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Bioorganic & Medicinal Chemistry Letters 15 (2005) 687–691
Bioorganic & Medicinal Chemistry Letters

Novel isoindoline compounds for potent and selective inhibition of prolyl dipeptidase DPP8

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Received 17 August 2004; revised 11 November 2004; accepted 11 November 2004
Available online 2 December 2004

Abstract—DPP8 is a prolyl dipeptidase homologous to DPP-IV, which is a drug target for Type II diabetes. The biological function of DPP8 is not known. To identify potent and selective chemical compounds against DPP8, we have synthesized a series of isoquinoline and isoindoline derivatives and have tested their inhibitory activity against DPP8, DPP-IV and DPP-II. Isoindoline derivatives were found to be more potent DPP8 inhibitors than isoquinoline derivatives. Isoindoline with a 1-(4,4′-difluor-benzhydryl)-piperazine group at the P2 site was observed to be a very potent DPP8 inhibitor, having an IC_{50} value of 14 nM with at least a 2500-fold selectivity over either DPP-IV or DPP-II. From SAR results, we speculate that the S1 site of DPP8 may be larger than that of DPP-IV, which would allow the accommodation of larger C-terminal residues, such as isoquinoline or isoindoline.

Dipeptidylpeptidase IV (DPP-IV, also known as CD26) (EC 3.4.14.5) is a drug target for Type II diabetes. It is a serine protease involved in the in vivo degradation of two insulin-sensing hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP).[1,2] By extending the duration of the action by GLP-1, animal studies preformed using DPP-IV inhibitors have demonstrated stimulated insulin secretion, inhibition of glucagon release, and slow gastric emptying. Each of these effects has benefits in the control of glucose homeostasis.[3] DPP-IV knockout mice and rats consistently display healthy phenotypes.[4] When presented with a high concentration of glucose, these animals show an improved glucose tolerance, enhanced insulin secretion, and an increased circulating GLP and GIP activity.[4] Therefore, selective and potent inhibitors of the DPP-IV protein are effective in the treatment of Type II diabetes.

DPP-IV belongs to the prolyl dipeptidase family, which also includes DPP-II, DPP8 and DPP9.[5] All these proteases show prolyl dipeptidase activity by cleaving the peptide bond at the penultimate proline residue.[5] The biological importance of these prolyl-cleaving peptidases is supported by the fact that their known in vivo substrates, that is, hormones, chemokines and neuropeptides, contain one or more proline residues, preventing their degradation by most proteases in vivo. As a result, the processing and degradation of these peptides requires the presence of these prolyl peptidases. Not surprisingly, some prolyl peptidases have been found to have important biological functions and are effective drug targets, for example, DPP-IV and prolyl oligopeptidase.[5,6]

The in vivo functions of prolyl dipeptidases, other than DPP-IV, are largely unknown. DPP-II is also known as quiescent cell proline dipeptidase (QPP) or DPP-VII (DPP7).[5,7] 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 in amino acid level with DPP-IV.[9] Even though the in vivo function of DPP8 is not known, the administration of selective DPP8/DPP9 inhibitors in animals results in severe

Keywords: DPP8; Isoindoline; Isoquinoline; Prolyl dipeptidase; Type II diabetes.
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doi:10.1016/j.bmcl.2004.11.023

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 to each enzyme for functional studies in vivo. In this work, we report on a systematic search for novel, potent and selective DPP8 inhibition by isoindoline compounds.

There are a number of DPP-IV inhibitors known (Fig. 1). The cyanopyrrolidine derivatives **1** and **2** are potent DPP-IV inhibitors developed by Novartis (Table 1).[11,12] Compound **1** (also known as NVP-LAF237) has progressed into mid-stage clinical trials and is showing positive and encouraging results in the treatment of Type II diabetes. Cyclohexylglycine-(2*S*)-cyanopyrolidine **3** is a potent DPP-IV inhibitor with an IC_{50} value of 12 nM.[13] However, a related compound lacking a nitrile group, isoleucylthiazolidide **4** (Ile-Thia, or P32/98), only shows modest DPP-IV inhibition potency, with an IC_{50} value of 1.66 μM.[14] This compound has been shown to be effective in improving glucose tolerance in both healthy and NIDDM patients.[15] During the course of investigating inhibitors targeting DPP-IV, we have identified two novel and potent DPP-IV inhibitors: compounds **5** and **6**, which have IC_{50} values of 63 and 274 nM, respectively. Structure–activity relationship (SAR) studies have been carried out on these compounds and the results will be published elsewhere.

