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Bioorganic & Medicinal Chemistry Letters 15 (2005) 2441–2445
Bioorganic & Medicinal Chemistry Letters

1-((*S*)-γ-Substituted prolyl)-(*S*)-2-cyanopyrrolidine as a novel series of highly potent DPP-IV inhibitors

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Received 23 February 2005; revised 9 March 2005; accepted 22 March 2005
Available online 12 April 2005

Abstract—1-(γ-Substituted prolyl)-(*S*)-2-cyanopyrrolidines were designed based on the predicted binding mode of the known DPP-IV inhibitor NVP-DPP728 and evaluated for their inhibitory activity. In structure–activity relationship study at the γ-position of proline, it became clear that compounds bearing (*S*)-stereochemistry were 20-fold more potent than the antipode. Of these compounds, the (3,4-dicyanophenyl)amino- and (3-chloro-4-cyanophenyl)amino-derivatives showed the highest inhibitory activity.

Dipeptidyl peptidase-IV (EC 3.4.14.5, DPP-IV) is a member of the serine protease family that recognizes an amino acid sequence having proline or alanine at the second position from the N-terminal and produces dipeptide.[1] DPP-IV is widely distributed in mammalian tissues and plays several physiological roles; in particular its role as a peptidase that rapidly inactivates glucagon-like peptide-1 (GLP-1) has drawn interest.[2] GLP-1 is secreted in response to meal ingestion and stimulates insulin secretion.[3] It has been suggested that potentiation and extension of the action of GLP-1 by DPP-IV inhibition would stimulate insulin secretion only after meals,[4] and DPP-IV inhibitors have therefore come to be seen as a potential new type of anti-diabetic agent free of side effects such as hypoglycemia and exhaustion of pancreatic beta-cells.

Although a number of DPP-IV inhibitors have been reported and classified into structural types, most of them are substrate analogs of the P_2–P_1 fragment.[5] Dipeptide inhibitors containing (*S*)-2-cyanopyrrolidine as a proline mimic at the P_1 site and an aliphatic amino acid at the P_2 site, for example, **1** (Fig. 1), have been reported as reversible and potent inhibitors.[6] Hughes et al. investigated a series of DPP-IV inhibitors, 1-(*N*-substituted glycyl)-2-cyanopyrrolidides, NVP-DPP728 (**2**), and NVP-LAF237 (**3**), the latter is under clinical trials as anti-diabetic agent (Fig. 1).[7–9] As the mode of NVP-DPP728 binding to DPP-IV, they estimated that hydrophobic interaction in the S_2 pocket stabilizes the P_2 site side-chain binding.[7] This description suggests that, when NVP-DPP728 displays inhibitory activity, the [2-(5-cyanopyridyl)amino]ethyl moiety assumes not an extended but a folded conformation, as shown in Figure 2a.

We hypothesized that analogs, which have a rigid conformation capable of interacting with the S_2 pocket of DPP-IV show more potent inhibitory activity. As prolyl-(*S*)-2-cyanopyrrolidine[6] (**4**) has a comparable inhibitory activity to NVP-DPP728, and as most of the proline derivatives are commercially available, we selected a proline structure for the P_2 site and introduced several functional groups at the γ-position. In this way we designed a series of 1-(γ-substituted prolyl)-(*S*)-2-cyanopyrrolidine compounds (Fig. 2b) as conformationally constrained analogs of NVP-DPP728 and evaluated them for inhibitory activity.

The (*R*)-substituted derivative **8** was synthesized from the *cis*-hydroxyproline **5** (Scheme 1). Conversion of the

Keywords: DPP-IV inhibitor; 2-Cyanopyrrolidine.
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doi:10.1016/j.bmcl.2005.03.077

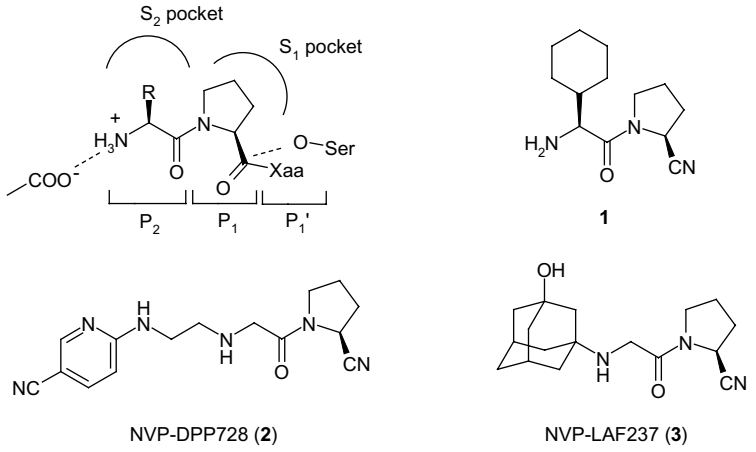


Figure 1.

