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Bioorganic & Medicinal Chemistry Letters 15 (2005) 3271–3275
Bioorganic & Medicinal Chemistry Letters

Glutamic acid analogues as potent dipeptidyl peptidase IV and 8 inhibitors

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Received 1 April 2005; revised 15 April 2005; accepted 22 April 2005
Available online 31 May 2005

Abstract—To find potent and selective inhibitors of dipeptidyl peptidase IV (DPP-IV), we synthesized a series of 2-cyanopyrrolidine with P2-site 4-substituted glutamic acid derivatives and tested their activities against DPP-IV, DPP8, and DPP-II. Analogues that incorporated a bulky substituent at the first carbon position of benzylamine or isoquinoline showed over 30-fold selectivity for DPP-IV over both DPP8 and DPP-II. From structure–activity relationship studies, we speculate that the S2 site of DPP8 might be similar to that of DPP-IV, while DPP-IV inhibitor with *N*-substituted glycine in the P2 site and/or with a moiety involving in hydrophobic interaction with the side chain of Phe357 might provide a better selectivity for DPP-IV over DPP8.

Dipeptidyl peptidase IV (DPP-IV, also known as CD26) (EC 3.4.14.5) is a drug target for type II diabetes. It is a prolyl dipeptidase involved in the in vivo degradation of two insulin-sensing hormones, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), by cleaving at the peptide bond of the penultimate position.[1,2] DPP-IV knockout mice and rats have consistently displayed healthy phenotypes.[3] When challenged with a high concentration of glucose, these animals show improved glucose tolerance, enhanced insulin secretions, and increased circulating active GLP-1 peptide.[3] By extending the duration of action of GLP-1, DPP-IV inhibitors stimulate insulin secretion, inhibit glucagon releases, and slow gastric emptying in animal models, each action a benefit in the control of glucose homeostasis.[4] Recent successes in clinical trials of several DPP-IV inhibitors have demonstrated that selective and potent inhibitors of DPP-IV protease are effective for the treatment of type II diabetes.[5]

DPP-IV belongs to the prolyl dipeptidase family, which includes DPP-II, DPP-IV, DPP8, and DPP9.[6] The in vivo functions of these prolyl dipeptidases, other than DPP-IV, are largely unknown. DPP-II is known as quiescent cell proline dipeptidase or DPP-VII (DPP7).[6,7] The inhibition of DPP-II has been shown to result in the apoptosis of quiescent T cells.[8] DPP8 is a cytoplasmic protease with a 51% homology with DPP-IV at the amino acid level.[9] The administration of selective DPP8/DPP9 inhibitors in animals results in severe toxic reactions, including alopecia, thrombocytopenia, anemia, enlarged spleen, multiple histological pathologies, and increased mortality.[10] In light of the physiological importance of prolyl cleaving enzymes and the toxicity associated with DPP8/DPP9, it is important to have selective inhibitors targeting DPP-IV for the treatment of type II diabetes.

In the current drug design of inhibitors, the selectivity of inhibitor over other closely related enzymes must be

Keywords: DPP-IV; DPP8.
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doi:10.1016/j.bmcl.2005.04.051

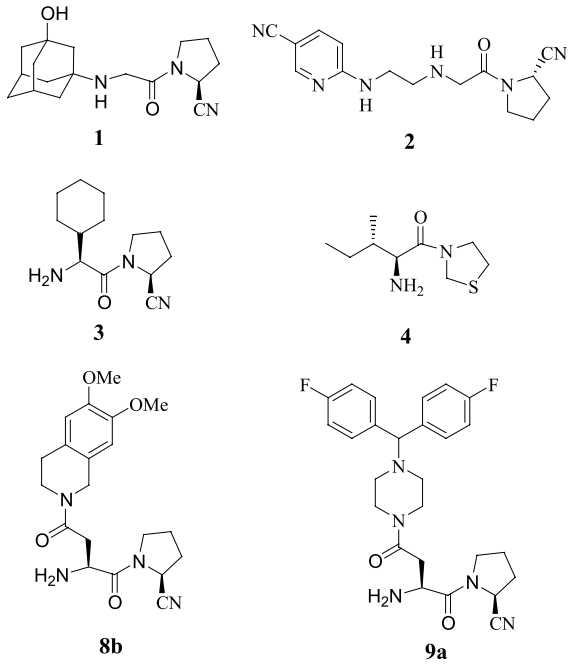


Figure 1. Inhibitors of DPP-IV.

