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Villhauer

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[54] **N-(SUBSTITUTED GLYCYL)-2-CYANOPYRROLIDINES, PHARMACEUTICAL COMPOSITIONS CONTAINING THEM AND THEIR USE IN INHIBITING DIPEPTIDYL PEPTIDASE-IV**

[75] Inventor: **Edwin Bernard Villhauer**, Morristown, N.J.

[73] Assignee: **Novartis AG**, Basle, Switzerland

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[51] **Int. Cl.**[7] **C07D 207/34**; C07D 207/42; C07D 401/06; C07D 405/10; C07D 409/06

[52] **U.S. Cl.** **544/333**; 544/326; 544/330; 546/208; 546/276.4; 546/279.1; 548/530; 548/540

[58] **Field of Search** 548/530, 540, 548/517; 546/276.4, 279.1, 208; 544/516, 330, 328, 333

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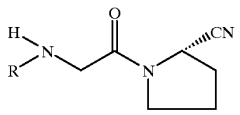
Primary Examiner—Floyd D. Higel
Attorney, Agent, or Firm—Joseph J. Borovian

[57] **ABSTRACT**

N-(N'-substituted glycyl)-2-cyanopyrrolidines of formula I

I



Compounds of formula I inhibit DPP-IV (dipeptidyl-peptidase-IV) activity. They are therefore indicated for use as pharmaceuticals in inhibiting DPP-IV and in the treatment of conditions mediated by DPP-IV, such as non-insulin-dependent diabetes mellitus, arthritis, obesity, osteoporosis and further conditions of impaired glucose tolerance.

11 Claims, No Drawings

N-(SUBSTITUTED GLYCYL)-2-CYANOPYRROLIDINES, PHARMACEUTICAL COMPOSITIONS CONTAINING THEM AND THEIR USE IN INHIBITING DIPEPTIDYL PEPTIDASE-IV

This application claims the benefit of Provisional Application number 60/030,570 filed on Nov. 7, 1996.

FIELD OF THE INVENTION

The present invention relates to the area of dipeptidyl peptidase-TV (DPP-IV) inhibition. DPP-IV is a serine protease which cleaves N-terminal dipeptides from a peptide chain containing, preferably, a proline residue in the penultimate position. Although the biological role of DPP-IV in mammalian systems has not been completely established, it is believed to play an important role in neuropeptide metabolism, T-cell activation, attachment of cancer cells to the endothelium and the entry of HIV into lymphoid cells.

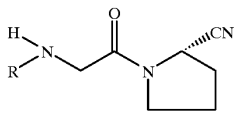
More recently, it was discovered that DPP-IV is responsible for inactivating glucagon-like peptide-1 (GLP-1). More particularly, DPP-IV cleaves the amino-terminal His-Ala dipeptide of GLP-1, generating a GLP-1 receptor antagonist, and thereby shortens the physiological response to GLP-1. Since the half-life for DPP-IV cleavage is much shorter than the half-life for removal of GLP-1 from circulation, a significant increase in GLP-1 bioactivity (5- to 10-fold) is anticipated from DPP-IV inhibition. Since GLP-1 is a major stimulator of pancreatic insulin secretion and has direct beneficial effects on glucose disposal, DPP-IV inhibition appears to represent an attractive approach for treating non-insulin-dependent diabetes mellitus (NIDDM).

SUMMARY OF THE INVENTION

The present invention provides new DPP-IV inhibitors which are effective in treating conditions mediated by DPP-IV. More particularly, the present invention relates to certain N-(substituted glycyl)-2-cyanopyrrolidines which inhibit DPP-IV. In addition, the present invention provides pharmaceutical compositions useful in inhibiting DPP-IV comprising a therapeutically effective amount of a N-(substituted glycyl)-2-cyanopyrrolidine disclosed herein. Moreover, the present invention provides a method of inhibiting DPP-IV comprising administering to a mammal in need of such treatment a therapeutically effective amount of a N-(substituted glycyl)-2-cyanopyrrolidine.