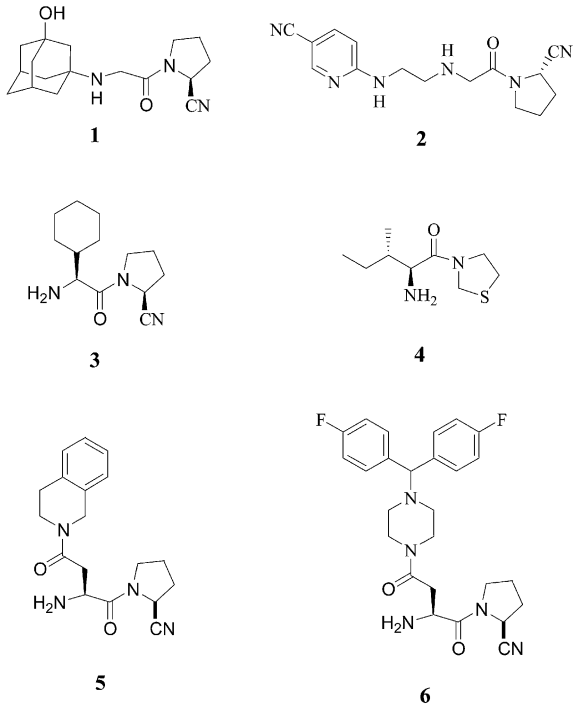


Figure 1. Inhibitors of DPP-IV.

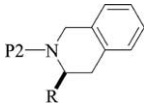
Table 1. Inhibition of DPP-IV, DPP8 and DPP-II by compounds **1**–**6**[a]

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
5	63	19	31,259
6	274	16	>100,000

[a] IC_{50} determination was carried out as described in Ref. 12.

There is a lack of structural information on the DPP8 protein. Due to the amino acid homology between DPP-IV and DPP8, we speculate that they may have a similar substrate-binding pocket. It is likely that some DPP-IV inhibitors may have an inhibitory effect on DPP8. Thus, the IC_{50} values for the inhibition of DPP8 for compounds **1**–**6**, were determined. As shown in Table 1, *N*-substituted glycines at the P2 site and the (2*S*)-cyanopyrrolidine at the P1 site (**1** and **2**) were weak DPP8 inhibitors, with IC_{50} values of 14.2 μM and 4.5 μM, respectively. However, α-carbon substituted glycine at the P2 site (**3**–**6**) dramatically improved the potency for DPP8 inhibition, showing IC_{50} values ranging from 19 to 364 nM. Thiazolidine (**4**) or (2*S*)-cyanopyrrolidine (**5** and **6**) at the P1 site yielded very potent DPP8 inhibitors, though the selectivity shown between DPP8 and DPP-IV was low (Table 1). Except for **1** and **6**, compounds **2**–**5** showed weak inhibition against DPP-II, with IC_{50} values ranging from 27 to 69 μM (Table 1).

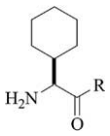
Table 2. Inhibitory activity of isoquinoline derivatives

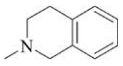
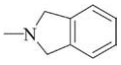


Compd	P2	R	DPP8 IC_{50} (nM)	DPP-IV IC_{50} (nM)	DPP-II IC_{50} (nM)
7	Chg[a]	H	3016	>100,000	>100,000
8	Chg[a]	CN	5962	>100,000	>100,000
9	PHg[b]	H	>100,000	>100,000	>100,000
10	3,4-Difluoro-Phe	H	>100,000	>100,000	>100,000
11	Pro	H	>100,000	>100,000	>100,000
12	Trp	CN	>100,000	13,000	>100,000

[a] Cha = cyclohexylglycine.
[b] PHg = phenylglycine.

