogen atom to which they are attached, a pyrrolidine or piperidine ring, which ring may further be fused with a benzenoid ring; Het is an optionally substituted aromatic nitrogen-containing heterocycle or benz-fused analogue thereof; X^1 is selected from –O–, -S- and –CH_2–; X^2 is selected from O and S; a is 2 or 3; b is 1, 2 or 3; c is 1 or 2; and y and z are 2, 3 or 4.

In one aspect, there is provided

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



1

wherein:

A is F or H;

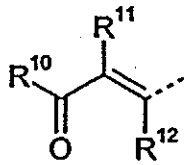
one of R^{1A} and R^{1B} is selected from H and CN and the other is H;

R^2 is selected from H, C_1 – C_8 alkyl, optionally substituted phenyl, optionally substituted benzyl and R^5; and

R^3 is selected from H, C_1 – C_8 alkyl, adamantyl, adamantylmethyl, adamantylethyl and Het-$NH(CH_2)_a$; or

R^2 and R^3 together constitute a chain of three or four methylene groups so as to form, together with the atoms to which they are attached, a pyrrolidine or piperidine ring, which ring may further be fused with a benzenoid ring;

R^4 is selected from H, R^6OCO, $H_2NCH(R^7)CO$, $H_2NCH(R^8)CONHCH(R^9)CO$, and a group according to general formula **2**;



2

R^5 is selected from CH_2R^{13}, $CH_2CH_2R^{13}$ and $C(R^{14})(R^{15})$-X^1-R^{16};

R^6 is selected from C_1 – C_6 alkyl, optionally substituted phenyl, optionally substituted

benzyl and $R^{17}CO_2C(R^{18})(R^{19})$;

R^7, R^8 and R^9 are each independently selected from the side chains of the proteinaceous amino acids;

R^{10} is selected from $C_1 - C_8$ alkyl, phenyl and O-($C_1 - C_8$ alkyl);

R^{11} is selected from H and $C_1 - C_8$ alkyl;

R^{12} is selected from H, $C_1 - C_8$ alkyl and phenyl;

R^{13} is selected from CO-N(R^{20})(R^{21}), N(R^{22})-C(=X^2)R^{23} and N(R^{22})(R^{24});

R^{14} and R^{15} are independently selected from H and methyl, or together are $-(CH_2)_z-$;

R^{16} is selected from $C_1 - C_8$ alkyl, optionally substituted phenyl, optionally substituted benzyl and $-(CH_2)_b$-R^{13};

R^{17} is selected from H and $C_1 - C_8$ alkyl;

R^{18} and R^{19} are independently selected from H and $C_1 - C_8$ alkyl, or together are $-(CH_2)_y-$;

R^{20} and R^{21} are independently selected from H, $C_1 - C_8$ alkyl, optionally substituted phenyl, optionally substituted phenylalkyl, Het and $-(CH_2)_c$Het, or R^{20} and R^{21} together constitute a chain of four or five methylene groups so as to form, together with the nitrogen atom to which they are attached, a pyrrolidine or piperidine ring, which ring may further be fused with a benzenoid ring;

R^{22} is selected from H and methyl;

R^{23} is selected from R^{25}, O-R^{25} and N(R^{26})(R^{27});

R^{24} is selected from optionally substituted phenyl, Het and $-CH_2$-Het;

R^{25} is selected from $C_1 - C_8$ alkyl, optionally substituted phenyl, optionally substituted phenylalkyl, Het and $-(CH_2)_cHet$;

R^{26} and R^{27} are independently selected from H, $C_1 - C_8$ alkyl, optionally substituted phenyl, optionally substituted phenylalkyl, Het and $-(CH_2)_cHet$, or R^{26} and R^{27} together constitute a chain of four or five methylene groups so as to form, together with the nitrogen atom to which they are attached, a pyrrolidine or piperidine ring, which ring may further be fused with a benzenoid ring;

Het is an aromatic nitrogen-containing heterocycle selected from pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, imidazolyl, pyrazolyl, thiazolyl, isothiazolyl, oxazolyl, isoxazolyl and benz-fused analogues of these, all of which may optionally be substituted on one or more carbon atoms, and where the substituents are selected from lower alkyl, hydroxy, lower alkyloxy, amino, lower alkylamino, di(lower alkyl)amino, fluoro, chloro, bromo, trifluoromethyl, nitro, cyano, carboxy and lower alkyloxycarbonyl groups;

X^1 is selected from –O-, -S- and $-CH_2-$;

X^2 is selected from O and S;

a is 2 or 3;

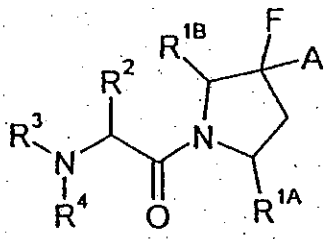
b is 1, 2 or 3;

c is 1 or 2; and

y and z are 2, 3 or 4; with the proviso that the compound is not Ile-3-fluoropyrrolidine.

DETAILED DESCRIPTION OF THE INVENTION

In a first aspect, the present invention comprises a series of novel compounds that are inhibitors of the enzyme DP-IV or prodrugs thereof and are useful for the treatment of certain human diseases. The compounds are described by general formula 1.



1

In general formula 1, the atom A may be either hydrogen (H) or fluorine (F). Preferably it is F. One of R^{1A} and R^{1B} may be a nitrile group (CN) and the other H. Alternatively both R^{1A} and R^{1B} may be H. In one preferred embodiment of the invention both R^{1A} and R^{1B} are H. In another preferred embodiment of the invention R^{1A} is CN and R^{1B} is H.

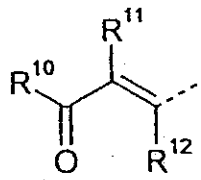
In one particularly preferred embodiment, A is F and both R^{1A} and R^{1B} are H. In another particularly preferred embodiment A is F, R^{1A} is CN and R^{1B} is H.

In one embodiment of the present invention R^2 is a group selected from H, $C_1 - C_8$ alkyl groups, an optionally substituted phenyl residue, an optionally substituted benzyl group and groups according to R^5. Suitable optional substituents on the phenyl residue or the benzyl group are lower alkyl groups, lower alkyloxy groups, halogen atoms selected from fluorine and chlorine atoms, hydroxyl groups, amino groups selected from NH_2, NH–(lower alkyl) and N(lower alkyl)$_2$, nitrile groups, nitro groups, CO_2H, CO_2–(lower alkyl), $CONH_2$, CONH–(lower alkyl) and CON(lower alkyl)$_2$. The phenyl residue or benzyl group may have up to three substituents, which may all be the same or may be different. In this embodiment, R^3 is a group selected from H, $C_1 - C_8$ alkyl groups, adamantyl, adamantylmethyl, adamantylethyl and a group according to Het-NH$(CH_2)_a$, where a is 2 or 3.

In a second embodiment of the present invention, R^2 and R^3 together constitute a chain of three or four methylene groups so as to form, together with the atoms to which they are attached, a pyrrolidine or piperidine ring. This ring may further be fused with a benzenoid ring so as to form an indoline, isoindoline, tetrahydroquinoline or tetrahydroisoquinoline moiety.

For those compounds according to the present invention that are direct inhibitors of DP-IV, R^4 is H. For those compounds according to the present invention that are prodrugs of these direct inhibitors, R^4 is selected from a group according to R^6OCO, a group

according to $H_2NCH(R^7)CO$, a group according to $H_2NCH(R^8)CONHCH(R^9)CO$, and a group according to general formula 2.



2

These prodrugs are converted into the corresponding direct inhibitors of DP-IV after administration to the patient.

The group R^5 is selected from a group according to CH_2R^{13}, a group according to $CH_2CH_2R^{13}$ and a group according to $C(R^{14})(R^{15})$-X^1-R^{16}, where X^1 is selected from –O–, –S– and $–CH_2–$.

The group R^6 is selected from C_1 – C_6 alkyl groups, an optionally substituted phenyl or benzyl group and a group according to $R^{17}CO_2C(R^{18})(R^{19})$. Suitable substituents on the phenyl or benzyl group are lower alkyl groups, lower alkyloxy groups, halogen atoms selected from fluorine and chlorine atoms, hydroxyl groups, amino groups selected from NH_2, NH–(lower alkyl) and N(lower alkyl)$_2$, nitrile groups, nitro groups, CO_2H, CO_2–(lower alkyl), $CONH_2$, CONH–(lower alkyl) and CON(lower alkyl)$_2$. The phenyl or benzyl group may have up to two substituents, which may be the same or different.

The groups R^7, R^8 and R^9 are each independently selected from the side chains of the proteinaceous amino acids. These amino acids and their side chains are enumerated in the Table below.

Alanine	$-CH_3$	Leucine	$-CH_2CH(CH_3)_2$
Arginine	$-(CH_2)_3NHC(=NH)NH_2$	Lysine	$-(CH_2)_4NH_2$
Asparagine	$-CH_2CONH_2$	Methionine	$-(CH_2)_2SCH_3$
Aspartic acid	$-CH_2CO_2H$	Phenylalanine	$-CH_2C_6H_5$
Cysteine	$-CH_2SH$	Serine	$-CH_2OH$
Glycine	$-H$	Threonine	$-CH(CH_3)OH$
Glutamic acid	$-(CH_2)_2CO_2H$	Tryptophan	$-CH_2C_8H_6N$
Glutamine	$-(CH_2)_2CONH_2$	Tyrosine	$-CH_2C_6H_4OH$
Histidine	$-CH_2C_3H_3N_2$	Valine	$-CH(CH_3)_2$
Isoleucine	$-CH(CH_3)CH_2CH_3$		

In general formula **2**, the group R^{10} is selected from $C_1 - C_8$ alkyl groups, phenyl and O-($C_1 - C_8$ alkyl) groups, the group R^{11} is selected from H and $C_1 - C_8$ alkyl groups, and the group R^{12} is selected from H, $C_1 - C_8$ alkyl groups and phenyl.

The group R^{13} is selected from a group according to $CO\text{-}N(R^{20})(R^{21})$, a group according to $N(R^{22})\text{-}C(=X^2)R^{23}$, where X^2 is selected from O and S, and a group according to $N(R^{22})(R^{24})$.

The groups R^{14} and R^{15} are independently selected from H and methyl, or together are $-(CH_2)_z-$, where z is 2, 3 or 4, so as to form, together with the carbon atom to which they are attached, a cyclopropane, cyclobutane or cyclopentane ring.

The group R^{16} is selected from $C_1 - C_8$ alkyl groups, an optionally substituted phenyl group, an optionally substituted benzyl group and groups according to $-(CH_2)_b\text{-}R^{13}$, where b is 1, 2 or 3. Suitable substituents on the phenyl or benzyl group are lower alkyl groups, lower alkyloxy groups, halogen atoms selected from fluorine and chlorine atoms, hydroxyl groups, amino groups selected from NH_2, NH–(lower alkyl) and N(lower alkyl)$_2$, nitrile groups, nitro groups, CO_2H, CO_2–(lower alkyl), $CONH_2$, CONH–(lower alkyl) and CON(lower alkyl)$_2$. The phenyl or benzyl group may have up to two substituents, which may be the same or different.

The group R^{17} is selected from H and $C_1 - C_8$ alkyl groups. The groups R^{18} and R^{19} are independently selected from H and $C_1 - C_8$ alkyl groups, or together are $-(CH_2)_y-$, where

y is 2, 3 or 4, so as to form, together with the carbon atom to which they are attached, a cyclopropane, cyclobutane or cyclopentane ring

The groups R^{20} and R^{21} may independently be selected from H, $C_1 - C_8$ alkyl groups, an optionally substituted phenyl group, an optionally substituted phenylalkyl group, a group according to Het and a group according to $-(CH_2)_c$Het, where c is 1 or 2. Suitable substituents on the phenyl or phenylalkyl group are lower alkyl groups, lower alkyloxy groups, halogen atoms selected from fluorine and chlorine atoms, hydroxyl groups, amino groups selected from NH_2, NH–(lower alkyl) and N(lower alkyl)$_2$, nitrile groups, nitro groups, CO_2H, CO_2–(lower alkyl), $CONH_2$, CONH–(lower alkyl) and CON(lower alkyl)$_2$. The phenyl or phenylalkyl group may have up to two substituents, which may be the same or different. Alternatively, the groups R^{20} and R^{21} may together constitute a chain of four or five methylene groups so as to form, together with the nitrogen atom to which they are attached, a pyrrolidine or piperidine ring, which ring may further be fused with a benzenoid ring so as to form an indoline, isoindoline, tetrahydroquinoline or tetrahydroisoquinoline moiety.