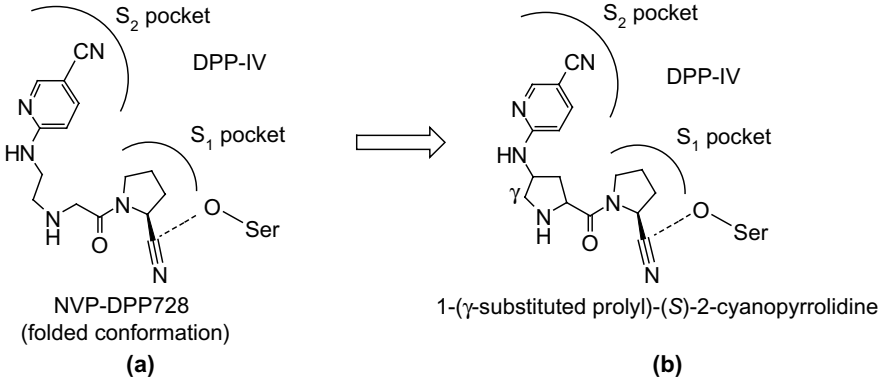
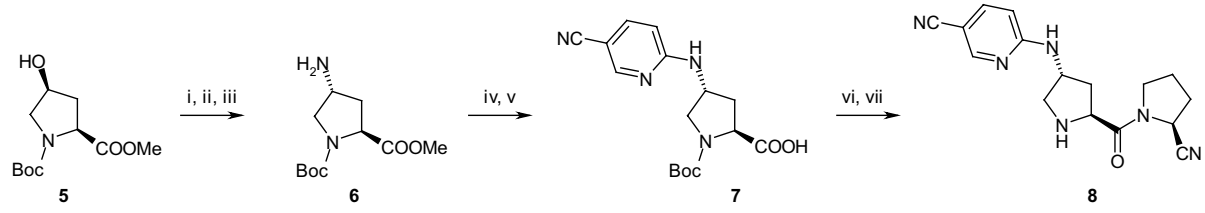


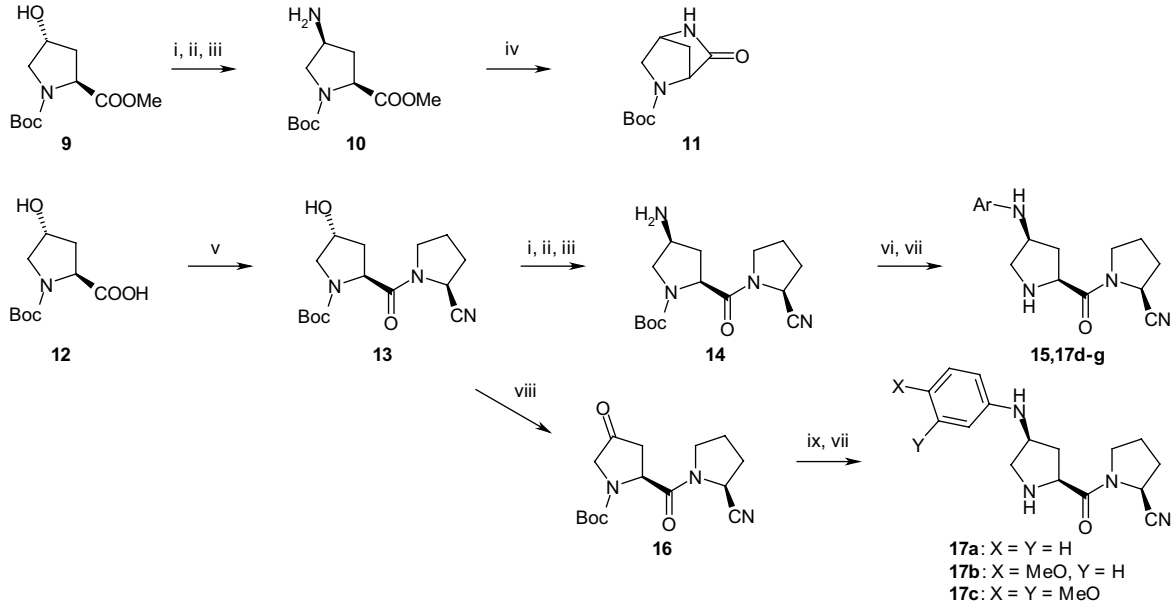
Figure 2. Design of 1-(γ-substituted prolyl)-(*S*)-2-cyanopyrrolidine.