DETAILED DESCRIPTION OF THE INVENTION

The instant invention relates to novel N-(substituted glycyl)-2-cyanopyrrolidines of formula I:



I

wherein R is:

a) $R_1R_{1a}N(CH_2)_m$- wherein
   R_1 is a pyridinyl or pyrimidinyl moiety optionally mono- or independently disubstituted with (C_{1-4})alkyl, (C_{1-4})alkoxy, halogen, trifluoromethyl, cyano or nitro; or phenyl optionally mono- or independently disubstituted with (C_{1-4})alkyl, (C_{1-4})alkoxy or halogen;

   R_{1a} is hydrogen or (C_{1-8})alkyl; and
   m is2or3;

b) (C_{3-12})cycloalkyl optionally monosubstituted in the 1-position with (C_{1-3})hydroxyalkyl;

c) $R_2(CH_2)_n$- wherein either
   R_2 is phenyl optionally mono- or independently di- or independently trisubstituted with (C_{1-4})alkyl, (C_{1-4})alkoxy, halogen or phenylthio optionally monosubstituted in the phenyl ring with hydroxymethyl; or is (C_{1-8})alkyl; a [3.1.1]bicyclic carbocyclic moiety optionally mono- or plurisubstituted with (C_{1-8})alkyl; a pyridinyl or naphthyl moiety optionally mono- or independently disubstituted with (C_{1-4})alkyl, (C_{1-4})alkoxy or halogen; cyclohexene; or adamantyl; and
   n is 1 to 3; or
   R_2 is phenoxy optionally mono- or independently disubstituted with (C_{1-4})alkyl, (C_{1-4})alkoxy or halogen; and
   n is 2 or 3;

d) $(R_3)_2CH(CH_2)_2$- wherein each R_3 independently is phenyl optionally mono- or independently disubstituted with (C_{1-4})alkyl, (C_{1-4})alkoxy or halogen;

e) $R_4(CH_2)_p$- wherein R_4 is 2-oxopyrrolidinyl or (C_{2-4})alkoxy and p is2to4;

f) isopropyl optionally monosubstituted in 1-position with (C_{1-3})hydroxyalkyl;

g) R_5 wherein R_5 is: indanyl; a pyrrolidinyl or piperidinyl moiety optionally substituted with benzyl; a [2.2.1]- or [3.1.1]bicyclic carbocyclic moiety optionally mono- or plurisubstituted with (C_{1-8})alkyl; adamantyl; or (C_{1-8})alkyl optionally mono- or independently plurisubstituted with hydroxy, hydroxymethyl or phenyl optionally mono- or independently disubstituted with (C_{1-4})alkyl, (C_{1-4})alkoxy or halogen;

in free form or in acid addition salt form.

The compounds of formula I can exist in free form or in acid addition salt form. Salt forms may be recovered from the free form in known manner and vice-versa. Acid addition salts may e.g. be those of pharmaceutically acceptable organic or inorganic acids. Although the preferred acid addition salts are the hydrochlorides, salts of methanesulfonic, sulfuric, phosphoric, citric, lactic and acetic acid may also be utilized.

The compounds of the invention may exist in the form of optically active isomers or diastereoisomers and can be separated and recovered by conventional techniques, such as chromatography.

"Alkyl" and "alkoxy" are either straight or branched chain, of which examples of the latter are isopropyl and tert-butyl.

R preferably is a), b) or e) as defined above. R_1 preferably is a pyridinyl or pyrimidinyl moiety optionally substituted as defined above. R_{1a} preferably is hydrogen. R_2 preferably is phenyl optionally substituted as defined above. R_3 preferably is unsubstituted phenyl. R_4 preferably is alkoxy as defined above. R_5 preferably is optionally substituted alkyl as defined above. m preferably is 2. n preferably is 1 or 2, especially 2. p preferably is 2 or 3, especially 3.

Pyridinyl preferably is pyridin-2-yl; it preferably is unsubstituted or monosubstituted, preferably in 5-position. Pyrimidinyl preferably is pyrimidin-2-yl. It preferably is unsubstituted or monosubstituted, preferably in 4-position. Preferred as substitutents for pyridinyl and pyrimidinyl are halogen, cyano and nitro, especially chlorine.

When it is substituted, phenyl preferably is monosubstituted; it preferably is substituted with halogen, preferably

chlorine, or methoxy. It preferably is substituted in 2-, 4- and/or 5-position, especially in 4-position.

(C_{3-12})cycloalkyl preferably is cyclopentyl or cyclohexyl. When it is substituted, it preferably is substituted with hydroxymethyl. (C_{1-4})alkoxy preferably is of I or 2 carbon atoms, it especially is methoxy. (C_{2-4})alkoxy preferably is of 3 carbon atoms, it especially is isopropoxy. Halogen is fluorine, chlorine, bromine or iodine, preferably fluorine, chlorine or bromine, especially chlorine. (C_{1-8})alkyl preferably is of 1 to 6, preferably I to 4 or 3 to 5, especially of 2 or 3 carbon atoms, or methyl. (C_{1-4}) alkyl preferably is methyl or ethyl, especially methyl. (C_{1-3})hydroxyalkyl preferably is hydroxymethyl.