The group R^{22} is selected from H and methyl. The group R^{23} is selected from a group according to R^{25}, a group according to O-R^{25} and a group according to N(R^{26})(R^{27}),. The group R^{24} is selected from an optionally substituted phenyl group, a group according to Het and a group according to $-CH_2$-Het. Suitable substituents on the phenyl group are lower alkyl groups, lower alkyloxy groups, halogen atoms selected from fluorine and chlorine atoms, hydroxyl groups, amino groups selected from NH_2, NH–(lower alkyl) and N(lower alkyl)$_2$, nitrile groups, nitro groups, CO_2H, CO_2–(lower alkyl), $CONH_2$, CONH–(lower alkyl) and CON(lower alkyl)$_2$. The phenyl group may have up to two substituents, which may be the same or different

The group R^{25} is selected from $C_1 - C_8$ alkyl groups, an optionally substituted phenyl group, an optionally substituted phenylalkyl group, a group according to Het and a group according to $-(CH_2)_c$Het. Suitable substituents on the phenyl or phenylalkyl group are lower alkyl groups, lower alkyloxy groups, halogen atoms selected from fluorine and chlorine atoms, hydroxyl groups, amino groups selected from NH_2, NH–(lower alkyl) and N(lower alkyl)$_2$, nitrile groups, nitro groups, CO_2H, CO_2–(lower alkyl), $CONH_2$, CONH–(lower alkyl) and CON(lower alkyl)$_2$. The phenyl or phenylalkyl group may have up to two substituents, which may be the same or different

The groups R^{26} and R^{27} may independently be selected from H, $C_1 - C_8$ alkyl groups, an optionally substituted phenyl group, an optionally substituted phenylalkyl group, a group according to Het and a group according to $-(CH_2)_c$Het. Suitable substituents on the phenyl or phenylalkyl group are lower alkyl groups, lower alkyloxy groups, halogen atoms selected from fluorine and chlorine atoms, hydroxyl groups, amino groups selected from NH_2, NH–(lower alkyl) and N(lower alkyl)$_2$, nitrile groups, nitro groups, CO_2H, CO_2–(lower alkyl), $CONH_2$, CONH–(lower alkyl) and CON(lower alkyl)$_2$. The phenyl or phenylalkyl group may have up to two substituents, which may be the same or different. Alternatively R^{26} and R^{27} may together constitute a chain of four or five methylene groups so as to form, together with the nitrogen atom to which they are attached, a pyrrolidine or piperidine ring, which ring may further be fused with a benzenoid ring so as to form an indoline, isoindoline, tetrahydroquinoline or tetrahydroisoquinoline moiety.

Het is an aromatic nitrogen-containing heterocyclic group selected from pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, imidazolyl, pyrazolyl, thiazolyl, isothiazolyl, oxazolyl, isoxazolyl and benz-fused analogues of these, such as for example quinolinyl, isoquinolinyl, quinoxallnyl, benzimidazolyl and the like, all of which may optionally be substituted on one or more carbon atoms, and where the substituents are selected from lower alkyl, hydroxy, lower alkyloxy, amino, lower alkylamino, di(lower alkyl)amino, fluoro, chloro, bromo, trifluoromethyl, nitro, cyano, carboxy and lower alkyloxycarbonyl groups;

In the context of the present document, the term "alkyl group", either by itself or in combinations such as "alkyloxy", includes linear, branched and cyclic saturated hydrocarbon groups. Examples of C_1 - C_8 alkyl groups include methyl, ethyl, propyl, *n*-octyl, 2,2,4-trimethylpentyl and bicyclo[2.2.2]octyl groups. Lower alkyl groups are alkyl groups with up to four carbon atoms, i.e. $C_1 - C_4$ alkyl groups such as methyl, ethyl, propyl, isopropyl, cyclopropyl, butyl, isobutyl, *tert*-butyl and cyclobutyl groups. The term "phenylalkyl group" includes lower alkyl groups with a phenyl substituent. Examples of phenylalkyl groups include benzyl, phenethyl, α-methylbenzyl and 4-phenylbutyl groups.

The compounds of general formula 1 may have one or more stereogenic centres and so can exhibit optical isomerism. All such isomers, including enantiomers, diastereomers and epimers are included within the scope of the invention. Furthermore, the invention

includes such compounds as single isomers and as mixtures, including racemates. Certain compounds according to general formula 1, including those with a heteroaryl group which carries a hydroxy or amino substituent, can exist as tautomers. These tautomers, either separately or as mixtures, are also considered to be within the scope of the invention.

The compounds according to general formula 1 wherein R^4 is H have at least one basic functional group. They can therefore form addition salts with acids. Other compounds according to general formula 1 wherein R^4 is not H may also have a basic functional group and so be able to form addition salts. Insofar as these addition salts are formed with pharmaceutically acceptable acids, they are included within the scope of the invention. Examples of suitable acids include acetic acid, trifluoroacetic acid, citric acid, fumaric acid, benzoic acid, pamoic acid, methanesulphonic acid, hydrochloric acid, nitric acid, sulphuric acid, phosphoric acid and the like.

Certain compounds according to general formula 1 have an acidic group and so are able to form salts with bases. Examples of such salts include the sodium, potassium and calcium salts, which are formed by the reaction of the acid with the corresponding metal hydroxide, oxide, carbonate or bicarbonate. Similarly, tetra-alkyl ammonium salts may be formed by the reaction of the acid with a tetra-alkyl ammonium hydroxide. Primary, secondary and tertiary amines, such as triethylamine, can form addition salts with the acid. A particular case of this would be an internal addition salt formed between an acidic group and the primary amine group of the same molecule, which is also called a zwitterion. Insofar as they are pharmaceutically acceptable, all these salts are included within the scope of the invention.

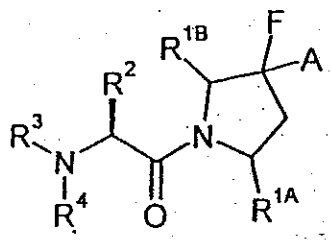
It is generally preferred that R^2 and R^3 should not both be H. In embodiments of the invention wherein R^2 is H, R^3 is preferably selected from adamantyl, adamantylmethyl, adamantylethyl and groups according to Het-$(CH_2)_a$-. More preferably it is a group according to Het-$(CH_2)_a$-, and most preferably it is such a group wherein a is 2 and Het is a 5-substituted-2-pyridyl moiety.

More preferred are those embodiments of the invention wherein R^3 is H and R^2 is selected from $C_1 - C_8$ alkyl groups, an optionally substituted phenyl residue, an optionally substituted benzyl group and groups according to R^5.

One particularly preferred embodiment of the invention is a compound wherein R^3 is H and R^2 is a $C_1 - C_8$ alkyl group.

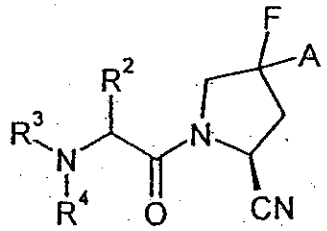
Another particularly preferred embodiment is a compound wherein R^3 is H and R^2 is a group according to R^5. More preferred still are those compounds wherein R^5 is either $CH_2CH_2R^{13}$ or $C(R^{14})(R^{15})$-X^1-R^{16}. Preferred compounds with R^5 as $CH_2CH_2R^{13}$ are those wherein R^{13} is CO-$N(R^{20})(R^{21})$. Preferred compounds with R^5 as $C(R^{14})(R^{15})$-X^1-R^{16} are those wherein R^{14} and R^{15} are either H or methyl and R^{16} is $-(CH_2)_b$-R^{13}, particularly those wherein R^{14} and R^{15} are both H, X^1 is CH_2 and b is 1 or 2, more particularly those wherein R^{13} is either $N(R^{22})$-$C(=X2)R^{23}$ or $N(R^{22})(R^{24})$, more particularly still those wherein R^{13} is $N(R^{22})$-$C(=X2)R^{23}$, R^{22} is H and X^2 is O, and most particularly those wherein R^{23} is Het.

Another preferred embodiment of the present invention is a compound according to general formula **1** wherein R^2 is other than H and the absolute stereochemistry is as shown in general formula **3**. In the conventional system of nomenclature this is the '*S*' configuration, except where R^2 is R^5, R^5 is $C(R^{14})(R^{15})$-X^1-R^{16} and X^1 is S, in which case it is the '*R*' configuration.



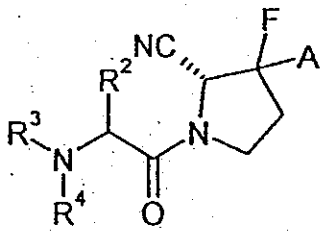
3

Another preferred embodiment of the present invention is a compound according to general formula **1** wherein R^{1A} is CN, R^{1B} is H and the absolute stereochemistry is as shown in general formula **4**. In the conventional system of nomenclature this is the '*S*' configuration.



4

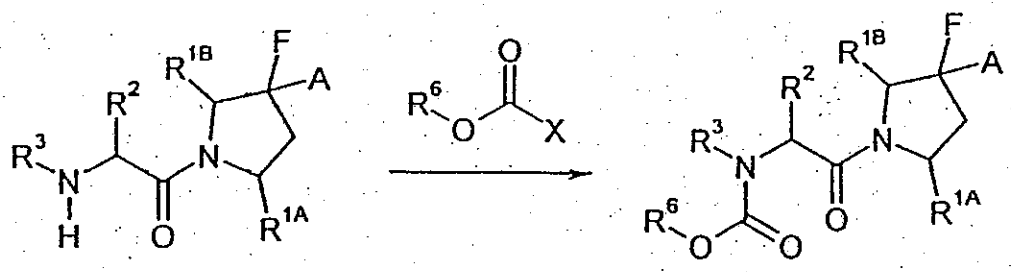
Another preferred embodiment of the present invention is a compound according to general formula 1 wherein R^{1A} is H, R^{1B} is CN and the absolute stereochemistry is as shown in general formula 5. In the conventional system of nomenclature this is the '*R*' configuration.



5

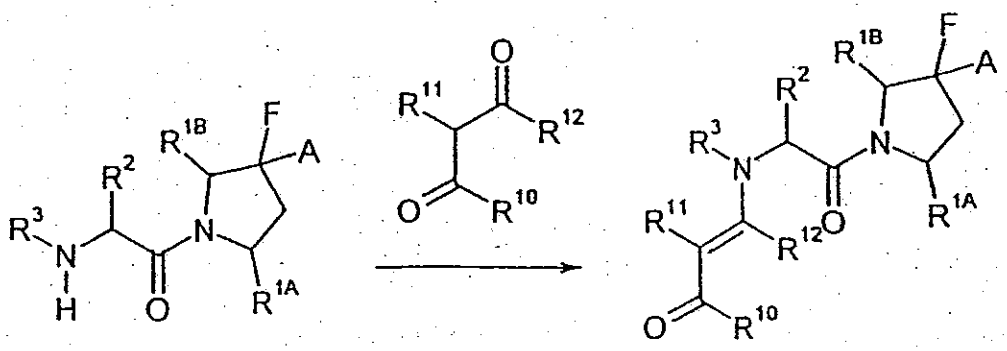
The compounds according to general formula 1 can be prepared using conventional synthetic methods.

Compounds wherein R^4 is other than H are generally accessible from the corresponding compounds wherein R^4 is H. When R^4 is R^6OCO- the desired compound can usually be prepared by the reaction of the amine functional group with a suitable carbonic acid derivative.