determined. The structures of a number of DPP-IV inhibitors have been published (Fig. 1). The cyanopyrrolidine derivatives **1** and **2** are potent DPP-IV inhibitors developed by Novartis (Table 1), with excellent selectivity over both DPP8 and DPP-II.[11,12] Compound **1** (also known as NVP-LAF237) has progressed to mid-stage clinical trials with positive and encouraging results for the treatment of type II diabetes. Cyclohexylglycine-(2*S*)-cyanopyrrolidine **3** is a potent DPP-IV inhibitor with an IC_{50} value of 12 nM,[13] but it lacks selectivity toward DPP8. The IC_{50} value of this compound for DPP8 is 27 nM (Table 1). A related compound lacking a nitrile group, isoleucylthiazolidide **4** (Ile-Thia, or P32/98), shows only modest potency with an IC_{50} of 1.66 μM toward DPP-IV inhibition,[14] while it is more potent against DPP8 with an IC_{50} value of 364 nM (Table 1). This compound is effective in improving glucose tolerance in healthy people and in people with type 2 diabetes.[15]

From our previous study, glutamic acid derivatives **8b** and **9a** are potent DPP-IV and DPP8 inhibitors.[16] In this paper, structure–activity relationship studies in this series are discussed. Introduction of suitable amines at the 4-position of glutamic acid as their P2 site produced some DPP-IV inhibitors with modest selectivity in regard to DPP8. These inhibitors were also tested against DPP-II. Since significant inhibition (IC_{50} <10 μM) was not observed, data are presented for DPP-IV and DPP8 only. Determination of IC_{50} values for inhibition of DPP-IV, DPP8, and DPP-II was carried out as described previously.[8,16]

Table 1. Inhibition of DPP-IV, DPP8, and DPP-II by compounds **1**–**4**, **8b**, and **9a**[a]

Compd	DPP-IV IC_{50} (nM)	DPP8 IC_{50} (nM)	DPP-II IC_{50} (nM)
1	51	14,219	>100,000
2	53	4573	26,520
3	12	27	39,873
4	1660	364	68,985
8b	63	19	31,259
9a	227	16	>100,000

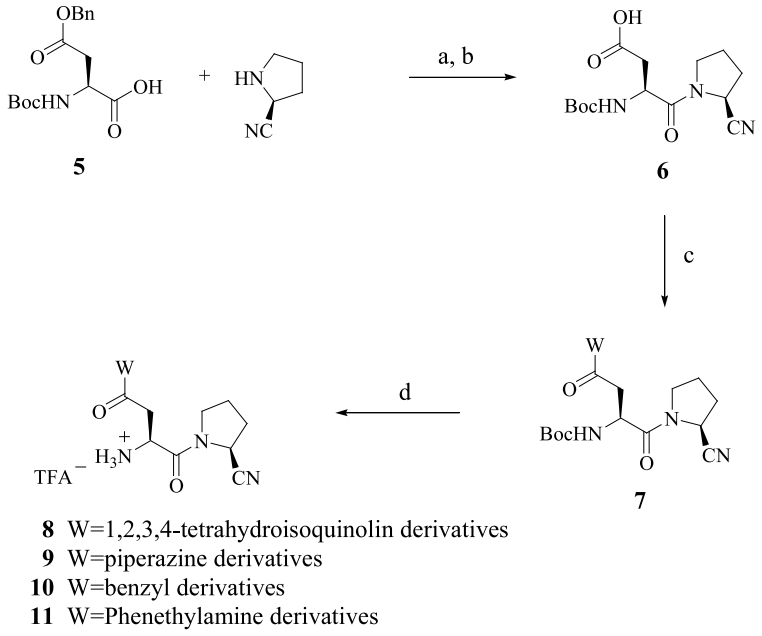
[a] IC_{50} determination was carried out as described in Refs. 8 and 16.

