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[(*S*)-γ-(4-Aryl-1-piperazinyl)-L-prolyl]thiazolidines as a novel series of highly potent and long-lasting DPP-IV inhibitors

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Abstract—In the search for an inhibitor of dipeptidyl peptidase IV (DPP-IV) highly potent both in vitro and in vivo, we synthesized a series of L-prolylthiazolidine-based DPP-IV inhibitors having 4-arylpiperazine or 4-arylpiperidine at the γ-position of the proline structure. Of these compounds, the 4-(5-nitro-2-pyridyl)piperazine analog **21e** showed a sub-nanomolar (IC_{50} = 0.92 nmol/L) DPP-IV inhibitory activity and a long-lasting in vivo DPP-IV inhibition profile.

Glucagon-like peptide (GLP)-1 is an incretin hormone that has multifaceted actions, including glucose-induced stimulation of insulin biosynthesis and secretion, regulation of gene expression, trophic effects on β cells, inhibition of food intake, and slowing of gastric emptying. These effects, which contribute to the normalization of elevated blood glucose and to the control of body weight, are tempered by the rapid enzymatic degradation of the peptide. Inhibition of dipeptidyl peptidase IV (DPP-IV) suppresses the degradation of many peptides, including GLP-1, thereby extending their bioactivity. Indeed, many DPP-IV inhibitors have been reported and several of them are undergoing late-stage clinical trials.[1–5] Recently, sitagliptin (**1**, MK-0431)[6] was approved as the first-in-class DPP-IV inhibitor for treatment of type 2 diabetes.

DPP-IV is a dipeptidase that recognizes an amino acid sequence having a proline or alanine residue at the P_1 position,[7] and many known DPP-IV inhibitors therefore have substituted pyrrolidines or thiazolidines as a proline mimetic in the P_1 part.[8] In particular, inhibitors possessing a (*S*)-2-cyanopyrrolidine moiety, for example NVP-DPP728 (**2**),[9–11] vildagliptin (**3**, LAF237),[11,12] and saxagliptin (**4**, BMS-477118)[13] (Fig. 1), interact with the Ser^{630} of the catalytic triad in DPP-IV and have highly potent inhibitory activities. We previously reported[14] that a series of [(*S*)-γ-(alylamino)-L-prolyl]-(*S*)-2-cyanopyrrolidine analogs (e.g., **5**) showed highly potent DPP-IV inhibitory activity. These compounds were, however, chemically unstable due to the intramolecular cyclization between the secondary amine of the proline and the electrophilic nitrile of the (*S*)-2-cyanopyrrolidine moiety. We also reported[15,16] that thiazolidide replacement (e.g., **6**) in this series conferred chemical stability, and that efforts to optimize the γ-alylamino moiety led to improved in vitro activity against DPP-IV (Fig. 2). The in vivo efficacy was nevertheless

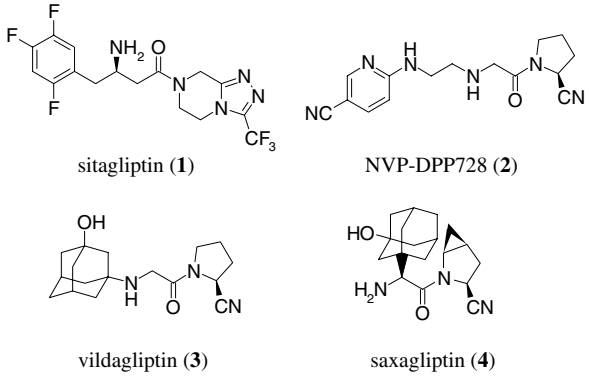

Figure 1. DPP-IV inhibitors.