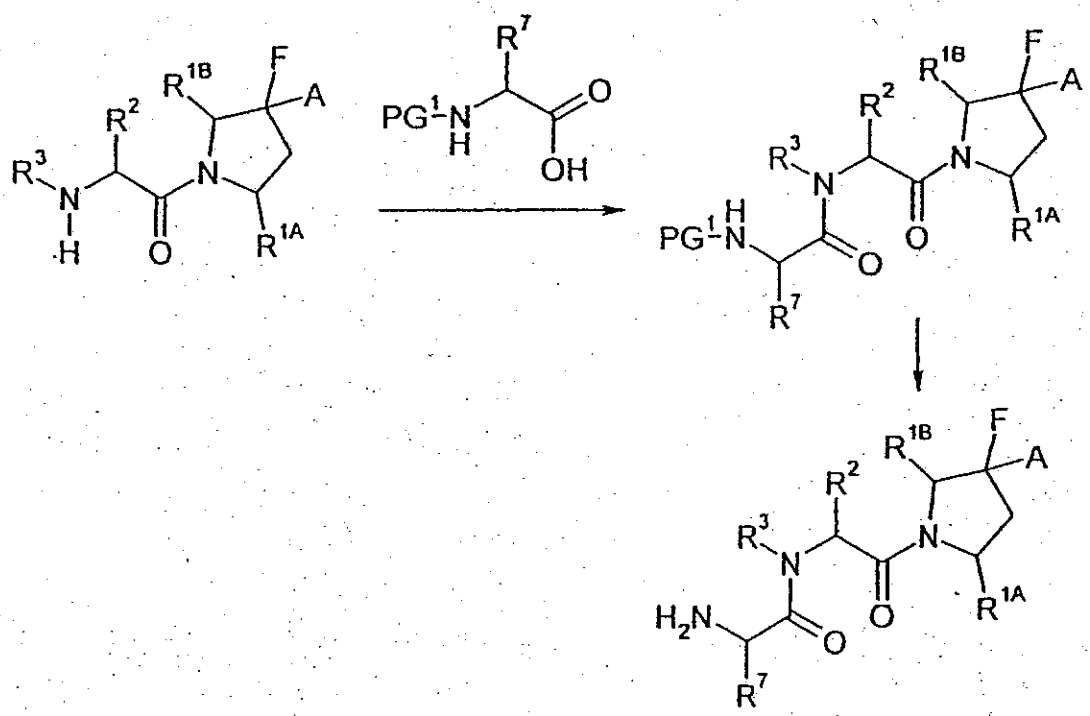


Here X is a leaving group such as a chlorine atom (Cl) or a *para*-nitrophenoxy group ($O_2NC_6H_4O$).

Compounds wherein R^4 is a group according to general formula 2 can be prepared by the reaction of the amine functional group with a 1,3-dicarbonyl compound such as a 1,3-diketone or a β-ketoester.

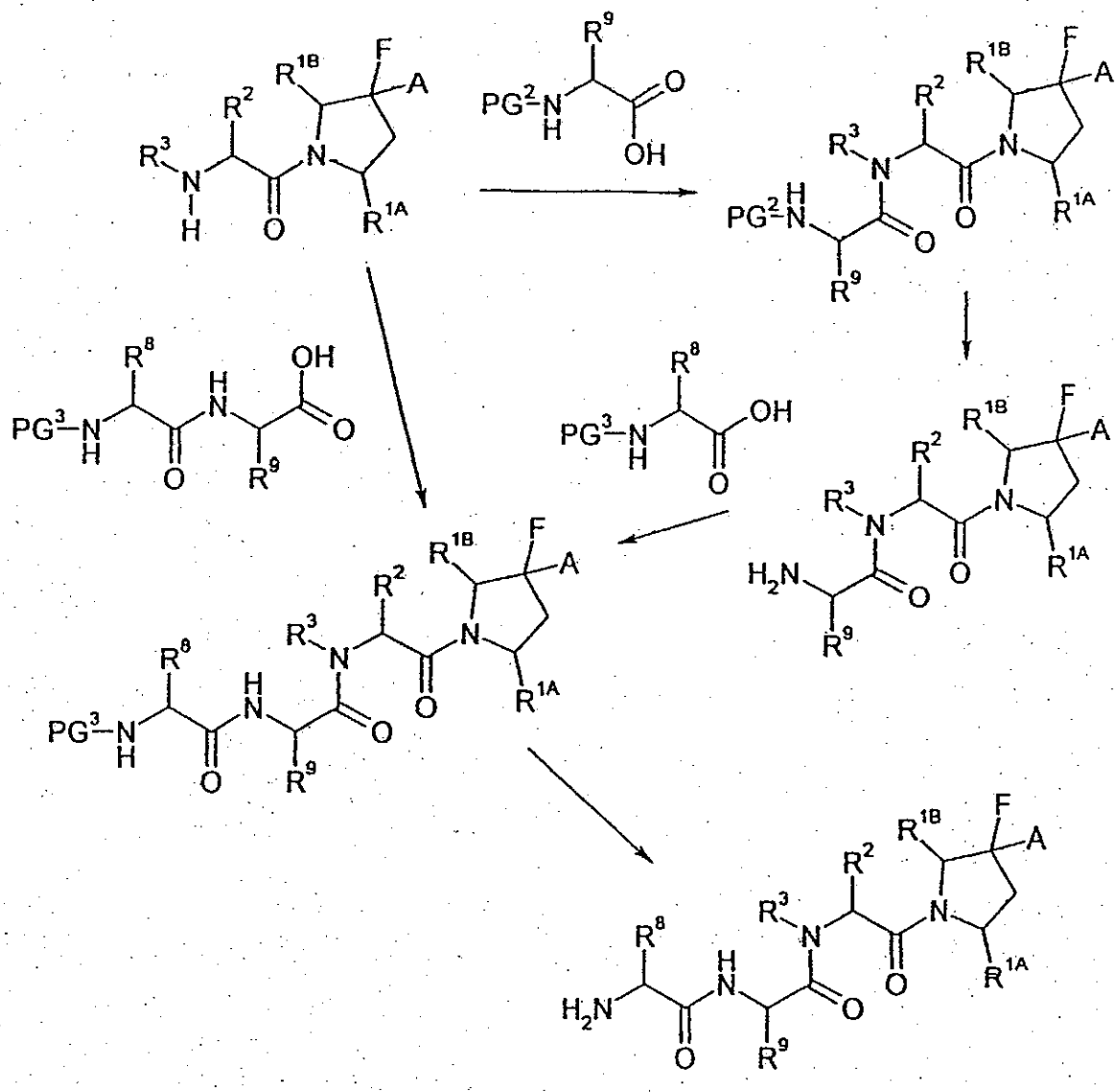


Compounds wherein R^4 is an amino acyl group $H_2NCH(R^7)CO$- can be prepared by the conventional methods of peptide synthesis.



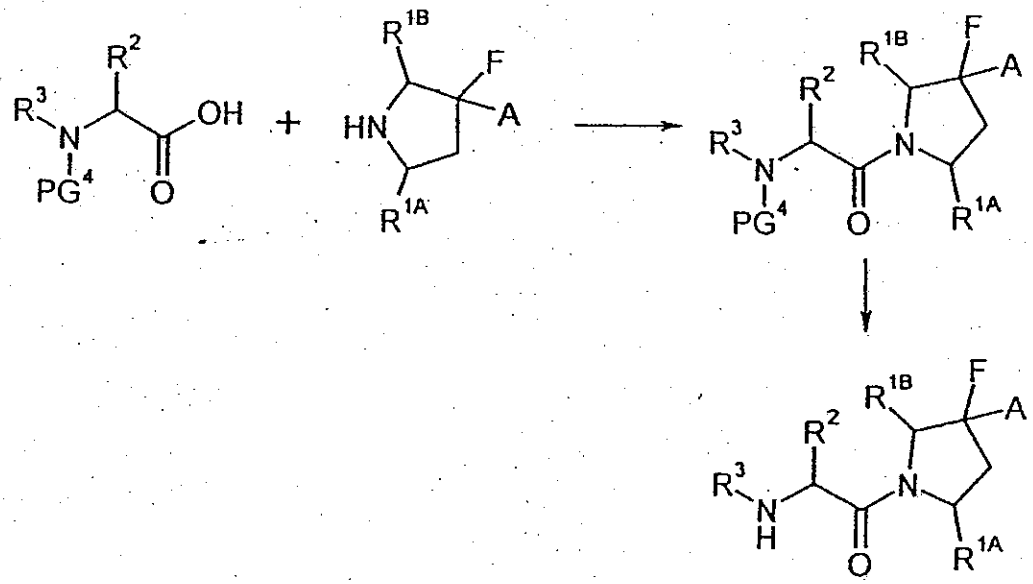
In a first step, the amine is reacted with a protected amino acid in the presence of a coupling agent. PG^1 is a protecting group such as tert-butyloxycarbonyl (BOC), benzyloxycarbonyl (Z) or 9-fluorenylmethyloxycarbonyl (Fmoc). The use of such groups is well known in the art. Where R^7 has a reactive functional group such as an amine or a carboxylic acid, this group will also be protected. In a second step the protecting group is removed.

Compounds wherein R^4 is a group $H_2NCH(R^8)CONHCH(R^9)CO$ - can also be prepared by the conventional methods of peptide synthesis.



Here again, PG^2 and PG^3 are protecting groups. The side chains R^8 and R^9 may also have protecting groups if necessary. The target compound may be assembled in a stepwise process or directly by coupling of a dipeptide fragment.

The most direct route to the compounds of the invention wherein R^4 is H is by the coupling of an appropriately functionalised and protected amino acid and a pyrrolidine derivative:



In some circumstances, such as when a large number of different compounds are to be made, it may be more convenient to prepare a compound that can serve as a common intermediate. For example, when a number of compounds are required wherein R^2 is $CH_2CH_2CON(R^{20})(R^{21})$, it is convenient to prepare a common intermediate with R^2 being $CH_2CH_2CO_2H$ and derivatise this by reaction with different amines.

The pyrrolidine derivatives are either known compounds or can be prepared by simple modification of published synthetic routes. These preparations are described in detail in the Examples.

In a second aspect, the present invention comprises a pharmaceutical composition for human therapeutic use. The composition is characterised in that it has, as an active agent, at least one of the compounds described above. Such a composition is useful in the treatment of human diseases. The composition will generally include one or more additional components selected from pharmaceutically acceptable excipients and pharmaceutically active agents other than those of the present invention.

The composition may be presented as a solid or liquid formulation, depending on the intended route of administration. Examples of solid formulations include pills, tablets, capsules and powders for oral administration, suppositories for rectal or vaginal administration, powders for nasal or pulmonary administration, and patches for transdermal or transmucosal (such as buccal) administration. Examples of liquid formulations include solutions and suspensions for intravenous, subcutaneous or

intramuscular injection and oral, nasal or pulmonary administration. A particularly preferred presentation is a tablet for oral administration. Another preferred presentation, particularly for emergency and critical care, is a sterile solution for intravenous injection.

The composition comprises at least one compound according to the preceding description. The composition may contain more than one such compound, but in general it is preferred that it should comprise only one. The amount of the compound used in the composition will be such that the total daily dose of the active agent can be administered n one to four convenient dose units. For example, the composition can be a tablet containing an amount of compound equal to the total daily dose necessary, said tablet to be taken once per day. Alternatively, the tablet can contain half (or one third, or one quarter) of the daily dose, to be taken twice (or three or four times) per day. Such a tablet can also be scored to facilitate divided dosing, so that, for example, a tablet comprising a full daily dose can be broken into half and administered in two portions. Preferably, a tablet or other unit dosage form will contain between 0.1mg and 1g of active compound. More preferably, it will contain between 1mg and 250mg.

The composition will generally include one or more excipients selected from those that are recognised as being pharmaceutically acceptable. Suitable excipients include, but are not limited to, bulking agents, binding agents, diluents, solvents, preservatives and flavouring agents. Agents that modify the release characteristics of the composition, such as polymers that selectively dissolve in the intestine ("enteric coatings") are also considered in the context of the present invention, to be suitable excipients.

The composition may comprise, in addition to the compound of the invention, a second pharmaceutically active agent. For example, the composition may include an anti-diabetic agent, a growth-promoting agent, an anti-inflammatory agent or an antiviral agent. However, it is generally preferred that the composition comprise only one active agent.

In a third aspect, the invention comprises a use for the compounds and compositions described above for the treatment of human diseases. This aspect can equally be considered to comprise a method of treatment for such diseases. The diseases susceptible to treatment are those wherein an inhibition of DP-IV or CD26 results in a clinical benefit either directly or indirectly. Direct effects include the blockade of T

lymphocyte activation. Indirect effects include the potentiation of peptide hormone activity by preventing the degradation of these hormones. Examples of diseases include, but are not limited to, auto-immune and inflammatory diseases such as inflammatory bowel disease and rheumatoid arthritis, growth hormone deficiency leading to short stature, polycystic ovary syndrome, impaired glucose tolerance and type 2 diabetes. Particularly preferred is the use of the compounds and compositions for the treatment of impaired glucose tolerance and type 2 diabetes, and equally a method of treatment of these diseases by the administration of an effective amount of a compound or composition as previously described.