A series of (2*S*)-cyanopyrrolidines with aspartic acid derivatives at the P2 site, compounds **8**–**11**, was synthesized. The synthetic strategy was as follows (Scheme 1). Compounds **8**–**11** were synthesized from BOC-L-aspartic acid 4-benzyl ester **5**; DCC coupling of **5** with (2*S*)-cyanopyrrolidine was followed by hydrogenation to give acid **6**. Compound **6** was then coupled with primary or secondary amines, 1,2,3,4-tetrahydroisoquinoline derivatives, piperazine derivatives, benzylamine derivatives, or phenylethylamine derivatives to yield **7**. Removal of the BOC protecting group of **7** with trifluoroacetic acid yielded the desired 4-substituted aspartic acid derivatives **8**–**11**, respectively.

Table 2 summarizes the inhibitory properties of 2-cyanopyrrolidine with aspartic acid derivative substituents at carbon 4. Generally, these inhibitors in Table 2 exhibited a similar range of activities against DPP-IV and DPP8. Lead compound **8a** inhibited DPP-IV with an IC_{50} of 99 nM, and DPP8 at 120 nM. The 6,7-dimethoxyl-substituted compound **8b** had slightly higher inhibitory activity for DPP-IV (IC_{50} = 63 nM), and inhibited DPP8 with an IC_{50} value of 19 nM, making it one of the most potent DPP8 inhibitors in our investigation. Introduction of a 2-hydroxylethyl group at the first carbon position of isoquinoline provided compound **8c**, which is still a potent DPP-IV inhibitor with an IC_{50} value of 45 nM. The selectivity index of compound **8c** shows 13-fold more selectivity than that of **8b**. This suggests that the alkyl substituent at first carbon position in the isoquinoline ring may confer selectivity.

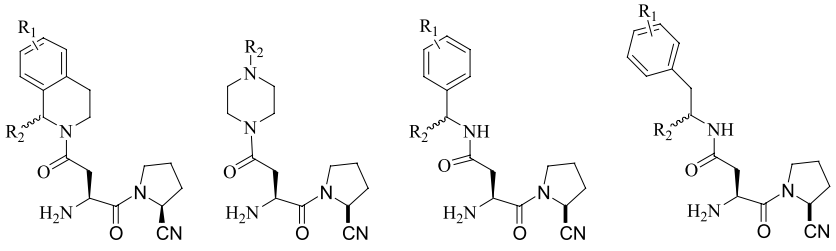
To explore this, further modification was carried out at the first carbon position. With a more bulky isopropyl substituent, compound **8d** preserved the high potency of **8c**, and showed some increase in selectivity with respect to DPP8. However, the benzyl substituent in compound **8e** neither improved potency nor enhanced the selectivity toward DPP-IV. As expected, increasing the bulk at the first carbon position of isoquinoline resulted in a dramatic improvement in selectivity; *tert*-butyl substituent **8f** was a 73 nM inhibitor of DPP-IV with 30-fold selectivity over DPP8. The 6-methoxyl analogue **8g** was approximately equipotent with the 6,7-dimethoxyl analogue **8f**, whereas the 7-methoxyl analogue **8h** showed a slight drop in potency. Both monomethoxyl substituted analogues **8g** and **8h** had reduced selectivities relative to the 6,7-dimethoxyl analogue **8f**.

The piperazine series of compounds **9a**–**c** was more potent as DPP8 inhibitors, with IC_{50} values ranging from 16 to 71 nM (Table 2). Among them,



Scheme 1. Synthesis of aspartic acid derivatives: (a) DCC, HOSu, 1,4-dioxane/CH_2Cl_2; (b) H_2, 5% Pd/C, MeOH; (c) DCC, CH_2Cl_2, various amines; (d) TFA, CH_2Cl_2.