Scheme 1. Reagents: (i) MsCl, Et_3N; (ii) NaN_3; (iii) H_2, $Pd/BaSO_4$; (iv) 2-chloro-5-cyanopyridine, Et_3N; (v) NaOH aq; (vi) (*S*)-2-cyanopyrrolidine·HCl, HOBt, WSCI, Et_3N; (vii) H^+.

mesylate of **5** to an azide compound and subsequent catalytic hydrogenation gave the *trans*-γ-amino proline derivative **6**. Reaction of **6** with 2-choloro-5-cyanopyridine in the presence of Et_3N at room temperature followed by alkaline hydrolysis gave the *trans*-γ-pyridylaminoproline compound **7**. The acid **7** was coupled with (*S*)-2-cyanopyrrolidine[6] and treated with HCl/AcOEt to afford the desired constrained analog of NVP-DPP728 (**8**).

The syntheses of (*S*)-substituted derivatives are shown in Scheme 2. The *cis*-amino proline **10** was prepared from the *trans*-hydroxyproline **9** and an attempt was made to introduce a pyridine moiety under the same conditions as described above. However, no reaction occurred at room temperature and intramolecular cyclization at high temperature resulted in the lactam **11**. In order to avoid this cyclization, a pyridyl group was introduced into the amide **14**. Amidation of the *trans*-hydroxyproline **12** with (*S*)-2-cyanopyrrolidine,[6] followed by transformation of the *trans*-hydroxy group to a *cis*-amino group, afforded the amine **14**. Reaction of the latter with 2-chloro-5-cyanopyridine or other electron-deficient aromatic halides and subsequent removal of the Boc group afforded the (*S*)-substituted derivatives **15** and **17d**–**g**. Meanwhile, the non-electron-deficient phenyl



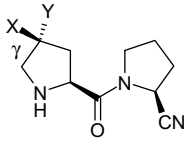
Scheme 2. Reagents: (i) MsCl, Et_3N; (ii) NaN_3; (iii) H_2, $Pd/BaSO_4$; (iv) 2-chloro-5-cyanopyridine, Et_3N; (v) (*S*)-2-cyanopyrrolidine·HCl, HOBt, WSCI, Et_3N; (vi) Ar–Cl or Ar–F, Et_3N or *N*-methylmorpholine; (vii) H^+; (viii) DMSO, SO_3–pyridine complex, Et_3N; (ix) Ar–NH_2, $NaBH_3CN$, AcOH.

derivatives **17a**–**c** were prepared by reductive amination of the ketone **16** with anilines and subsequent deprotection.

Table 1 summarizes the DPP-IV inhibitory effects of the conformationally restricted NVP-DPP728 analogs represented by 1-prolyl-(*S*)-2-cyanopyrrolidines having a (5-cyano-2-pyridyl)amino group at the γ-position of the proline moiety.[10] Compound **15**, bearing (*S*)-stereochemistry at the γ-position, showed 5–10-fold more potent inhibitory activity than NVP-DPP728 or the non-substituted derivative **4**.[6] The (*R*)-isomer **8** was less potent than the (*S*)-isomer **15**. This result indicates that the pyridylamino group of the (*S*)-isomer **15** is able to adapt to the S_2 pocket of DPP-IV, whereas that of the (*R*)-isomer **8** cannot achieve interaction at this site. As expected, conformationally restricted analogs, which mimic the constrained structure of NVP-DPP728 showed more potent inhibitory effect. These findings indicate that the 1-((*S*)-γ-substituted prolyl)-(*S*)-2-cyanopyrrolidine has a structure suited to DPP-IV inhibition.

Next, the effect of substituents at the γ-position of the proline moiety was investigated (Table 2). Introduction of an amino or cyclohexylamino group (**18**, **19**) resulted in a decrease in DPP-IV inhibitory activity compared to the (5-cyano-2-pyridyl)amino compound **15**.

Table 1.