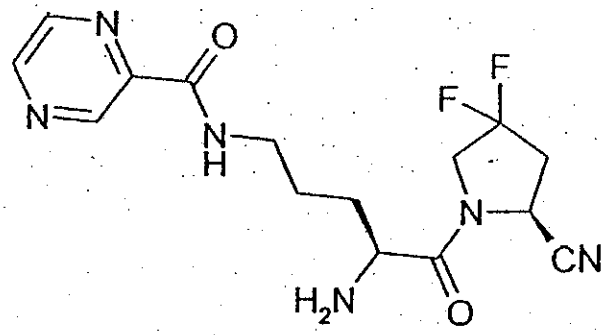
The precise details of the treatment, including the dosing regimen, will be established by the attending physician taking into account the general profile of the patient and the severity of the disease. For diseases such as inflammatory bowel disease that have acute phases of active disease separated by quiescent periods, the physician may select a relatively high dose during the acute phase and a lower maintenance dose for the quiescent period. For chronic diseases such as type 2 diabetes and impaired glucose tolerance, the dosing may need to be maintained at the same level for an extended period. A dosing schedule of one to four tablets per day, each comprising between 0.1mg and 1g (and preferably between 1mg and 250mg) of active compound might be typical in such a case.

The invention is further illustrated with the following non-limiting Examples.

EXAMPLES

EXAMPLE 1

(2*S*)-4,4-Difluoro-1-[*N*$^{\omega}$-(pyrazinyl-2-carbonyl)-L-ornithinyl]-pyrrolidine-2-carbonitrile trifluoroacetate



1A. Methyl (2*S*)-*N*-(*tert*-butyloxycarbonyl)-4-pyrrolidone-2-carboxylate

N-(*tert*-Butyloxycarbonyl)-L-4-*trans*-hydroxyproline methyl ester (2.5g, 10.2mmol) was dissolved in CH_2Cl_2 (70ml). Dess-Martin periodinane (5.0g, 12.1mmol) was added and the mixture was stirred for 3 hours at room temperature. The solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (300ml). The solution was washed with sat. $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give a colourless oil. The residue was purified by flash chromatography (eluant: 10% ethyl acetate, 90% pet. ether 60-80) to give a colourless oil identified as methyl (2*S*)-*N*-(*tert*-butyloxycarbonyl)-4-pyrrolidone-2-carboxylate (2.4g, 9.7mmol,95%).

1B. Methyl (2*S*)-*N*-(*tert*-butyloxycarbonyl)-4,4-difluoropyrrolidine-2-carboxylate

Methyl (2*S*)-*N*-(*tert*-butyloxycarbonyl)-4-pyrrolidone-2-carboxylate (2.3g, 9.3mmol) was dissolved in CH_2Cl_2 (70ml). (Diethylamino)sulphur trifluoride (4.5g, 27.9mmol) was added to this solution at 0°C and the mixture was stirred for 18 hours at 0°C to room temperature. The reaction mixture was carefully poured into sat. $NaHCO_3$ (100ml) and the mixture was stirred for 15min then extracted with CH_2Cl_2. The organic extract was washed with water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give an orange oil. The residue was purified by flash chromatography (eluant: 10% ethyl acetate, 90% pet. ether 60-80) to give a colourless oil identified as methyl (2*S*)-*N*-(*tert*-butyloxycarbonyl)-4,4-difluoropyrrolidine-2-carboxylate (2.4g, 8.9mmol,96%).

1C. (2*S*)-*N*-(*tert*-Butyloxycarbonyl)-4,4-difluoropyrrolidine-2-carboxylic acid

Methyl (2*S*)-*N*-(*tert*-butyloxycarbonyl)-4,4-difluoropyrrolidine-2-carboxylate (2.2g, 8.3mmol) was dissolved in THF (100ml). Aqueous lithium hydroxide (1M, 10.6ml, 10.6mmol) was added. The mixture was stirred for 3 hours at room temperature then diluted with ethyl acetate (150ml), washed with 1M HCl, water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give an orange oil. The residue was purified by flash chromatography (eluant: 95% chloroform, 4% methanol, 1% acetic acid) to give an orange oil identified as (2*S*)-*N*-(*tert*-butyloxycarbonyl)-4,4-difluoropyrrolidine-2-carboxylic acid (2.1g, 8.3mmol, 100%).

1D. (2*S*)-*N*-(*tert*-Butyloxycarbonyl)-4,4-difluoropyrrolidine-2-carboxamide

(2*S*)-*N*-(*tert*-Butyloxycarbonyl)-4,4-difluoropyrrolidine-2-carboxylic acid (1.0g, 4.0mmol) was dissolved in CH_2Cl_2/DMF (9:1, 50ml). To this solution at 0°C was added 1-hydroxybenzotriazole hydrate (1.1g, 8.1mmol) and water-soluble carbodiimide (960mg,

4.8mmol). The mixture was stirred for 1 hour at 0°C then ammonia (35%, 5ml) was added. The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (200ml). The solution was washed with 0.3M $KHSO_4$, sat. $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil. The residue was purified by flash chromatography (eluant: 85% ethyl acetate, 15% pet. ether 60-80) to give a colourless oil identified as (2*S*)-*N*-(*tert*-butyloxycarbonyl)-4,4-difluoropyrrolidine-2-carboxamide (945mg, 3.8mmol, 95%).

1E. (2*S*)-1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]-4,4-difluoropyrrolidine-2-carboxamide

(2*S*)-*N*-(*tert*-Butyloxycarbonyl)-4,4-difluoropyrrolidine-2-carboxamide (130mg, 0.54mmol) was dissolved in 4M HCl/dioxan (30ml). The solution was stirred for 1 hour at room temperature then the solvent was removed *in vacuo* and the residue was dissolved in CH_2Cl_2 /DMF (9:1, 20ml). To this solution at 0°C was added N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithine (180mg, 0.53mmol), 1-hydroxybenzotriazole hydrate (90mg, 0.67mmol) and water-soluble carbodiimide (136mg, 0.65mmol). The mixture was stirred for 15 mins at 0°C then the pH was adjusted to pH8 with *N*-methylmorpholine. The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 0.3M $KHSO_4$, sat. $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil. The residue was purified by flash chromatography (eluant: 92% chloroform, 8% methanol) to give a white solid identified as (2*S*)-1-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]-4,4-difluoropyrrolidine-2-carboxamide (195mg, 0.41mmol, 77%).

1F. (2*S*)-1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]-4,4-difluoropyrrolidine-2-carbonitrile

(2*S*)-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]-4,4-difluoro-pyrrolidine-2-carboxamide (175mg, 0.37mmol) was dissolved in dry THF (30ml). This solution was cooled to 0°C then triethylamine (75mg, 0.75mmol) was added followed by trifluoroacetic anhydride (190mg, 0.9mmol). The mixture was stirred for 5min then the pH was adjusted to pH9 with triethylamine. The mixture was stirred for a further 30min then diluted with ethyl acetate (150ml), washed with water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil. The residue was purified by flash

chromatography (eluant: 70% ethyl acetate, 30% pet. ether 60-80) to give a white solid identified as (2*S*)-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]-4,4-difluoropyrrolidine-2-carbonitrile (148mg, 0.33mmol, 88%).

1G. (2*S*)-4,4-Difluoro-1-[N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]pyrrolidine-2-carbonitrile trifluoroacetate

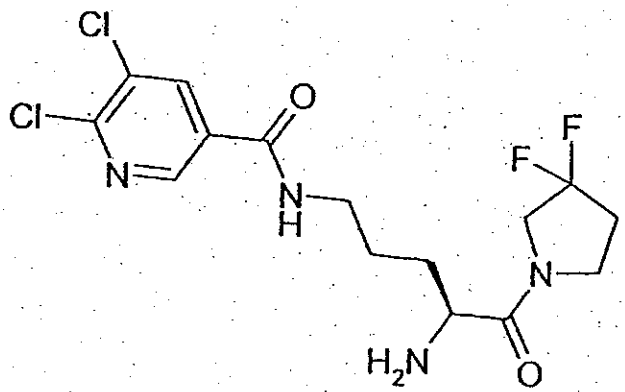
(2*S*)-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]-4,4-difluoro-pyrrolidine-2-carbonitrile (135mg, 0.3mmol) was dissolved in trifluoroacetic acid (10ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo* to give a colourless oil identified as (2*S*)-4,4-difluoro-1-[N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]-pyrrolidine-2-carbonitrile trifluoroacetate (140mg, 0.3mmol, 100%).

$[M+H]^+ = 353.1$

^{1}H NMR (CD_3OD): δ 1.74-1.82 (2H,m), 1.90-2.02 (2H,m), 2.82-2.89 (2H,m), 3.30-3.32 (1H,m), 3.51 (2H,t,J=6.7Hz), 4.12 (2H,t,J=11.9Hz), 4.25-4.29 (1H,m), 4.88 (2H,s), 5.09-5.14 (1H,m), 8.67-8.68 (1H,m), 8.7 (1H,d,J=2.5Hz), 9.23 (1H,d,J=1.4Hz) ppm.

EXAMPLE 2

1-[N^{ω}-(5,6-Dichloronicotinoyl)-L-ornithinyl]-3,3-difluoropyrrolidine hydrochloride



2A. 1-(*tert*-Butyloxycarbonyl)-3-pyrrolidone

(3*R*)-1-(*tert*-Butyloxycarbonyl)-3-hydroxypyrrolidine (980mg, 5.3mmol) was dissolved in CH_2Cl_2 (40ml). Dess-Martin periodinane (2.5g, 5.8mmol) was added. The mixture was stirred for 3 hours at room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (300ml). The solution was washed with sat. $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give a colourless oil. The residue was purified by flash chromatography (eluant: 20% ethyl acetate, 80%

pet. ether 60-80) to give a colourless oil identified as 1-(*tert*-butyloxycarbonyl)-3-pyrrolidone (842mg, 4.6mmol, 87%).

2B. 1-(*tert*-Butyloxycarbonyl)-3,3-difluoropyrrolidine

1-(*tert*-Butyloxycarbonyl)-3-pyrrolidone (810mg, 4.4mmol) was dissolved in CH_2Cl_2 (30ml). (Diethylamino)sulphur trifluoride (2.2g, 13.7mmol) was added to this solution at 0 °C. The mixture was stirred for 18 hours at 0°C to room temperature then carefully poured into sat. $NaHCO_3$ (100ml). The mixture was stirred for 15min then extracted with CH_2Cl_2. The organic extract was washed with water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give an orange oil. The residue was purified by flash chromatography (eluant: 10% ethyl acetate, 90% pet. ether 60-80) to give a colourless oil identified as 1-(*tert*-butyloxycarbonyl)-3,3-difluoropyrrolidine (580mg, 2.8mmol, 64%).

2C. 3,3-Difluoropyrrolidine hydrochloride

1-(*tert*-Butyloxycarbonyl)-3,3-difluoropyrrolidine (540mg, 2.6mmol) was dissolved in 4M HCl/dioxan (30ml). The solution was stirred for 1 hour at room temperature then the solvent was removed *in vacuo* to give an off white solid identified as 3,3-difluoropyrrolidine hydrochloride (370mg, 2.6mmol, 100%).

2D. N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithine *tert*-butyl ester

N^{α}-(*tert*-Butyloxycarbonyl)-L-ornithine *tert*-butyl ester hydrochloride (650mg, 2.0mmol) was dissolved in CH_2Cl_2 /DMF (9:1, 40ml). To this solution at 0°C was added 5,6 dichloronicotinic acid (383mg, 2.0mmol), 1-hydroxybenzotriazole hydrate (459mg, 3.0mmol) and water-soluble carbodiimide (461mg, 2.4mmol). The mixture was stirred for 15 mins at 0°C then the pH was adjusted to pH8 with *N*-methylmorpholine. The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (100ml). The solution was washed with 0.3M $KHSO_4$, sat. $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil. The residue was purified by flash chromatography (eluant: 50% ethyl acetate, 50% pet. ether 60-80) to give a white solid identified as N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithine *tert*-butyl ester (660mg, 1.42mmol, 71%).

2E. N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithine

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithine *tert*-butyl ester (650mg, 1.40mmol) was dissolved in trifluoroacetic acid/dichloromethane (1:1, 20ml). The mixture was stirred for 2 hours at room temperature then the solvent was removed *in vacuo*. The residue was dissolved in dioxan (20ml) and aqueous potassium hydrogen carbonate (1M, 10ml) and di-*tert*-butyl dicarbonate (327mg,1.5mmol) were added. The mixture was stirred for 18 hours at room temperature then the dioxan was removed *in vacuo*. The residue was diluted with water, washed with diethyl ether, acidified to pH2 with 1M HCl and extracted with chloroform. The organic extract was washed with water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give a colourless oil identified as N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithine (530mg, 1.34mmol, 96%).

2F. 1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithinyl]-3,3-difluoropyrrolidine

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithine (98mg, 0.24mmol) was dissolved in CH_2Cl_2 (20ml). To this solution at 0°C was added 3,3-difluoropyrrolidine hydrochloride (36mg, 0.25mmol), PyBOP (139mg, 0.27mmol) and triethylamine (60mg, 0.6mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 0.3M $KHSO_4$, sat. $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give an orange oil. The residue was purified by flash chromatography (eluant: 60% ethyl acetate, 40% pet. ether 60-80) to give a colourless oil identified as 1-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithinyl]-3,3-difluoropyrrolidine (79mg, 0.16mmol, 68%).