Keywords: DPP-IV inhibitor; Thiazolidine; 4-Arylpiperazine.
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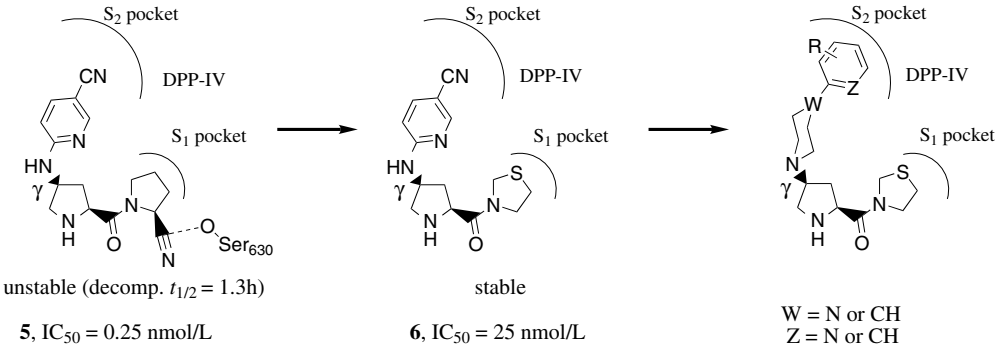


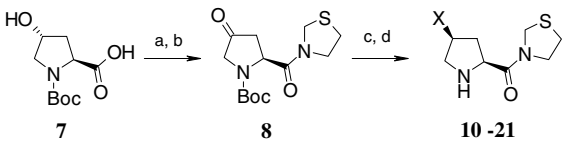
Figure 2. Design of (*S*)-γ-substituted L-prolylthiazolidines as DPP-IV inhibitors.

still insufficient. We focused further on γ-substituents of the proline moiety at the P_2 position in the search for highly potent DPP-IV inhibitor both in vitro and in vivo. Here we describe how the 4-aryl-1-piperazine or 4-aryl-1-piperidine substitution at the γ-position of proline resulted in not only increased potency, but also improved in vivo efficacy.

The synthesis of a series of cyclic amine substituted prolylthiazolidine compounds is shown in Scheme 1. The key intermediate **8** was prepared by coupling the N-Boc-*trans*-4-hydroxy-L-proline **7** with thiazolidine, followed by DMSO-oxidation, as previously reported.[14,15] Reductive amination of the ketone **8** with a variety of cyclic amines afforded only cis-configuration intermediates and subsequent removal of the Boc group yielded analogs **10**–**21**.

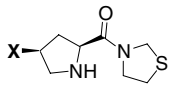
Table 1 summarizes the DPP-IV inhibitory activity[17] of L-prolylthiazolidide DPP-IV inhibitors bearing a representative cyclic amino group at their γ-position. Substitution of a 5- or 6-membered cyclic amino group undoubtedly increased the activity compared to the non-substituted compound **9**. Of these compounds, the 4-phenylpiperazine analog **14** exhibited almost 50-fold more potent activity. We therefore next examined the substituent of the phenyl ring of compound **14**. The results are shown in Table 2.

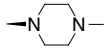
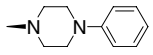
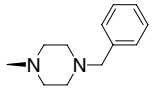
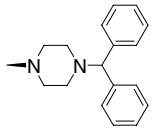
4-Methoxy (**17a**) or 4-fluoro (**17b**) substituents were similar to **14**, while 4-chloro (**17c**) or 4-bromo (**17f**) substituents led to a slight improvement in potency. Comparison of potency according to the substituted position on the phenyl ring indicated that the 3-chloro (**17c**) substituent was superior to the 2-chloro (**17e**) or the 4-chloro (**17c**) analog. A more significant improvement was seen with the 4-nitro (**17g**) substituent



Scheme 1. Reagents: (a) thiazolidine, HOBt, EDC, DMF; (b) DMSO, SO_3-pyridine complex; (c) amine X, $NaBH(OAc)_3$, AcOH, 1,2-dichloroethane; (d) H^+.