A [3.1.1]bicyclic carbocyclic moiety optionally substituted as defined above preferably is bicyclo[3.1.1]hept-2-yl optionally disubstituted in 6-position with methyl, or bicyclo [3.1.1]hept-3-yl optionally trisubstituted with one methyl in 2-position and two methyl groups in 6-position. A [2.2.1] bicyclic carbocyclic moiety optionally substituted as defined above preferably is bicyclo[2.2.1]hept-2-yl.

Naphthyl preferably is 1-naphthyl. Cyclohexene preferably is cyclohex-1-en-1-yl. Adamantyl preferably is 1- or 2-adamantyl.

A pyrrolidinyl or piperidinyl moiety optionally substituted as defined above preferably is pyrrolidin-3-yl or piperidin-4yl. When it is substituted it preferably is N-substituted.

A preferred group of compounds of the invention is the compounds of formula I wherein R is R' (compounds Ia), whereby R' is:

- R_1'$NH(CH_2)_2$- wherein R_1' is pyridinyl optionally mono- or independently disubstituted with halogen, trifluoromethyl, cyano or nitro; or unsubstituted pyrimidinyl;
- (C_{3-7})cycloalkyl optionally monosubstituted in 1-position with (C_{1-3})hydroxyalkyl;
- R_4'$(CH_2)_3$- wherein R_4' is (C_{2-4})alkoxy; or
- R_5, wherein R_5 is as defined above;

in free form or in acid addition salt form.

More preferred compounds of the invention are those compounds of formula I wherein R is R" (compounds Ib), whereby R" is:

- R_1"$NH(CH_2)_2$- wherein R_1" is pyridinyl mono- or independently disubstituted with halogen, trifluoromethyl, cyano or nitro;
- (C_{4-6})cycloalkyl monosubstituted in 1-position with (C_{1-3})hydroxyalkyl;
- R_4'$(CH_2)_3$- wherein R_4' is as defined above; or
- R_5' wherein R_5' is a [2.2.1]- or [3.1.1]bicyclic carbocyclic moiety optionally mono- or plurisubstituted with (C_{1-8})alkyl; or adamantyl;

in free form or in acid addition salt form.

Even more preferred compounds of the invention are the compounds of formula I wherein R is R'" (compounds Ic), whereby R'" is:

- R_1"$NH(CH_2)_2$- wherein R_1" is as defined above;
- (C_{4-6})cycloalkyl monosubstituted in 1-position with hydroxymethyl;
- R_4'$(CH_2)_3$- wherein R_4' is as defined above; or
- R_5" wherein R_5" is adamantyl;

in free form or in acid addition salt form.

A further group of compounds of the invention is compounds Ip, wherein R is R^P, which is:

a) $R_1{}^P NH(CH_2)_2$- wherein $R_1{}^P$ is a pyridinyl or pyrimidinyl moiety optionally mono- or independently disubstituted with halogen, trifluoromethyl, cyano or nitro;

b) (C_{3-7})cycloalkyl optionally monosubstituted in 1-position with (C_{1-3})hydroxyalkyl;

c) $R_2{}^P(CH_2)_2$- wherein $R_2{}^P$ is phenyl optionally mono- or independently di- or independently trisubstituted with halogen or (C_{1-3})alkoxy;

d) $(R_3{}^P)_2CH(CH_2)_2$- wherein each $R_3{}^P$ independently is phenyl optionally monosubstituted with halogen or (C_{1-3})alkoxy;

e) $R_4(CH_2)_3$- wherein R_4 is as defined above; or

f) isopropyl optionally monosubstituted in 1-position with (C_{1-3})hydroxyalkyl;

in free form or in pharmaceutically acceptable acid addition salt form.