Table 3. Structure–activity relationship at C-terminal P1 site

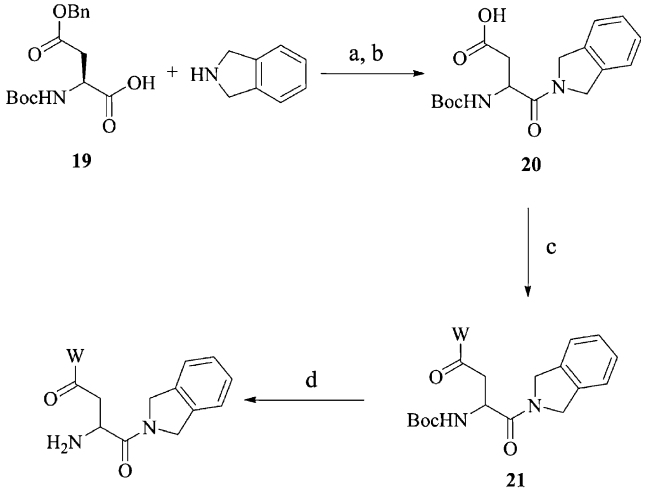


Compd	R	DPP8 IC_{50} (nM)	DPP-IV IC_{50} (nM)	DPP-II IC_{50} (nM)
3		27	12	39,873
7		3016	>100,000	>100,000
13		1448	31,000	27,357
14		78	1034	>100,000
15		24	15,000	16,520

To improve on the selectivity and to maintain the potency for DPP8, we replaced the P1 site of compound **3** with isoquinoline, generating (*S*)-cyclohexylglycine isoquinoline **7**. Compound **7** inhibited DPP8, showing an IC_{50} value of 3 μM, and no detectable DPP-IV or DPP-II inhibitory activity (Table 2). The addition of a nitrile group on isoquinoline, forming the 3-cyano derivative **8**, did not improve potency, nor enhance the selectivity towards DPP8. Therefore, isoquinoline compounds conferred moderate selectivity towards DPP8, with no DPP-IV or DPP-II inhibitory activity detected. Due to the weak activity of compounds **7** and **8**, we focused further optimization on the N-terminal P2 site. Phenylglycine at the P2 site with isoquinoline **9** resulted in a complete loss of inhibitory activity against DPP8, DPP-IV, and DPP-II. Similarly, compounds with 3,4-difluoro-Phe **10** or Pro **11** groups at the P2 site did not inhibit any of these proteases. A Trp group at the P2 site with 3-cyanoisoquinoline **12** did not exhibit DPP8 or DPP-II inhibition, although it did exhibit a weak inhibitory activity towards DPP-IV, showing an IC_{50} value of 13 μM.

Isoquinoline at P1 site may not be the optimum site for DPP8 inhibition, and further modification on the P1 site was therefore carried out. First, we replaced the isoquinoline by other ring structures, piperidine **13**, pyrrolidine **14** and isoindoline **15** (Table 3). Compared to isoquinoline **7**, piperidine **13** was twice as active towards DPP8 inhibition, but retained a weak DPP-IV and DPP-II inhibitory activity. Upon decreasing the ring size to a five-member ring, the pyrrolidine **14** gave an IC_{50} value of 78 nM and 1 μM against DPP8 and DPP-IV, respectively, with no inhibitory activity observed against DPP-II.[14] The selectivity index (SI) of **14** for DPP8 versus DPP-IV inhibition was about 13-fold. Introduction of isoindoline at the P1 site **15** greatly improved DPP8 inhibition, showing an IC_{50} value of 24 nM. Moreover, the SI value increased to 625- and 688-fold over DPP-IV and DPP-II, respectively. Thus, compound **15** is a potent DPP8 inhibitor, with a good selectivity against either DPP-IV or DPP-II .

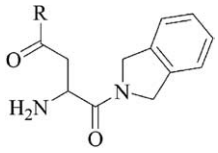
Next, we aimed to improve further the selectivity of the isoindoline compound **15** over DPP-IV and DPP-II, preferably by eliminating any detectable inhibitory activity. Since compounds **5** and **6** exhibited potent DPP8 inhibition with an aspartic acid derivative at the P2 site (Table 1), a series of isoindolines with aspartic acid derivatives at the P2 site, compounds **16**–**18**, were synthesized (Scheme 1). The synthetic strategy was as follows. Compounds **16**–**18** were synthesized from

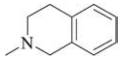
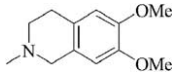
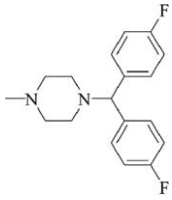