2G. 1-[N^{ω}-(5,6-Dichloronicotinoyl)-L-ornithinyl]-3,3-difluoropyrrolidine hydrochloride

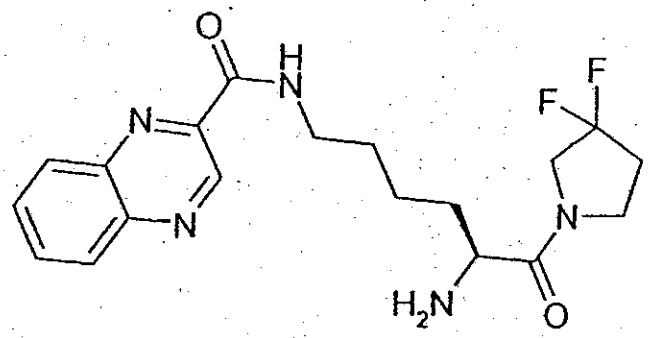
1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithinyl]-3,3-difluoro-pyrrolidine (68mg, 0.14mmol) was dissolved in 4M HCl/dioxan (20ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo* to give a colourless oil identified as 1-[N^{ω}-(5,6-dichloronicotinoyl)-L-ornithinyl]-3,3-difluoro-pyrrolidine hydrochloride (49mg, 0.117mmol, 83%).

$[M+H]^+$ = 395.1

^1H NMR (CD_3OD): δ 1.28-1.34 (2H,m), 1.72-1.76 (2H,m), 1.85-1.92 (2H,m), 2.25-2.71 (2H,m), 3.30-3.41 (2H,m), 3.87-4.30 (6H,m), 8.36-8.39 (1H,m), 8.73-8.79 (1H,m) ppm.

EXAMPLE 3

3,3-Difluoro-1-[N^{ω}-(2-quinoxaloyl)-L-lysinyl]pyrrolidine hydrochloride



3A. N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(2-quinoxaloyl)-L-lysine methyl ester

N^{α}-(*tert*-Butyloxycarbonyl)-L-lysine methyl ester acetate (640mg, 2.0mmol) was dissolved in CH_2Cl_2 (40ml). To this solution at 0°C was added 2-quinoxaloyl chloride (385mg, 2.0mmol) and triethylamine (60mg, 0.6mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (100ml). The solution was washed with 0.3M $KHSO_4$, sat. $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil. The residue was purified by flash chromatography (eluant: 65% ethyl acetate, 35% pet. ether 60-80) to give a white solid identified as N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(2-quinoxaloyl)-L-lysine methyl ester (580mg, 1.40mmol, 70%).

3B. N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(2-quinoxaloyl)-L-lysine

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(2-quinoxaloyl)-L-lysine methyl ester (570mg, 1.37mmol) was dissolved in THF (50ml). Aqueous lithium hydroxide (1M, 2ml, 2.0mmol) was added. The mixture was stirred for 3 hours at room temperature then the reaction mixture was diluted with ethyl acetate (150ml), washed with 1M HCl, water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give a white solid identified as N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(2-quinoxaloyl)-L-lysine (440mg, 1.1mmol, 80%).

3C. 1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(2-quinoxaloyl)-L-lysinyl]-3,3-difluoro-pyrrolidine

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(2-quinoxaloyl)-L-lysine (95mg, 0.24mmol) was dissolved in CH_2Cl_2 (20ml). To this solution at 0°C was added 3,3-difluoropyrrolidine hydrochloride (34mg, 0.24mmol), PyBOP (145mg, 0.28mmol) and triethylamine (60mg, 0.6mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 0.3M $KHSO_4$, sat. NaHCO3, water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give an orange oil. The residue was purified by flash chromatography (eluant: 60% ethyl acetate, 40% pet. ether 60-80) to give a colourless oil identified as 1-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(2-quinoxaloyl)-L-lysinyl]-3,3-difluoro-pyrrolidine (87mg, 0.18mmol, 75%).

3D. 3,3-Difluoro-1-[N^{ω}-(2-quinoxaloyl)-L-lysinyl]pyrrolidine hydrochloride

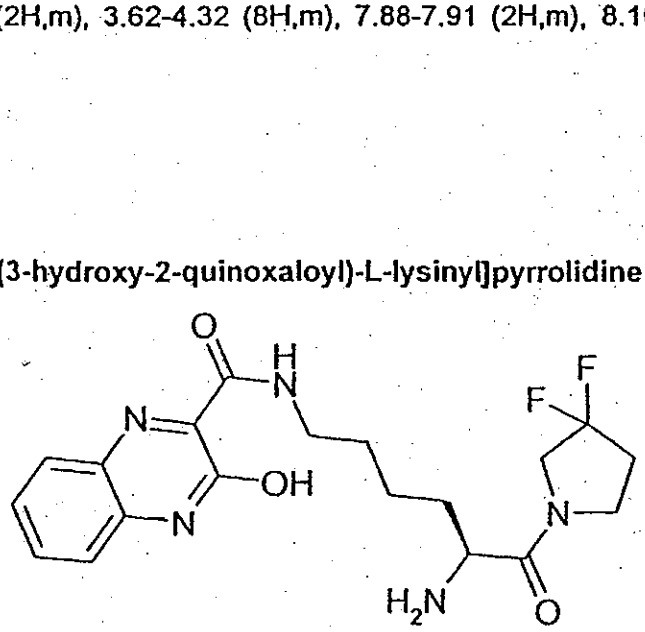
1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(2-quinoxaloyl)-L-lysinyl]-3,3-difluoropyrrolidine (87mg, 0.18mmol) was dissolved in 4M HCl/dioxan (20ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo* to give a colourless oil identified as 3,3-difluoro-1-[N^{ω}-(2-quinoxaloyl)-L-lysinyl]pyrrolidine hydrochloride (75mg, 0.18mmol, 100%).

$[M+H]^+ = 392.3$

^{1}H NMR (CD_3OD): δ 1.51-1.59 (2H,m), 1.70-1.78 (2H,m), 1.81-1.90 (2H,m), 2.37-2.58 (2H,m), 3.51-3.59 (2H,m), 3.62-4.32 (8H,m), 7.88-7.91 (2H,m), 8.10-8.21 (2H,m), 9.41 (1H,s) ppm.

EXAMPLE 4

3,3-Difluoro-1-[N^{ω}-(3-hydroxy-2-quinoxaloyl)-L-lysinyl]pyrrolidine hydrochloride



4A. 1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-lysinyl]-3,3-difluoropyrrolidine

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-lysine (1.14g, 2.4mmol) was dissolved in CH_2Cl_2 /DMF (9:1, 100ml). To this solution at 0°C were added 1-hydroxybenzotriazole hydrate (394mg, 2.9mmol), water-soluble carbodiimide (680mg, 3.4mmol), 3,3-difluoropyrrolidine hydrochloride (380mg, 2.43mmol) and triethylamine (400mg, 4mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (200ml). The solution was washed with 0.3M $KHSO_4$, sat. $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography (eluant: 65% ethyl acetate, 35% pet. ether 60-80) to give a white solid identified as 1-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-lysinyl]-3,3-difluoropyrrolidine (1.0g, 1.8mmol, 75%).

4B. 1-[N^{α}-(*tert*-Butyloxycarbonyl)-L-lysinyl]-3,3-difluoropyrrolidine

1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-lysinyl]-3,3-difluoropyrrolidine (1.01g, 1.8mmol) was dissolved in THF (20ml). Diethylamine (5ml) was added. The mixture was stirred for 3 hours at room temperature then the solvent was removed *in vacuo* and the residue was purified by flash chromatography (eluant: 90% chloroform, 7% methanol, 3% triethylamine) to give a pale yellow oil identified as 1-[N^{α}-(*tert*-butyloxycarbonyl)-L-lysinyl]-3,3-difluoropyrrolidine (598mg, 1.78mmol, 99%).

4C. 1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(3-hydroxy-2-quinoxaloyl)-L-lysinyl]-3,3-difluoropyrrolidine

1-[N^{α}-(*tert*-Butyloxycarbonyl)-L-lysinyl]-3,3-difluoropyrrolidine (147mg, 0.44mmol) was dissolved in CH_2Cl_2 (20ml). To this solution at 0°C was added 3-hydroxy-2-quinoxaline-carboxylic acid (83mg, 0.44mmol), PyBOP (274mg, 0.53mmol) and triethylamine (100mg, 1.0mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 0.3M $KHSO_4$, sat. $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give an orange oil. The residue was purified by flash chromatography (eluant: 96% dichloromethane, 4% methanol) to give a yellow gummy solid identified as 1-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(3-hydroxy-2-quinoxaloyl)-L-lysinyl]-3,3-difluoropyrrolidine (106mg, 0.21mmol, 47%).

4D. 3,3-Difluoro-1-[N^{ω}-(3-hydroxy-2-quinoxaloyl)-L-lysinyl]pyrrolidine hydrochloride

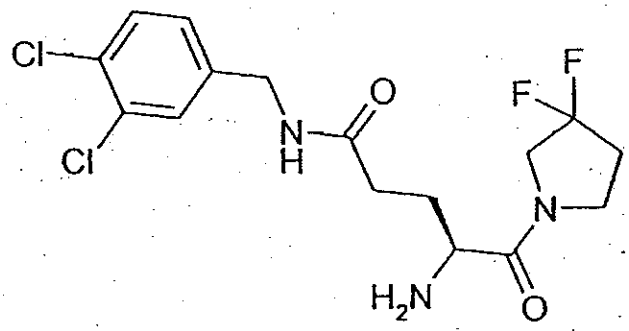
1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(3-hydroxy-2-quinoxaloyl)-L-lysinyl]-3,3-difluoro-pyrrolidine (106mg, 0.3mmol) was dissolved in 4M HCl/dioxan (20ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo* to give a colourless oil identified as 3,3-difluoro-1-[N^{ω}-(3-hydroxy-2-quinoxaloyl)-L-lysinyl]-pyrrolidine hydrochloride (66mg, 0.15mmol, 50%).

$[M+H]^{+}$ = 408.1

^{1}H NMR (CD_3OD): δ 1.85-1.87 (6H,m), 2.3-2.7 (2H,br m), 3.29-3.31 (6H,m), 3.4-3.7 (5H,br m), 7.35-7.5 (2H,m), 7.6-7.8 (1H,m), 7.9-8.0 (1H,m) ppm.

EXAMPLE 5

1-[N^{ω}-(3,4-Dichlorobenzyl)glutaminyl]-3,3-difluoropyrrolidine hydrochloride



5A. 1-[*N*-(*tert*-Butyloxycarbonyl)-O^{ω}-methylglutamyl]-3,3-difluoropyrrolidine

N-(*tert*-Butyloxycarbonyl)-O^{ω}-methylglutamic acid (462mg, 1.04mmol) was dissolved in CH_2Cl_2 /DMF (9:1, 20ml). To this solution at 0°C were added 1-hydroxybenzotriazole hydrate (192mg, 1.25mmol), water-soluble carbodiimide (277mg, 1.46mmol), 3,3-difluoropyrrolidine hydrochloride (150mg, 1.04mmol) and triethylamine (200mg, 2.0mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70mL). The solution was washed with 0.3M $KHSO_4$, sat. $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography (eluant: 40% ethyl acetate, 60% pet. ether 60-80) to give a colourless oil identified as 1-[*N*-(*tert*-butyloxycarbonyl)-O^{ω}-methylglutamyl]-3,3-difluoropyrrolidine (362mg, 1.03mmol, 99%).

5B. 1-[*N*-(*tert*-Butyloxycarbonyl)glutamyl]-3,3-difluoropyrrolidine

1-[*N*-(*tert*-Butyloxycarbonyl)-O^{ω}-methylglutamyl]-3,3-difluoropyrrolidine (362mg, 1.03mmol) was dissolved in dioxan (5mL). Aqueous lithium hydroxide (1M, 2.5ml, 2.5mmol) was added. The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70mL). The solution was washed with 1M $KHSO_4$, water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give a colourless oil identified as 1-[*N*-(*tert*-butyloxycarbonyl)glutamyl]-3,3-difluoropyrrolidine (200mg, 0.66mmol, 58%).