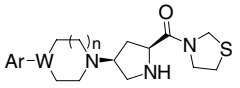
Table 1. Inhibition of ((*S*)-γ-substituted-prolyl)thiazolidines



Compound	X	DPP-IV inhibition IC_{50} (nmol/L)	
		Human	Rat
9	H	538	607
10		127	121
11		47.6	74.4
12		13.9	18.9
13		57.5	81.1
14		10.9	17.6
15		30.5	38.3
16		21.0	31.4

(IC_{50} = 1.6 nmol/L), which was 7-fold more potent than the unsubstituted phenyl analog **14**. Similar improvement was found in electron-withdrawing 4-cyano (**17h**) and 4-trifluoromethyl (**17i**) analogs. Moreover, disubstitution of the electron-withdrawing group increased potency compared with monosubstitution (**17c** vs **17j**, **17h** vs **17k**). The 4-phenylpiperidine analogs **18** and **19** also had a strong potency and produced similar SAR results to the piperazine analogs. These results suggested that the piperazine and the piperidine play linker roles and arrange the aryl moiety in the appropriate space in the S_2 pocket. Replacement of the piperazine linker in this series with a homopiperazine led to a slight decrease in potency (**17g** vs **20**). In light of the SAR results

Table 2. Inhibition of ((*S*)-γ-phenylpiperazinylproly1) thiazolidines



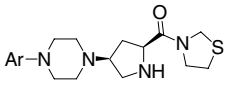
Compound	Ar	W	*n*	DPP-IV inhibition, IC_{50} (nmol/L)	
				Human	Rat
14	Ph	N	1	10.9	17.6
17a	4-MeOPh	N	1	13.5	16.9
17b	4-FPh	N	1	12.2	17.5
17c	4-ClPh	N	1	8.7	10.3
17d	3-ClPh	N	1	4.1	7.2
17e	2-ClPh	N	1	10.8	10.9
17f	4-BrPh	N	1	8.2	9.0
17g	4-NO_2Ph	N	1	1.6	2.2
17h	4-CNPh	N	1	4.3	4.7
17i	4-CF_3Ph	N	1	6.8	7.2
17j	3,4-Cl_2Ph	N	1	3.0	4.2
17k	3,4-CN_2Ph	N	1	1.3	1.8
18	Ph	C	1	5.6	9.6
19	4-NO_2Ph	C	1	2.3	3.3
20	4-NO_2Ph	N	2	2.4	2.9

from Table 2, it appears that the introduction of the electron-withdrawing group to the phenyl ring is desirable to improve potency.

These results encouraged us to incorporate a pyridine substitution as an electron-deficient aromatic ring into the piperazine scaffold (Table 3). As expected, each of the pyridine analogs had a highly potent activity. Most noteworthy among them was the 5-nitro-2-pyridine analog **21e**, which provided a sub-nanomolar activity (IC_{50} = 0.92 nmol/L) despite the lack of an electrophilic trap at the P_1 position. Comparison of potency according to the substituted position on the pyridine ring indicated the superiority of the 4-cyano position (**21g** vs **21d**, **21h**). From the many SAR studies of DPP-IV inhibitors reported by other groups, it seems that the S_2 pocket in DPP-IV is able to accommodate a large substituent.[18] It was hypothesized that the 4-arylpiperazine group in this series filled the S_2 pocket better than our prototype compound **6** (Fig. 1) and as a result compensated for the potency lost due to the lack of an electrophilic trap at the P_1 position.

Evaluation of plasma DPP-IV activity after oral dosing of the compounds can be used to predict the efficacy of antihyperglycemic activity and the pharmacokinetic profile. The representative compounds **17k** and **21e** and the prototype compound **6** were tested for in vivo DPP-IV inhibition. Each compound was administered orally to Wistar rats at a dose of 10 μmol/kg and the plasma DPP-IV activity was evaluated ex vivo.[19] As shown in Figure 3, compound **6** showed only 70% maximum inhibition of plasma DPP-IV activity and its efficacy diminished within 5h, whereas the maximum inhibition of both compounds **17k** and **21e** reached about 95% within 30 min, with more than 60% inhibition of DPP-IV persisting for 9 h after oral dosing. These fast-onset and long-lasting DPP-IV inhibitory activities were most likely due to the increased potency