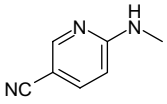
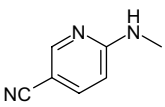
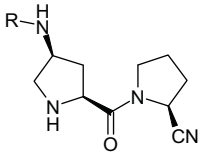
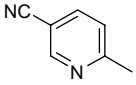
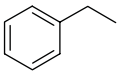
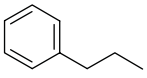
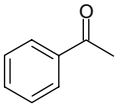
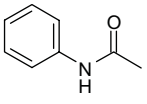
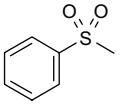
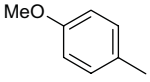
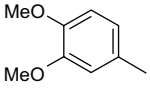
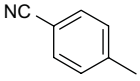
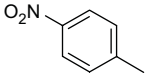
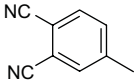
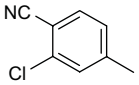
Compound no.	Configuration at γ position	X	Y	DPP-IV inhibition, IC_{50} (nmol/L)	
				Human	Rat
15	*S*		H	0.25	0.27
8	*R*	H		6.6	6.7
4	—	H	H	2.9	2.9
NVP-DPP728 (**2**)				1.4	2.3

Table 2.



Compound no.	R	DPP-IV inhibition, IC_{50} (nmol/L)	
		Human	Rat
15		0.25	0.27
18	H	9.7	9.1
19		3.0	3.6
17a		0.53	0.53
20		4.6	3.0
21		1.9	1.5
22		5.5	3.8
23		1.5	1.1
24		6.7	3.9
17b		0.33	0.28
17c		0.28	0.24
17d		0.19	0.26
17e		0.18	0.17
17f		0.13	0.17
17g		0.13	0.15

Replacement of the cyanopyridine moiety of **15** with a benzene ring had little effect (**17a**). We therefore explored further modification of phenyl-containing compounds. First we examined the effect of a spacer between the phenyl and the nitrogen atom at the γ-position (**17a**, **20**–**24**). Insertion of all the spacers tested resulted in decreased DPP-IV inhibitory activity. We next examined the influence of substituents on the benzene ring. Substitution of an electron-donating methoxy group (**17b**) or an electron-withdrawing cyano or nitro group (**17d**, **17e**) both resulted in ca. 2–3-fold more potent activity. In these structure–activity relationship studies, no correlation was observed between the electronic effect of the substituents on the benzene ring and inhibitory activity. It is possible that they have some hydrophobic interaction with the S_2 pocket. In investigation of NVP-DPP728, it has been reported that electron-withdrawing substituents on the 2-aminopyridine ring do not appear to be essential.[8] Of the compounds investigated in the present study, compounds **17f** and **17g**, bearing 3,4-dicyanophenyl and 3-chloro-4-cyanophenyl groups, showed the highest inhibitory activity.

Rasmussen et al. reported the crystal structure of DPP-IV in complex with a valine–pyrrolidide inhibitor and indicated that the isopropyl side chain of this inhibitor points into a large cavity.[11] Given the presence of a large cavity in the S_2 pocket, it could hold a hydrophobic binding site interacting with the γ-substituent. Further optimization of these proline-containing inhibitors, focused on the binding site in the cavity, is in progress.

In conclusion, we designed a series of 1-(γ-substituted prolyl)-(*S*)-2-cyanopyrrolidine compounds as novel DPP-IV inhibitors and investigated their inhibitory activity. In structure–activity relationship study at the γ-position of proline, it became clear that compounds bearing (*S*)-stereochemistry were more potent than the antipode. Of these compounds, 1-[(*S*)-γ-[(3,4-dicyanophenyl)amino]prolyl]-(*S*)-2-cyanopyrrolidine (**17f**) and 1-[(*S*)-γ-[(3-chloro-4-cyanophenyl)amino]prolyl]-(*S*)-2-cyanopyrrolidine (**17g**) showed the highest inhibitory activity.

Acknowledgements

We wish to thank Dr. Tohru Nakajima and Dr. Takao Kondo for their insight and guidance during the course of this work.

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10. The DPP-IV inhibitory activity of human plasma and rat plasma was measured by fluorescence assay using Gly-Pro-MCA (Peptide Institute Inc.) as a DPP-IV-specific fluorescent substrate. Reaction solutions containing 20 μL of human or rat plasma (10-fold diluted solution), 20 μL of fluorescent substrate (100 μmol/L), 140 μL of buffer (0.003% Brij-35 containing PBS), and 20 μL of test substrate (of various concentrations) were incubated at room temperature for 60 min using a 96-well flat-bottom microtiter plate. The measured fluorescent intensity (excitation 360 nm/emission 465 nm, SPECTRA FLUOR, TECAN) was taken as the DPP-IV activity. The inhibitory rate relative to the solvent addition group was calculated and IC_{50} values determined by logistic analysis.
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