5C. 1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(3,4-dichlorobenzyl)glutaminyl]-3,3-difluoro-pyrrolidine

1-[*N*-(*tert*-Butyloxycarbonyl)glutamyl]-3,3-difluoropyrrolidine (100mg, 0.30mmol) was dissolved in CH_2Cl_2 /DMF (9:1, 20ml). To this solution at 0°C were added 1-hydroxybenzotriazole hydrate (53mg, 0.36mmol), water-soluble carbodiimide (80mg, 0.42mmol), 3,4-dichlorobenzylamine (53mg, 0.4mmol) and triethylamine (61mg, 0.6mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (200ml). The solution was washed with 0.3M $KHSO_4$, sat. $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography (eluant: 75% ethyl acetate, 25% pet. ether 60-80) to give a white solid identified as 1-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(3,4-dichlorobenzyl)glutaminyl]-3,3-difluoropyrrolidine (144mg, 0.29mmol, 100%).

5D. 1-[N^{ω}-(3,4-Dichlorobenzyl)glutaminyl]-3,3-difluoropyrrolidine hydrochloride

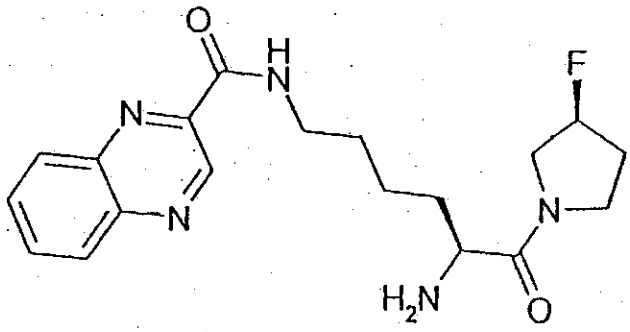
1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(3,4-dichlorobenzyl)glutaminyl]-3,3-difluoropyrrolidine (144mg, 0.29mmol) was dissolved in 4M HCl/dioxan (20ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo* to give a white solid identified as 1-[N^{ω}-(3,4-dichlorobenzyl)glutaminyl]-3,3-difluoropyrrolidine hydrochloride (120mg, 0.28mmol, 100%).

$[M+H]^+$ = 394.0, 395.7

^1H NMR (CD_3OD): δ 2.00-2.20 (2H,m), 2.30-2.50 (4H,m), 3.25-3.35 (3H,m), 3.60-4.20 (4H,m), 4.20-4.40 (3H,m), 7.20-7.30 (1H,m), 7.40-7.50 (2H,m) ppm

EXAMPLE 6

(3*S*)-3-Fluoro-1-[N^{ω}-(2-quinoxaloyl)-L-lysinyl]pyrrolidine hydrochloride



6A. (3*S*)-1-(*tert*-Butyloxycarbonyl)-3-fluoropyrrolidine

(3*R*)-N-(*tert*-Butyloxycarbonyl)-3-hydroxypyrrolidine (1.0g, 5.34mmol) was dissolved in CH_2Cl_2 (30ml). (Diethylamino)sulphur trifluoride (860g, 5.34mmol) was added to this solution at -78 °C. The mixture was stirred for 18 hours at -78 °C to room temperature then the reaction mixture was carefully poured into sat. $NaHCO_3$ (100ml) and stirred for 15min and extracted with CH_2Cl_2. The organic extract was washed with water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give an orange oil. The residue was purified by flash chromatography (eluant: 28% ethyl acetate, 72% pet. ether 60-80) to give a colourless oil identified as (3*S*)-1-(*tert*-butyloxycarbonyl)-3-fluoropyrrolidine (507mg, 2.67mmol, 50%).

6B. (3*S*)-3-Fluoropyrrolidine hydrochloride

(3*S*)-1-(*tert*-Butyloxycarbonyl)-3-fluoropyrrolidine (507mg, 2.68mmol) was dissolved in 4M HCl/dioxan (30ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo* to give an off-white solid identified as (3*S*)-3-fluoro-pyrrolidine hydrochloride (320mg, 2.6mmol, 95%).

6C. (3*S*)-1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(2-quinoxaloyl)-L-lysinyl]-3-fluoro-pyrrolidine

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(2-quinoxaloyl)-L-lysine (50mg, 0.124mmol) was dissolved in CH_2Cl_2 (20ml). To this solution at 0°C was added (3*S*)-3-fluoropyrrolidine hydrochloride (17mg, 0.136mmol), 1-hydroxybenzotriazole hydrate (20mg, 0.149mmol), water-soluble carbodiimide (35mg, 0.17mmol) and triethylamine (30mg, 0.3mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 0.3M $KHSO_4$, sat. $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated *in*

vacuo to give an orange oil. The residue was purified by flash chromatography (eluant: 60% ethyl acetate, 40% pet. ether 60-80) to give a colourless oil identified as (3*S*)-1-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(2-quinoxaloyl)-L-lysinyl]-3-fluoropyrrolidine (50mg, 0.107mmol, 86%).

6D. (3*S*)-3-Fluoro-1-[N^{ω}-(2-quinoxaloyl)-L-lysinyl]pyrrolidine hydrochloride

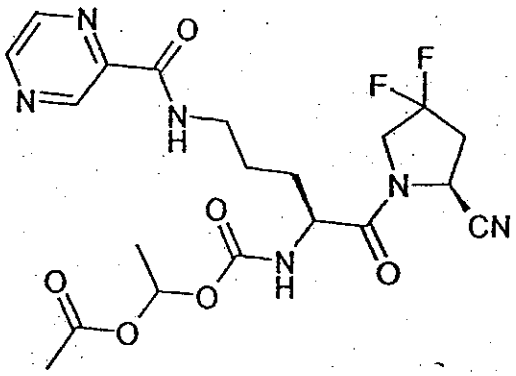
(3*S*)-1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(2-quinoxaloyl)-L-lysinyl]-3-fluoropyrrolidine (50mg, 0.105mmol) was dissolved in 4M HCl/dioxan (10ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo* to give an off-white solid identified as (3*S*)-3-fluoro-1-[N^{ω}-(2-quinoxaloyl)-L-lysinyl]pyrrolidine hydrochloride (43mg, 0.105mmol, 100%).

$[M+H]^+ = 374.0$

^{1}H NMR (CD_3OD): δ 1.53-1.57 (2H,m), 1.72-1.75 (2H,m), 1.92-1.94 (2H,m), 2.21-2.31 (1H,m), 3.43-4.01 (8H,m), 4.16-4.18 (1H,m), 5.19-5.39 (1H,m), 7.96-7.97 (2H,m), 8.16-8.21 (2H,m), 9.41(1H,s) ppm.

EXAMPLE 7

(2*S*)-1-[N^{α}-(1'-Acetoxyethoxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]-4,4-difluoropyrrolidine-2-carbonitrile



A solution of (2*S*)-1-[N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]-4,4-difluoropyrrolidine-2-carbonitrile trifluoroacetate (40mg, 0.066mmol), α-acetoxyethyl *p*-nitrophenyl carbonate (28mg, 0.11 mmol; prepared according to Alexander *et al.*, J. Med. Chem. 31, 318, 1988) and triethylamine (20mg, 0.2mmol) in dichloromethane (25ml) was stirred at room temperature for 18 hours, then evaporated *in vacuo*. The residue taken up in ethyl acetate (70ml). The solution was washed with sat $NaHCO_3$, water and brine, dried

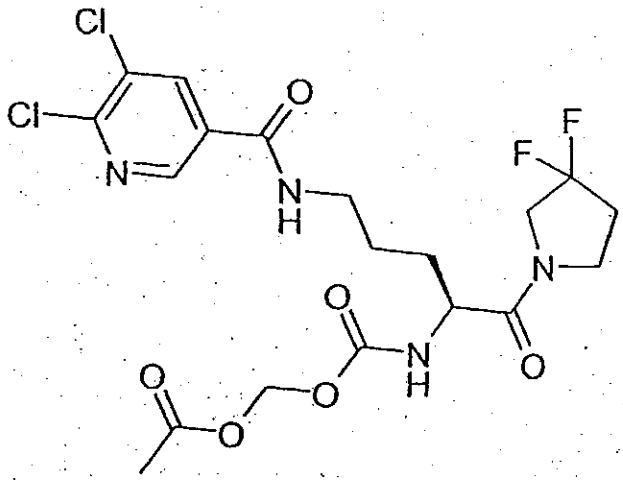
(Na_2SO_4) and evaporated. The residue was purified by flash chromatography (eluant 98% chloroform, 2%methanol) to give a white solid identified as (2*S*)-1-[N^{α}-(1'-acetoxyethoxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]pyrrolidine-2-carbonitrile (26mg, 0.053mmol, 62%).

$[M+H]^+$ = 483.1

^{1}H NMR ($CDCl_3$): δ 1.41-1.46 (3H,m), 1.72-1.83 (4H,m), 2.01-2.05 (3H,m), 2.68-2.74 (2H,m), 3.49-3.58 (2H,m), 4.03-4.11 (2H,m), 4.41-4.43 (1H,m), 4.94-4.98 (1H,m), 5.56 (1H,d,J = 8.6Hz), 6.73-6.76 (1H,m), 7.90-7.93 (1H,m), 8.51-8.52 (1H,m), 8.75 (1H,d,J = 2.4Hz), 9.37 (1H,d,J = 1.4Hz) ppm.

EXAMPLE 8

1-[N^{α}-(Acetoxymethoxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithinyl]-3,3-difluoropyrrolidine



1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithinyl]-3,3-difluoro-pyrrolidine (88mg, 0.18mmol) was dissolved in 4M HCl/dioxan (20ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane (25ml), acetoxymethyl *p*-nitrophenyl carbonate (60mg, 0.24mmol; prepared according to Alexander *et al.*, J. Med. Chem. 31, 318, 1988) and triethylamine (60mg, 0.6mmol) were added, and the mixture was stirred at room temperature for 18 hours. The solution was evaporated *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with sat $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography (eluant 80% ethyl acetate, 20% pet. Ether 60-80) to give a white solid

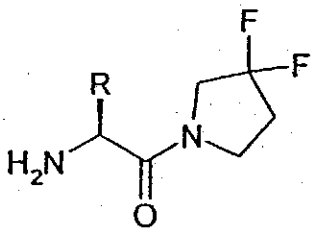
identified as 1-[N^{α}-acetoxymethoxycarbonyl-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithinyl]-3,3-difluoropyrrolidine (64mg, 0.126mmol, 71%).

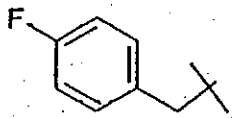
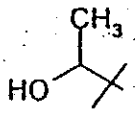
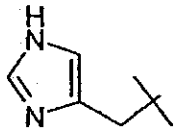
$[M+H]^+$ = 512.8

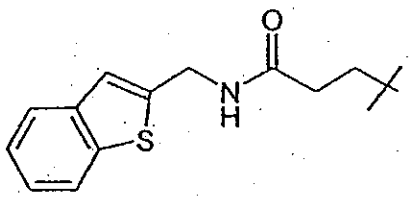
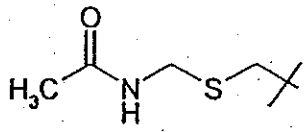
^{1}H NMR ($CDCl_3$): δ 1.66-1.78 (4H,m), 2.01 (3H,s), 2.36-2.67 (2H,m), 3.49-3.53 (2H,m), 3.63-3.87 (4H,m), 4.25-4.70 (1H,m), 5.62-5.65 (1H,m), 5.72-5.76 (1H,m), 5.97-6.01 (1H,m), 6.85-7.09 (1H,m), 8.26 (1H,d,J = 2Hz), 8.61 (1H,d,J = 2.2Hz) ppm.

The following compounds were prepared using analogous methods.