Table 3. Inhibition of ((*S*)-γ-pyridylpiperazinylprolyl) thiazolidines



Compound	Ar	DPP-IV inhibition, IC_{50} (nmol/L)	
		Human	Rat
21a	2-Pyridyl	2.7	3.5
21b	4-Pyridyl	3.1	3.9
21c	5-Cl-2-pyridyl	2.5	3.1
21d	5-CN-2-pyridyl	1.6	1.9
21e	5-NO_2-2-pyridyl	0.92	1.1
21f	5-CF_3-2-pyridyl	1.5	1.8
21g	4-CN-2-pyridyl	1.0	1.4
21h	3-CN-2-pyridyl	1.2	1.2

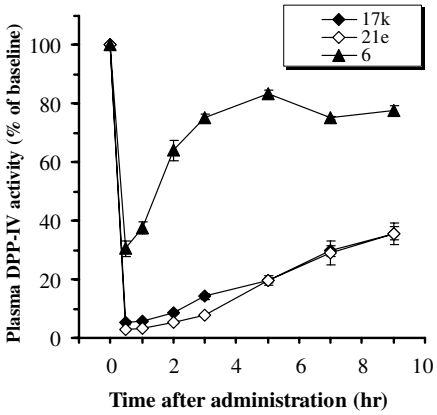


Figure 3. Effects of **17k**, **21e**, and **6** on plasma DPP-IV activity (% change of baseline) in Wistar rat. Each compound was orally administered at a single dose of 10 μmol/kg at 0 h. Data are expressed as means ± SEM (*n* = 3).

and good PK profile of the 4-arylpiperazine analogs (PK profile not evaluated).

In conclusion, the present report details our program of optimizing γ-substituted L-prolylthiazolidine-based DPP-IV inhibitors by introducing 4-arylpiperazine, which resulted in highly potent and long-lasting inhibitors. SAR study revealed that the introduction of an electron-deficient 4-arylpiperazine scaffold was desirable for improved potency, as demonstrated by compounds **21a**–**h**. In particular, compound **21e** proved exceptionally potent in vitro (IC_{50} = 0.92 nmol/L) despite the lack of an electrophilic trap at the P_1 position. Moreover, this compound showed in vivo DPP-IV inhibition of fast-onset and of longer duration than that of the prototype compound **6**. Further efforts focused on the aromatic ring in this arylpiperazine scaffold and its predicted interaction mode with DPP-IV will be reported in due course.