16 W=1,2,3,4-tetrahydroisoquinoline
17 W=6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline
18 W=1-(4,4'-difluor-benzhydryl)-piperazine

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

Table 4. Inhibitory activity and selectivity of isoindoline derivatives



Compd	R	DPP8 IC_{50} (nM)	DPP-IV IC_{50} (nM)	DPP-II IC_{50} (nM)
16		555	>100,000	20,891
17		150	>100,000	>100,000
18		14	>100,000	38,377

Boc-L-aspartic acid 4-benzyl ester **19**; the DCC coupling of **19** with isoindoline was followed by hydrogenation to give acid **20**. Compound **20** was then coupled with the primary or secondary amines, 1,2,3,4-tetrahydroisoquinoline, 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, or 1-(4,4′-difluor-benzhydryl)-piperazine to yield **21**. Removal of the Boc protecting group of **21** with trifluoroacetic acid yielded the desired 4-substituted aspartic acid derivatives **16**, **17** and **18**, respectively.

As shown in Table 4, by changing 2-cyanopyrrolidine to isoindoline in compounds **16**–**18**, the selectivity for DPP8 increased dramatically. All these isoindoline compounds showed varying degrees of DPP8 inhibition with no detectable DPP-IV inhibitory activity. Compounds **16** and **18** showed very weak DPP-II inhibitory activity, while **17** did not inhibit DPP-II at all. Among these compounds, compound **18** was a very potent inhibitor for DPP8, with an IC_{50} value of 14 nM: a 2500-fold selectivity over DPP-IV and DPP-II.

The crystal structure of the DPP8 protein has yet to be elucidated. Our results suggest that the S1 site of DPP8 is larger than that of DPP-IV, and can thus allow larger moieties, such as isoquinoline **7** and **8** and isoindoline **15**–**18**, to be accommodated. As a result, larger P1 groups with either isoindoline **15**–**18** or isoquinoline derivatives **7** and **8** were more potent inhibitors towards DPP8 than DPP-IV. Consistently, smaller sized moieties, such as the five-member ring pyrrolidine derivatives **3**–**6** and **14**, inhibited DPP8 as well as DPP-IV, while the six-membered ring piperidine **13** inhibited DPP8 by a magnitude of twenty times better than it did DPP-IV.

In summary, to find potent and selective chemical compounds against prolyl dipeptidase DPP8, we have synthesized a series of isoquinoline and isoindoline derivatives, and have tested their inhibitory activity against DPP8, DPP-IV and DPP-II. Isoquinoline derivatives are weak DPP8 inhibitors, with IC_{50} values of 3–6 μM, though they generally lack any activity against DPP-IV or DPP-II. Excitingly, isoindoline derivatives are very potent DPP8 inhibitors, with IC_{50} values below 20 nM. Isoindoline **18** with 1-(4,4′-difluor-benzhydryl)-piperazine at the P2 site is a very potent DPP8 inhibitor, with an IC_{50} value at 14 nM: a 2500-fold selectivity over either DPP-IV or DPP-II. From the SAR studies presented in this paper, we speculate 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.

Acknowledgements

We would like to thank Dr. Yu-Sheng Chao for his support and encouragement, and Dr. Peter Nell for critically reading the manuscript and making suggestions. 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.2004.11.023.

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12. The IC_{50} determinations were performed as described in Ref. 8 and were measured at least twice with independent triplicate samples. The data obtained were reproducible with less than 15% difference in the independent experiments. The standard deviation in each data point was less than 15% of the IC_{50} value presented. Sigma plots were used to obtain the IC_{50} values. DPP-IV and DPP8 were purified as described by Chen et al. in 'Protein Expression and Purification', 2004; Vol. 35, pp 142–146. The Purification of DPP-II was carried out as described in Ref. 8 with the modification. The detailed expression and purification of DPP-II will be described elsewhere. The buffers used in the DPP-IV, DPP8 and DPP-II assays were 2 mM Tris–HCl at pH 8.0, PBS buffer and 125 mM of sodium phosphate buffer at pH 5.5, respectively. The substrates used for DPP-IV and DPP8 were Gly-Pro-pNA at concentrations of 0.5 mM and 2.5 mM, respectively, while the substrate used for DPP-II was Lys-Ala-pNA at concentration of 1 mM.
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