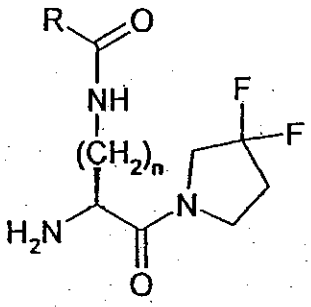
EXAMPLES 9-22

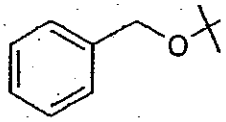
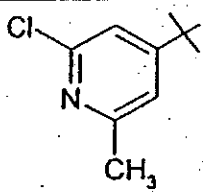
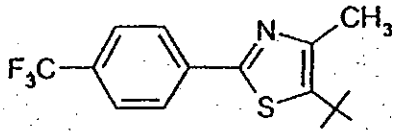
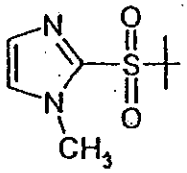


Ex No	R
9	Isopropyl
10	*n*-Butyl
11	*sec*-Butyl
12	*tert*-Butyl
13	Cyclohexyl
14	Benzyl
15	
16	$CH_3S(CH_2)_2$
17	$HOCH_2$
18	
19	
20	HO_2CCH_2

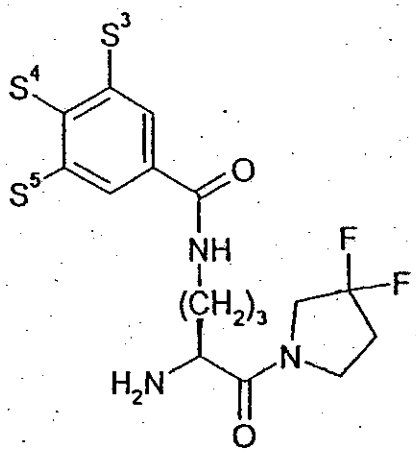
Ex No	R
21	
22	

EXAMPLES 23-29



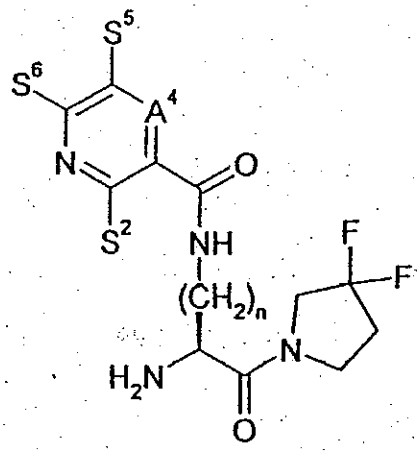
Ex No	n	R
23	3	
24	4	
25	4	NH_2
26	3	
27	4	
28	3	
29	3	

EXAMPLES 30-36



Ex No	S^3	S^4	S^5
31	CN	H	H
32	NO_2	H	H
33	Cl	H	Cl
34	H	Cl	H
35	Cl	H	H
36	CH_3	H	H

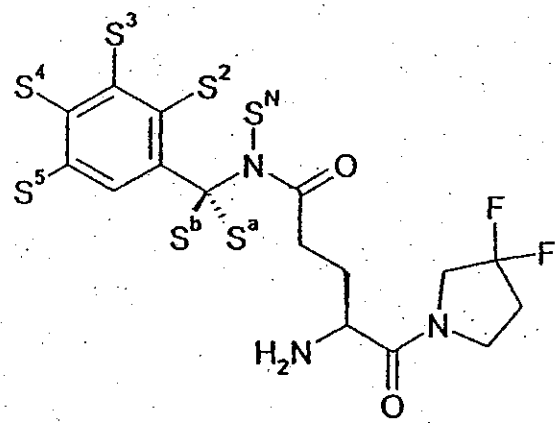
EXAMPLES 37-61



Ex No	n	S^2	A^4	S^5	S^6
37	3	H	CH	H	Cl
38	3	H	CH	H	CH_3
39	3	H	CH	H	CF_3
40	3	Cl	CH	H	Cl
41	3	Cl	CH	H	CH_3

Ex No	n	S^2	A^4	S^5	S^6
42	3	CH_3	CH	H	CF_3
43	3	H	N	-CH=CH-CH=CH-	
44	3	H	N	H	CH_3
45	3	H	CH	-CH=CH-CH=CH-	
46	3	H	CH	Br	H
47	3	H	CH	H	SH
48	3	H	CH	H	CN
49	3	OH	N	-CH=CH-CH=CH-	
50	3	Cl	CH	H	H
51	4	CO_2H	CH	H	H
52	4	H	CH	Cl	OH
53	4	H	C(Cl)	$-C(CH_3)=N-N(CH_3)-$	
54	4	H	CH	Cl	Cl
55	4	H	CH	-CH=CH-CH=CH-	
56	4	H	CH	Br	H
57	4	H	CH	CH_3	H
58	4	H	CH	H	SH
59	4	H	CH	H	CN
60	4	H	CH	H	CF_3
61	4	H	N	H	CH_3

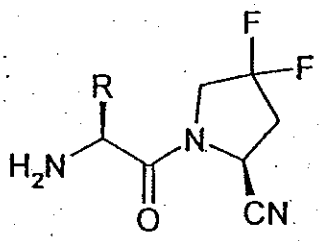
EXAMPLES 62-84



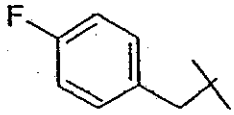
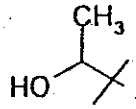
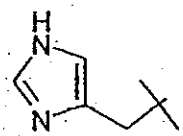
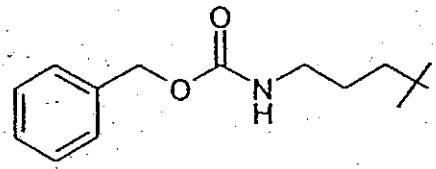
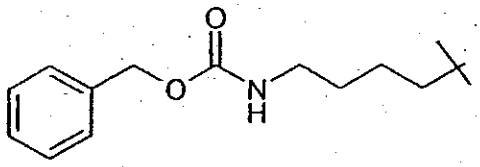
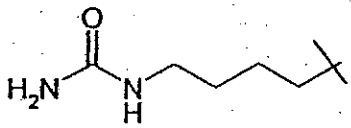
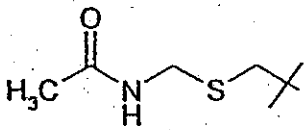
Ex No	S^a	S^b	S^N	S^2	S^3	S^4	S^5
62	H	H	H	Cl	H	H	H

Ex No	S^a	S^b	S^N	S^2	S^3	S^4	S^5
63	H	H	H	H	F	H	H
64	H	H	H	H	CF_3	H	H
65	H	H	H	H	H	F	H
66	H	H	H	H	H	Cl	H
67	H	H	H	H	CF_3	H	CF_3
68	H	H	H	H	Br	H	H
69	H	H	H	H	I	H	H
70	H	H	H	H	NO_2	H	H
71	H	H	H	H	H	NO_2	H
72	H	H	H	H	Cl	H	H
73	H	H	H	H	Cl	F	H
74	H	H	H	H	H	CH_3SO_2	H
75	H	H	-CH_2-CH_2-		H	H	H
76	H	H	H	CH_3SO_2	H	H	H
77	H	H	H	CH_3SO_2NHCO	H	H	H
78	H	H	H	H	H_2NCO	H	H
79	H	H	H	-CH=CH-CH=CH-		H	H
80	CH_3	H	H	H	H	H	H
81	H	CH_3	H	H	H	H	H
82	H	H	H	H	Cl	H	Cl
83	H	H	H	H	CH_3CO	H	H
84	H	H	H	H	CH_3	H	H

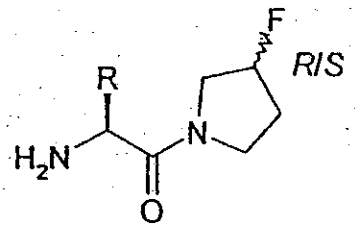
EXAMPLES 85-100

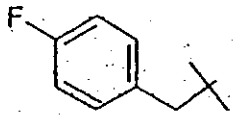
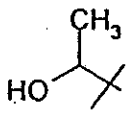
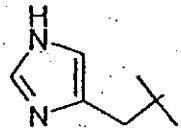


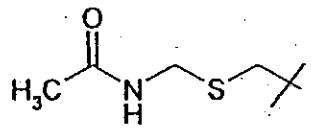
Ex No	R
85	Isopropyl
86	*n*-Butyl
87	*sec*-Butyl

Ex No	R
88	*tert*-Butyl
89	Cyclohexyl
90	Benzyl
91	
92	$CH_3S(CH_2)_2$
93	$HOCH_2$
94	
95	
96	HO_2CCH_2
97	
98	
99	
100	

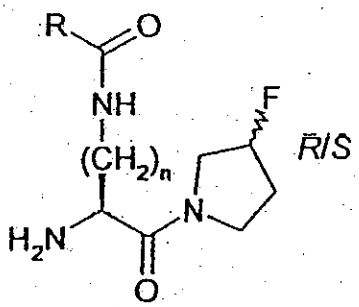
EXAMPLES 101-126

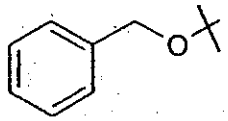
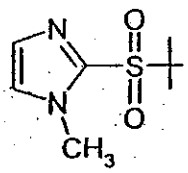


Ex No	*R/S*	R
101	R	Isopropyl
102	S	
103	R	*n*-Butyl
104	S	
105	R	*sec*-Butyl
106	S	
107	R	*tert*-Butyl
108	S	
109	R	Cyclohexyl
110	S	
111	R	Benzyl
112	S	
113	R	
114	S	
115	R	$CH_3S(CH_2)_2$
116	S	
117	R	$HOCH_2$
118	S	
119	R	
120	S	
121	R	
122	S	
123	R	HO_2CCH_2
124	S	

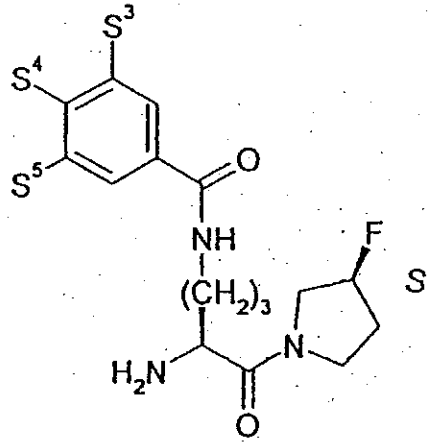
Ex No	*R/S*	R
125	R	
126	S	

EXAMPLES 127-134



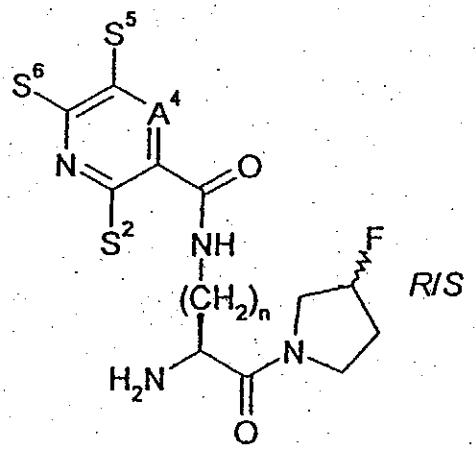
Ex No	*R/S*	n	R
127	*R*	3	
128	*R*	4	
129	*S*	3	
130	*S*	4	
131	*R*	4	NH_2
132	*S*		
133	*R*	3	
134	*S*		

EXAMPLES 135-139



Ex No	S^3	S^4	S^5
135	CN	H	H
136	NO_2	H	H
137	Cl	H	Cl
138	H	Cl	H
139	Cl	H	H

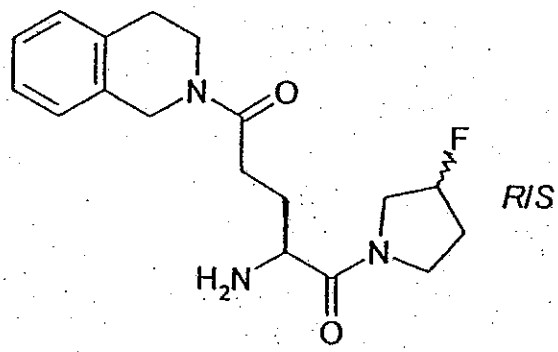
EXAMPLES 140-164



Ex No	*R/S*	n	S^2	A^4	S^6	S^6
140	*S*	3	H	CH	H	Cl
141	*S*	3	OH	CH	H	CH_3
142	*S*	3	H	CH	H	OH
143	*S*	3	H	CH	H	CH_3
144	*S*	3	H	CH	Cl	OH
145	*S*	3	H	C(Cl)	$-C(CH_3)=N-N(CH_3)-$	

Ex No	*R/S*	n	S^2	A^4	S^5	S^6
146	*S*	3	H	CH	Cl	Cl
147	*R*	3	H	CH	Cl	Cl
148	*S*	3	Cl	CH	H	Cl
149	*S*	3	Cl	CH	H	CH_3
150	*S*	3	H	N	-CH=CH-CH=CH-	
151	*S*	3	H	N	H	CH_3
152	*S*	3	OH	N	-CH=CH-CH=CH-	
153	*S*	3	Cl	CH	H	H
154	*S*	4	CO_2H	CH	H	H
155	*S*	4	H	CH	Cl	OH
156	*S*	4	H	C(Cl)	-C(CH_3)=N-N(CH_3)-	
157	*S*	4	H	CH	Cl	Cl
158	*S*	4	H	CH	-CH=CH-CH=CH-	
159	*S*	4	H	CH	Br	H
160	*S*	4	H	CH	Cl	OH
161	*S*	4	OH	CH	-CH=CH-CH=CH-	
162	*S*	4	H	CH	CH_3	H
163	*S*	4	H	CH	H	SH
164	*R*	4	H	N	-CH=CH-CH=CH-	

EXAMPLES 165-166



Ex No	*R/S*
165	*R*
166	*S*

EXAMPLE 167

Determination of activity

Compounds were assayed as inhibitors of DP-IV according to the methods described in WO95/15309. All the compounds described in the foregoing Examples were competitive inhibitors of DP-IV with K_i values less than 300nM, except for the compounds of Examples 7 and 8. These two compounds are prodrugs and do not show significant inhibition of DP-IV at concentrations up to 5μM.