Table 2. Inhibition of DPP-IV and DPP8 by compounds **8**–**11**



Compd	R_1	R_2	DPP-IV IC_{50} (nM)	DPP8 IC_{50} (nM)
8a	H	H	99	120
8b	6,7-$(OMe)_2$	H	63	19
8c	6,7-$(OMe)_2$	$-(CH_2)_2OH$[a]	45	174
8d	6,7-$(OMe)_2$	Isopropyl[a]	47	439
8e	6,7-$(OMe)_2$	Benzyl[a]	97	220
8f	6,7-$(OMe)_2$	*tert*-Butyl[a]	73	2196
8g	6-OMe	*tert*-Butyl[a]	72	1439
8h	7-OMe	*tert*-Butyl[a]	195	2712
9a	H	$-CH(4\text{-}FC_6H_5)_2$	227	16
9b	H	Nicotinonitrile	87	28
9c	H	Benzoyl	123	71
10a	H	H	238	389
10b	H	Benzyl[a]	252	2327
10c	H	Ethyl[a]	140	1412
10d	H	Isopropyl[a]	212	3055
10e	H	*tert*-Butyl[a]	251	13,217
11a	3,4-OMe	H	116	177
11b	H	$-CH_2OMe$[a]	182	2110
11c	H	Isopropyl[a]	305	1597

[a] The first carbon position of isoquinoline, benzylamine, or phenethylamine is racemate.

1-(4,4′-difluorobenzhydryl)piperazine **9a** showed the greatest potency to DPP8 (IC_{50} = 16 nM), having 14-fold selectivity over DPP-IV. A similar trend was observed for the nicotinonitrile analogue **9b** and the

benzoyl analogue **9c**, which were also more potent as DPP8 inhibitors, although selectivity over DPP-IV was relatively low.

Compared with the above isoquinoline series of compounds **8**, phenyl-substituted aliphatic amine extensions, such as the benzylamine derivatives **10** and the phenethylamine derivatives **11**, showed a slight drop in potency (DPP-IV IC_{50} = 116–305 nM, Table 2). Several alkyl substitutions at the first carbon position of benzylamine and phenethylamine, compounds **10b–e** and **11b,c**, were explored, and showed little effect in improving potency compared with unsubstituted analogues **10a** and **11a**, respectively. Nevertheless, increasing the size of the alkyl substituent again resulted in dramatic improvements in the selectivity profile of these compounds. Unsubstituted analogue **10a** showed similar inhibition against DPP-IV and DPP8, and ethyl substituted analogue **10c** or isopropyl substituted analogue **10d** exhibited >10-fold selectivity over DPP8. A more profound improvement was obtained with the more bulky *tert*-butyl-substituent in analogue **10e**, which showed 53-fold selectivity for DPP-IV over DPP8, and exhibited no significant DPP8 inhibition (IC_{50} = 13 μM), although its IC_{50} value for DPP-IV was a moderate 251 nM. This analogue represents the most selective DPP-IV inhibitor among the 4-substituted aspartic acid series of inhibitors.

To explore the mechanism of the inhibition, computer-modeling study was carried out to investigate a possible interaction between these inhibitors and DPP-IV. The predicted binding mode of compound **8a** at the active site of DPP-IV is shown in Figure 2. The (2*S*)-cyanopyrrolidine moiety of inhibitor occupies the narrow hydrophobic S1 pocket of the enzyme and the nitrile group is correctly positioned to interact with the active Ser630 to form an imidate intermediate.[17]

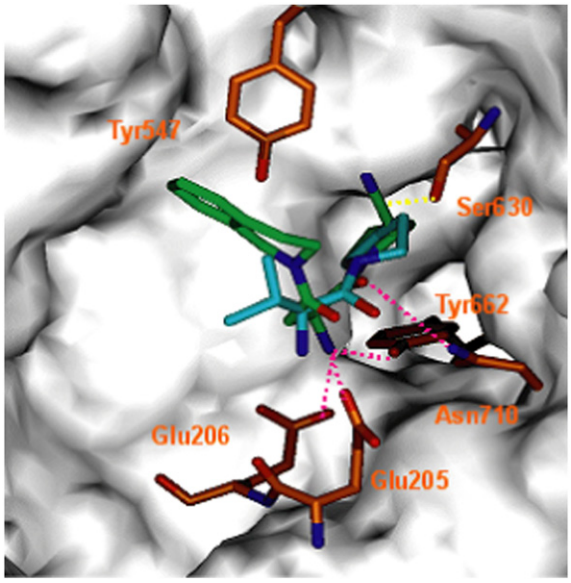


Figure 2. The binding mode of compound **8a** is predicted by computer model.