A further group of compounds of the invention is compounds Is, wherein R is R^s, which is:

a) $R_1{}^s R_{1a}{}^s(CH_2)_{ms}$- wherein $R_1{}^s$ is pyridinyl optionally mono- or independently disubstituted with chlorine, trifluoromethyl, cyano or nitro; pyrimidinyl optionally monosubstituted with chlorine or trifluoromethyl; or phenyl; $R_{1a}{}^s$ is hydrogen or methyl; and ms is 2or3;

b) (C_{3-12})cycloalkyl optionally monosubstituted in 1-position with hydroxymethyl;

c) $R_2{}^s(CH_2)_{ms}$- wherein either
$R_2{}^s$ is phenyl optionally mono- or independently di- or independently trisubstituted with halogen, alkoxy of 1 or 2 carbon atoms or phenylthio monosubstituted in the phenyl ring with hydroxymethyl; (C_{1-6})alkyl; 6,6-dimethylbicyclo[3.1.1]hept-2-yl; pyridinyl; naphthyl; cyclohexene; or adamantyl; and
ns is 1 to 3; or
$R_2{}^s$ is phenoxy; and ns is 2;

d) (3,3-diphenyl)propyl;

e) $R_4{}^s(CH_2)_{ps}$wherein $R_4{}^s$ is 2-oxopyrrolidin-1 -yl or isopropoxy and ps is2or3;

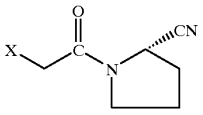
f) isopropyl optionally monosubstituted in 1-position with hydroxymethyl;

g) $R_5{}^s$ wherein $R_5{}^s$ is: indanyl; a pyrrolidinyl or piperidinyl moiety optionally N-substituted with benzyl; bicyclo[2.2.1]hept-2-yl; 2,6,6trimethylbicyclo-[3.1.1] hept-3-yl; adamantyl; or (C_{1-8})alkyl1 optionally mono- or independently disubstituted with hydroxy, hydroxymethyl or phenyl;

in free form or in acid addition salt form.

The compounds of the invention may be prepared by a process which comprises coupling a reactive (2-cyanopyrrolidino)carbonylmethylene compound with an appropriate substituted amine; more particularly, for the preparation of the compounds of formula I it comprises reacting a compound of formula II

II



wherein X is a reactive group,
with a compound of formula III

NH_2R III

wherein R is as defined above,
and recovering the resultant compound of formula I in free form or in acid addition salt form.

X preferably is a halogen such as bromine, chlorine or iodine.

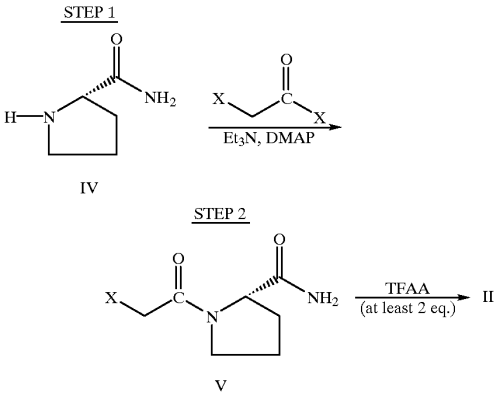
The process of the invention may be effected in conventional manner.

The compound of formula II is preferably reacted with at least 3 equivalents of a primary amine of formula III. The reaction is conveniently conducted in the presence of an inert, organic solvent, preferably a cyclic ether such as tetrahydrofuran. The temperature preferably is of from about 0° to about 35° C., preferably between about 0° and about 25° C.

The compounds of the invention may be isolated from the reaction mixture and purified in conventional manner, e.g. by chromatography.

The starting materials may also be prepared in conventional manner.

The compounds of formula II may e.g. be prepared by the following two-step reaction scheme:

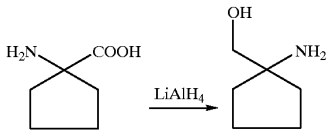


Step 1 involves the reaction of the pyrrolidine of formula IV with a slight molar excess of a haloacetylhalide such as bromoacetylbromide or chloroacetylchloride and triethylamine and a catalytic amount of dimethylaminopyridine (DMAP). The reaction conveniently is conducted in the presence of an inert, organic solvent, preferably a chlorinated, aliphatic hydrocarbon such as methylene chloride, at a temperature of from about 0° to about 25° C., preferably at a temperature between about 0° and about 15° C.

Step 2 concerns the dehydration of the compound of formula V, prepared in Step 1, with at least 2 equivalents of trifluoroacetic anhydride (TFAA). The dehydration preferably is conducted in the presence of an inert, organic solvent such as tetrahydrofuran or a chlorinated, aliphatic hydrocarbon such as methylene chloride, at a temperature of from about 0° to about 25° C., preferably at a temperature between about 0° and about 15° C.