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References and notes

1. Kieffer, T. J.; Habener, J. F. *Endocr. Rev.* **1999**, *20*, 876.
2. Drucker, D. J. *Diabetes* **1998**, *47*, 159.
3. Holst, J. J. *Curr. Med. Chem.* **1999**, *6*, 1005.
4. Conarello, S. L.; Li, Z.; Ronan, J.; Roy, R. S.; Zhu, L.; Jiang, G.; Liu, F.; Woods, J.; Zycband, E.; Moller, D. E.; Thornberry, N. A.; Zhang, B. B. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6825.
5. Nielsen, L. L. *Drug Discovery Today* **2005**, *10*, 703.
6. Kim, D.; Wang, L.; Beconi, M.; Eiermann, G. J.; Fisher, M. H.; He, H.; Hickey, G. J.; Kowalchick, J. E.; Leiting, B.; Lyons, K.; Marsilio, F.; McCann, M. E.; Patel, R. A.; Petrov, A.; Scapin, G.; Patel, S. B.; Roy, R. S.; Wu, J. K.; Wyvratt, M. J.; Zhang, B. B.; Zhu, L.; Thornberry, N. A.; Weber, A. E. *J. Med. Chem.* **2005**, *48*, 141.
7. Heins, J.; Welker, P.; Schönlein, C.; Born, I.; Hartrodt, B.; Neubert, K.; Tsuru, D.; Barth, A. *Biochim. Biophys. Acta* **1988**, *954*, 161.
8. For comprehensive reviews on DPP-IV inhibitors, see: (a) Villhauer, E. B.; Coppola, G. M.; Hughes, T. E. *Annu. Rep. Med. Chem.* **2001**, *36*, 191; (b) Weber, A. E. *J. Med. Chem.* **2004**, *47*, 4135.
9. Hughes, T. E.; Mone, M. D.; Russell, M. E.; Weldon, S. C.; Villhauer, E. B. *Biochemistry* **1999**, *38*, 11597.
10. Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Dunning, B. E.; Mangold, B. L.; Mone, M. D.; Russell, M. E.; Weldon, S. C.; Hughes, T. E. *J. Med. Chem.* **2002**, *45*, 2362.
11. Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Burkey, B. F.; Dunning, B. E.; Prasad, K.; Mangold, B. L.; Russell, M. E.; Hughes, T. E. *J. Med. Chem.* **2003**, *46*, 2774.
12. McIntyre, J. A.; Castańer, J. *Drugs Future* **2004**, *29*, 887.
13. Augeri, D. J.; Robl, J. A.; Betebenner, D. A.; Magnin, D. R.; Khanna, A.; Robertson, J. G.; Wang, A.; Simpkins, L. M.; Taunk, P.; Huang, Q.; Han, S.-P.; Abboa-Offei, B.; Cap, M.; Xin, L.; Tao, L.; Tozzo, E.; Welzel, G. E.; Egan, D. M.; Marcinkeviciene, J.; Chang, S. Y.; Biller, S. A.; Kirby, M. S.; Parker, R. A.; Hamann, L. G. *J. Med. Chem.* **2005**, *48*, 5025.
14. Sakashita, H.; Kitajima, H.; Nakamura, M.; Akahoshi, F.; Hayashi, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2441.
15. Sakashita, H.; Akahoshi, F.; Kitajima, H.; Tsutsumiuchi, R.; Hayashi, Y. *Bioorg. Med. Chem.* **2006**, *14*, 3662.
16. Sakashita, H.; Akahoshi, F.; Yoshida, T.; Kitajima, H.; Hayashi, Y.; Ishii, S.; Takashina, Y.; Tsutsumiuchi, R.; Ono, S. *Bioorg. Med. Chem.* **2007**, *15*, 641.
17. 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.
18. (a) Xu, J.; Wei, L.; Mathvink, R. J.; Edmondson, S. D.; Eiermann, G. J.; He, H.; Leone, J. F.; Leiting, B.; Lyons, K. A.; Marsilio, F.; Patel, R. A.; Patel, S. B.; Petrov, A.; Scapin, G.; Wu, J. K.; Thornberry, N. A.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5373; (b) Edmondson, S. D.; Mastracchio, A.; Mathvink, R. J.; He, J.; Harper, B.; Park, Y.-J.; Beconi, M.; Salvo, J. D.; Eiermann, G. J.; He, H.; Leiting, B.; Leone, J. F.; Levorse, D. A.; Lyons, K.; Patel, R. A.; Patel, S. B.; Petrov, A.; Scapin, G.; Shang, J.; Roy, R. S.; Smith, A.; Wu, J. K.; Xu, S.; Zhu, B.; Thornberry, N. A.; Weber, A. E. *J. Med. Chem.* **2006**, *49*, 3614; (c) Tsai, T.-Y.; Coumar, M. S.; Hsu, T.; Hsieh, H.-P.; Chien, C.-H.; Chen, C.-T.; Chang, C.-N.; Lo, Y.-K.; Wu, S.-H.; Huang, C.-Y.; Huang, Y.-W.; Wang, M.-H.; Wu, H.-Y.; Lee, H.-J.; Chen, X.; Chao, Y.-S.; Jiaang, W.-T. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3268.
19. Male Wistar rats (7–9 weeks of age) fasted overnight were used. Each compound of **17k**, **21e**, and **6** was dissolved in 0.5% hydroxypropylmethyl-cellulose and administered orally at a dose of 10 μmol/kg. At pre-administration and at 0.5, 1, 2, 3, 5, 7, and 9 h after administration, 0.1 mL of blood was collected from the jugular vein. After centrifugation, 10 μL of plasma was diluted 10-fold using buffer (0.003% Brij-35 containing PBS). 20 μL of the diluted plasma was used instead of 20 μL of the test substrate for the determination of DPP-IV inhibitory activity by fluorescence as described above.