The P2-carbonyl oxygen forms hydrogen bonds to Asn710 and Arg125. The free amino terminus of the P2-residue forms hydrogen bonds with the side chain of Glu205, Glu206, and Tyr662. In addition to hydrogen-bonding interactions, the phenyl ring of 1,2,3,4-tetrahydroisoquinoline is stacked against the side chain of Tyr547 to produce hydrophobic interactions. Superimposition of the modeling structure of compound **8a** with the published X-ray structure of substrate analogue valine-pyrrolidine (blue) shows these predicted interactions are consistent with the literature reports (Fig. 2).[18]

The crystal structure of the DPP8 protein has yet to be elucidated. An earlier report from our laboratories speculates that the S1 site of DPP8 may be larger than that of DPP-IV, allowing the accommodation of larger C-terminal residues, such as isoquinoline or isoindoline, at the P1 site.[16] In this study, our results suggest that the S2 pocket of DPP-IV might be similar to DPP8. Thus, it is likely that some DPP-IV inhibitors may have an inhibitory effect on DPP8. Furthermore, proline mimetic inhibitors, for which the P2 residues are α-carbon substituted glycine derivatives (**3**, **4**, **8–10**), exhibited poor selectivities. However, DPP-IV inhibitors with *N*-substituted glycine in the P2 site, such as NVP LAF-237 (**1**), NVP DPP-728 (**2**),[17b] or with an aromatic moiety involving in hydrophobic interaction with the side chain of Phe357, such as β-amino acid MK-0431,[19] provided excellent selectivity over DPP8. Nevertheless, we still found some selective 4-substituted aspartic acid 2-cyanopyrrolidine inhibitors, such as *tert*-butyl substitution at first carbon position of the isoquinoline **8f** and the benzylamine **10e**, that selectively inhibited DPP-IV by a magnitude of >30-fold better than DPP8.

In summary, by introducing a series of primary or secondary amines to the 4-position of aspartic acid with P1-site 2-cyanopyrrolidine, novel chemical compounds that inhibit prolyl dipeptidases DPP-IV and DPP8 have been examined. In general, this class of inhibitors shows modest selectivity profile over DPP8. We found that isoquinoline derivatives **8c–d** are potent DPP-IV inhibitors (IC_{50} <50 nM), but less selective over DPP8 (<10-fold). Interestingly, the bulky *tert*-butyl-substituted isoquinoline derivative **8f** shows 30-fold selectivity over DPP8, and maintains excellent potency for DPP-IV with an IC_{50} value of 73 nM. Phenyl-substituted piperazine series **9** are potent DPP8 inhibitors, with compound **9a** the most potent (DPP8 IC_{50} = 16 nM). However, phenyl-substituted aliphatic derivatives **10** and **11** are less potent as DPP-IV and DPP8 inhibitors. Notable among these is the *tert*-butyl-substituted analogue **10e**, which shows 53-fold selectivity for DPP-IV over DPP8, with moderate DPP-IV inhibition (IC_{50} = 251 nM). From the structure–activity relationship studies, we speculate that the S2 pocket of DPP8 might be similar to that of DPP-IV, while DPP-IV inhibitor with *N*-substituted glycine in the P2 site, and/or with a moiety involving hydrophobic interaction with the side chain of Phe357 might provide a more selective DPP-IV inhibitor.

Acknowledgments

We would like to thank Dr. Yu-Sheng Chao for his support and encouragement. The study was financially supported by National Health Research Institutes, Taiwan.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.04.051.

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