Insofar as its preparation is not particularly described herein, a compound used as starting material is known or may be prepared from known compounds in known manner or analogously to known methods or analogously to methods described in the Examples.

For example, the primary amine compounds of formula III are known and may be prepared by procedures documented in the literature. More particularly,: a) 1-hydroxymethylcyclopentylamine can be prepared by the reduction of 1-amino-1-cyclopentane carboxylic acid with lithium aluminum hydride as set forth below:



The reduction is conducted in the presence of an inert, organic solvent, preferably a cyclic ether such as tetrahydrofuran, at the reflux temperature of the solvent for a period of between 14 and 24 hours. (b) 2-[(5-chloropyridin-2-yl)amino]ethylamine can be prepared by refluxing a mixture of 2,5-dichloropyridine with ethylenediamine in an oil bath for a period of between 6 and 12 hours. (c) Similarly, 2-[(5-trifluoromethylpyridin-2-yl) amino]ethylamine can be prepared by refluxing a mixture of 2-chloro-5-trifluoromethyl pyridine with ethylenediamine in an oil bath for a period of between 6 and 12 hours. (d) 2-[(5-cyanopyridin-2-yl)amino]-ethylamine can be prepared by stirring a mixture of 2-chloropyridine-5-carbonitrile and ethylenediamine at a temperature between 20° and 30° C., for a period of between 4 and 6 hours. (e) 2-[(pyrimidin-2-yl)amino]ethylamine can be prepared by adding ethylenediamine to ice-bath cooled 2-chloropyrimidine and allowing the mixture to react at a temperature between 20° and 30° C., for a period of between 12 and 20 hours. (f) 1-amino-1-cyclohexanemethanol can be prepared by the reduction of 1-amino-1-cyclohexane carboxylic acid with lithium aluminum hydride. The reduction is conducted in the presence of an inert, organic solvent, preferably a cyclic ether such as tetrahydrofuran, at the reflux temperature of the solvent for a period of between 14 and 24 hours. (g) 2(3-aminopropylamino)-5-cyanopyridine can be prepared by refluxing a mixture of 2,5-dichloropyridine with 1,3 propyl diamine in an oil bath for a period of between 6 and 12 hours. Alternatively, the above examples (a) through (g) may be carried out at room temperature.

The instant invention also includes pharmaceutical compositions useful in inhibiting DPP-IV comprising a pharmaceutically acceptable carrier or diluent and a therapeutically effective amount of a compound of formula 1, or a pharmaceutically acceptable acid addition salt thereof.

In still another embodiment, the instant invention provides a method of inhibiting DPP-IV comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound of formula I, or a pharmaceutically acceptable acid addition salt thereof.

In a further embodiment, the instant invention provides a method of treating conditions mediated by DPP-IV inhibition comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound of formula I above, or a pharmaceutically acceptable acid addition salt thereof.

As indicated above, all of the compounds of formula 1, and their corresponding pharmaceutically acceptable acid addition salts, are useful in inhibiting DPP-IV. The ability of the compounds of formula I, and their corresponding pharmaceutically acceptable acid addition salts, to inhibit DPP-IV may be demonstrated employing the Caco-2 DPP-IV Assay which measures the ability of test compounds to inhibit DPP-IV activity from human colonic carcinoma cell extracts. The human colonic carcinoma cell line Caco-2 was obtained from the American Type Culture Collection (ATCC HTB 37). Differentiation of the cells to induce DPP-IV expression was accomplished as described by Reisher, et al. in an article entitled "Increased expression of . . . intestinal

cell line Caco-2" in Proc. Natl. Acad. Sci., Vol. 90, pgs. 5757–5761 (1993). Cell extract is prepared from cells solubilized in 10 mM Tris-HCl, 0.15 M NaCl, 0.04 t.i.u. aprotinin, 0.5% nonidet-P40, pH 8.0, which is centrifuged at 35,000 g for 30 min. at 4° C. to remove cell debris. The assay is conducted by adding 20 μg solubilized Caco-2 protein, diluted to a final volume of 125 pi in assay buffer (25 mM Tris-HCl pH 7.4, 140 mM NaCl, 10 mM KCl, 1% bovine serum albumin) to microtiter plate wells. The reaction is initiated by adding 25 μl of 1 mM substrate (H-Alanine-Proline-pNA; pNA is p-nitroaniline). The reaction is run at room temperature for 10 minutes after which time a 19 μl volume of 25% glacial acetic acid is added to stop the reaction. Test compounds are typically added as 30 μl additions and the assay buffer volume is reduced to 95 μl. A standard curve of free p-nitroaniline is generated using 0–500 μM solutions of free pNA in assay buffer. The curve generated is linear and is used for interpolation of substrate consumption (catalytic activity in nmoles substrate cleaved/min). The endpoint is determined by measuring absorbance at 405 nm in a Molecular Devices UV Max microfiter plate reader. The potency of the test compounds as DPP-IV inhibitors, expressed as IC_{-50}, is calculated from 8-point, dose-response curves using a 4-parameter logistic function.