EXAMPLE 168

Determination of activity *in vivo*

The anti-diabetic action of selected compounds was demonstrated in Zucker obese rats using a standard oral glucose tolerance test. Control rats were given a solution of glucose by oral gavage, and plasma glucose levels were determined. These rats demonstrated a significant hyperglycaemia. Compounds according to the present invention were dissolved in glucose solution at various concentrations, such that the rats could be given varying doses of the compound simultaneously with the glucose challenge. The hyperglycaemic excursion was reduced in a dose-dependent manner in animals receiving between 0.1 and 100 mg/kg of DP-IV inhibitor.

EXAMPLE 169

Pharmaceutical formulation

Tablets containing 100mg of the compound of Example 1 as the active agent are prepared from the following:

Compound of Example 1	200.0g
Corn starch	71.0g
Hydroxypropylcellulose	18.0g
Carboxymethylcellulose calcium	13.0g
Magnesium stearate	3.0g
Lactose	195.0g
Total	*500.0g*

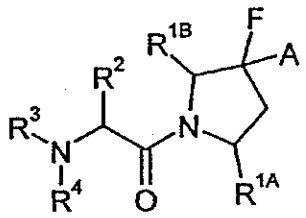
The materials are blended and then pressed to give 2000 tablets of 250mg, each containing 100mg of the compound of Example 1.

The above demonstrates that the compounds according to the present invention are inhibitors of DP-IV or prodrugs thereof and would accordingly be expected to be useful as therapeutic agents for the treatment of impaired glucose tolerance, type II diabetes, and other diseases where inhibition of this enzyme leads to an improvement in the underlying pathology or the symptoms.

The present invention is further defined in the following Claims.

CLAIMS

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



1

wherein:

A is F or H;

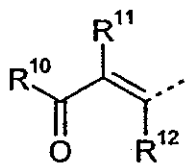
one of R^{1A} and R^{1B} is selected from H and CN and the other is H;

R^2 is selected from H, C_1 – C_8 alkyl, optionally substituted phenyl, optionally substituted benzyl and R^5; and

R^3 is selected from H, C_1 – C_8 alkyl, adamantyl, adamantylmethyl, adamantylethyl and Het-$NH(CH_2)_a$; or

R^2 and R^3 together constitute a chain of three or four methylene groups so as to form, together with the atoms to which they are attached, a pyrrolidine or piperidine ring, which ring may further be fused with a benzenoid ring;

R^4 is selected from H, R^6OCO, $H_2NCH(R^7)CO$, $H_2NCH(R^8)CONHCH(R^9)CO$, and a group according to general formula **2**;



2

R^5 is selected from CH_2R^{13}, $CH_2CH_2R^{13}$ and $C(R^{14})(R^{15})$-X^1-R^{16};

R^6 is selected from C_1 – C_6 alkyl, optionally substituted phenyl, optionally substituted

benzyl and $R^{17}CO_2C(R^{18})(R^{19})$;

R^7, R^8 and R^9 are each independently selected from the side chains of the proteinaceous amino acids;

R^{10} is selected from $C_1 - C_8$ alkyl, phenyl and O-($C_1 - C_8$ alkyl);

R^{11} is selected from H and $C_1 - C_8$ alkyl;

R^{12} is selected from H, $C_1 - C_8$ alkyl and phenyl;

R^{13} is selected from $CO\text{-}N(R^{20})(R^{21})$, $N(R^{22})\text{-}C(=X^2)R^{23}$ and $N(R^{22})(R^{24})$;

R^{14} and R^{15} are independently selected from H and methyl, or together are $-(CH_2)_z-$;

R^{16} is selected from $C_1 - C_8$ alkyl, optionally substituted phenyl, optionally substituted benzyl and $-(CH_2)_b\text{-}R^{13}$;

R^{17} is selected from H and $C_1 - C_8$ alkyl;

R^{18} and R^{19} are independently selected from H and $C_1 - C_8$ alkyl, or together are $-(CH_2)_y-$;

R^{20} and R^{21} are independently selected from H, $C_1 - C_8$ alkyl, optionally substituted phenyl, optionally substituted phenylalkyl, Het and $-(CH_2)_c$Het, or R^{20} and R^{21} together constitute a chain of four or five methylene groups so as to form, together with the nitrogen atom to which they are attached, a pyrrolidine or piperidine ring, which ring may further be fused with a benzenoid ring;

R^{22} is selected from H and methyl;

R^{23} is selected from R^{25}, $O\text{-}R^{25}$ and $N(R^{26})(R^{27})$;

R^{24} is selected from optionally substituted phenyl, Het and $-CH_2$-Het;

R^{25} is selected from $C_1 - C_8$ alkyl, optionally substituted phenyl, optionally substituted phenylalkyl, Het and $-(CH_2)_c$Het;

R^{26} and R^{27} are independently selected from H, $C_1 - C_8$ alkyl, optionally substituted phenyl, optionally substituted phenylalkyl, Het and $-(CH_2)_c$Het, or R^{26} and R^{27} together constitute a chain of four or five methylene groups so as to form, together with the nitrogen atom to which they are attached, a pyrrolidine or piperidine ring, which ring may further be fused with a benzenoid ring;

Het is an aromatic nitrogen-containing heterocycle selected from pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, imidazolyl, pyrazolyl, thiazolyl, isothiazolyl, oxazolyl, isoxazolyl and benz-fused analogues of these, all of which may optionally be substituted on one or more carbon atoms, and where the substituents are selected from lower alkyl, hydroxy, lower alkyloxy, amino, lower alkylamino, di(lower alkyl)amino, fluoro, chloro, bromo, trifluoromethyl, nitro, cyano, carboxy and lower alkyloxycarbonyl groups;

X^1 is selected from –O-, -S- and $-CH_2-$;

X^2 is selected from O and S;

a is 2 or 3;

b is 1, 2 or 3;

c is 1 or 2; and

y and z are 2, 3 or 4; with the proviso that the compound is not Ile-3-fluoropyrrolidine.

2 A compound according to Claim 1, or a pharmaceutically acceptable salt thereof, wherein R^{1A} and R^{1B} are both H.

3 A compound according to Claim 1, or a pharmaceutically acceptable salt thereof,

wherein R^{1A} is CN and R^{1B} is H.

4 A compound according to Claim 1, or a pharmaceutically acceptable salt thereof, wherein R^{1A} is H and R^{1B} is CN.

5 A compound according to any preceding Claim, or a pharmaceutically acceptable salt thereof, wherein A is F.

6 A compound according to any one of claims 1 to 4, or a pharmaceutically acceptable salt thereof, wherein A is H.

7 A compound according to any preceding Claim, or a pharmaceutically acceptable salt thereof, wherein R^4 is H.

8 A compound according to any preceding Claim, or a pharmaceutically acceptable salt thereof, wherein R^3 is H.

9 A compound according to any one of claims 1 to 7, or a pharmaceutically acceptable salt thereof, wherein R^2 is H and R^3 is selected from adamantyl, adamantylmethyl, adamantylethyl and Het-$NH(CH_2)_a$.

10 A compound according to Claim 9, or a pharmaceutically acceptable salt thereof, wherein R^3 is Het-$NH(CH_2)_a$.

11 A compound according to Claim 10, or a pharmaceutically acceptable salt thereof, wherein a is 2 and Het is 5-substituted-2-pyridyl.

12 A compound according to any one of claims 1 to 7, or a pharmaceutically acceptable salt thereof, wherein R^3 is H and R^2 is selected from C_1 – C_8 alkyl, optionally substituted phenyl, optionally substituted benzyl and R^5.

13 A compound according to Claim 12, or a pharmaceutically acceptable salt thereof, wherein R^2 is C_1 – C_8 alkyl.

14 A compound according to Claim 12, or a pharmaceutically acceptable salt thereof,

wherein R^2 is R^5.

15 A compound according to Claim 14, or a pharmaceutically acceptable salt thereof, wherein R^5 is selected from $CH_2CH_2R^{13}$ and $C(R^{14})(R^{15})$-X^1-R^{16}.

16 A compound according to Claim 15, or a pharmaceutically acceptable salt thereof, wherein R^5 is $CH_2CH_2R^{13}$ and R^{13} is CO-$N(R^{20})(R^{21})$.

17 A compound according to Claim 15, or a pharmaceutically acceptable salt thereof, wherein R^5 is $C(R^{14})(R^{15})$-X^1-R^{16}, R^{14} and R^{15} are independently selected from H and methyl, and R^{16} is $-(CH_2)_b$-R^{13}.

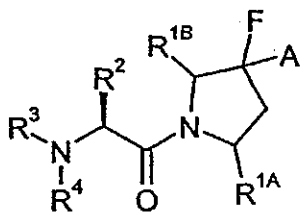
18 A compound according to Claim 17, or a pharmaceutically acceptable salt thereof, wherein R^{14} and R^{15} are both H, X^1 is CH_2 and b is 1 or 2.

19 A compound according to Claim 18, or a pharmaceutically acceptable salt thereof, wherein R^{13} is selected from $N(R^{22})$-$C(=X^2)R^{23}$ and $N(R^{22})(R^{24})$.

20 A compound according to Claim 19, or a pharmaceutically acceptable salt thereof, wherein R^{13} is $N(R^{22})$-$C(=X^2)R^{23}$, R^{22} is H and X^2 is O.

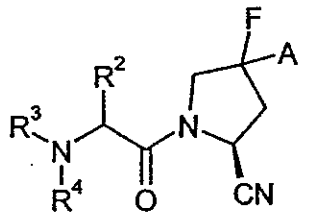
21 A compound according to Claim 20, or a pharmaceutically acceptable salt thereof, wherein R^{23} is Het.

22 A compound according to Claim 1 wherein R^2 is other than H and the absolute stereochemistry is as shown in general formula 3.



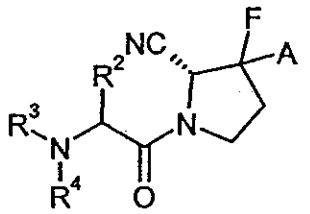
3

23 A compound according to Claim 1 wherein R^{1A} is CN, R^{1B} is H and the absolute stereochemistry is as shown in general formula **4**.



4

24 A compound according to Claim 1 wherein R^{1A} is H, R^{1B} is CN and the absolute stereochemistry is as shown in general formula **5**.



5

25 A pharmaceutical composition for human therapeutic use comprising at least one compound according to any preceding Claim, or a pharmaceutically acceptable salt thereof.

26 A composition according to Claim 25 for the treatment of type 2 diabetes or impaired glucose tolerance.

27 A composition according to Claim 25 for the treatment of growth hormone deficiency or polycystic ovary syndrome.

28 A composition according to Claim 25 for the treatment of auto-immune and inflammatory diseases.

29 The use of a compound according to any one of claims 1 to 24, or a pharmaceutically acceptable salt thereof, for the preparation of a pharmaceutical composition for the treatment of type 2 diabetes, impaired glucose tolerance, growth hormone deficiency, polycystic ovary syndrome, and auto-immune and inflammatory diseases.

30 At least one optical isomer of a compound according to any one of claims 1 to 21.

31 A tautomer of a compound according to any one of claims 1 to 24.

32. A compound substantially as described herein with reference to any example thereof, with the proviso that the compound is not Ile-3-fluoropyrrolidine.

33. A composition which includes a compound substantially as described herein with reference to any example thereof with the proviso that the compound is not Ile-3-fluoropyrrolidine.

34. The use of a compound substantially as described herein with reference to any example thereof for the preparation of a pharmaceutical composition for the treatment of type 2 diabetes, impaired glucose tolerance, growth hormone deficiency, polycystic ovary syndrome, and auto-immune and inflammatory diseases with the proviso that the compound is not Ile-3-fluoropyrrolidine.

35. At least one optical isomer of a compound substantially as described herein with reference to any example thereof with the proviso that the compound is not Ile-3-fluoropyrrolidine.

36. A tautomer of a compound substantially as described herein with reference to any example thereof with the proviso that the compound is not Ile-3-fluoropyrrolidine.