The following IC_{50}s were obtained:

Compound	Caco-2 DPP-IV (nM)
Ex. 1	36
Ex. 2	176
Ex. 3	22
Ex. 4	140
Ex. 5	26
Ex. 6	50
Ex. 7A	165
Ex. 8	8
Ex. 7B	175
Ex. 9A	990
Ex. 7C	290
Ex. 9C	295
Ex. 10	54
Ex. 11	215
Ex. 7D	382
Ex. 7E	388
Ex. 12	279
Ex. 13	227
Ex. 14	110
Ex. 15	150
Ex. 16	130
Ex. 17	60
Ex. 18	100
Ex. 19	120
Ex. 20	90
Ex. 21	390
Ex. 22	150
Ex. 23	50
Ex. 24	70
Ex. 25	140
Ex. 26	170
Ex. 27	310
Ex. 28	90
Ex. 29	130
Ex. 30	650
Ex. 31	500
Ex. 32	150
Ex. 33	10
Ex. 34	37
Ex. 35	130
Ex. 36	160
Ex. 37	220
Ex. 38	50
Ex. 39	380
Ex. 40	240
Ex. 41	140
Ex. 42	240
Ex. 43	850
Ex. 44	5
Ex. 45	700
Ex. 46	150
Ex. 47	10
Ex. 48	35
Ex. 49	12
Ex. 50	23
Ex. 51	250
Ex. 52	20
Ex. 53	860
Ex. 54	240
Ex. 55	270
Ex. 56	350
Ex. 57	470
Ex. 58	50
Ex. 59	390
Ex. 60	600
Ex. 61	310
Ex. 62	270
Ex. 63	46
Ex. 64	220
Ex. 65	80
Ex. 66	60

The ability of the compounds of formula I, and their corresponding pharmaceutically acceptable acid addition salts, to inhibit DPP-IV may also be demonstrated by measuring the effects of test compounds on DPP-IV activity in human and rat plasma employing a modified version of the assay described by Kubota, et al. in an article entitled "Involvement of dipeptidylpeptidase IV in an in vivo immune response" in Clin. Exp. Immunol., Vol. 89, pgs. 192–197 (1992). Briefly, five μl of plasma are added to 96-well flat-bottom mictotiter plates (Falcon), followed by the addition of 5 μl of 80 mM $MgCl_2$ in incubation buffer (25 mM HEPES, 140 mM NaCl, 1% RIA-grade BSA, pH 7.8). After a 5 min. incubation at room temperature, the reaction is initiated by the addition of 10 μl of incubation buffer containing 0.1 mM substrate (H-Glycine-Proline-AMC; AMC is 7-amino4-methylcoumarin). The plates are covered with aluminum foil (or kept in the dark) and incubated at room temperature for 20 min. After the 20 min. reaction, fluorescence is measured using a CytoFluor 2350 fluorimeter (Excitation 380 nm Emission 460 nm; sensitivity setting 4). Test compounds are typically added as 2 μl additions and the assay buffer volume is reduced to 13 μl. A fluorescence-concentration curve of free AMC is generated using 0–50 μM solutions of AMC in assay buffer. The curve generated is linear and is used for interpolation of substrate consumption (catalytic activity in nmoles substrate cleaved/min). As with the previous assay, the potency of the test compounds as DPP-IV inhibitors, expressed as IC_{50}, is calculated from 8-point, dose-response curves using a 4 parameter logistic function.

The following IC_{50}s were obtained:

Compound	human plasma DPP-IV (nM)	rat plasma DPP-IV (nM)
Ex. 1	27	22
Ex. 3	7	6
Ex. 4	40	23
Ex. 5	37	18
Ex. 6	22	32
Ex. 8	12	11
Ex. 10	51	19
Ex. 12	95	38

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