Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3268–3272

Substituted pyrrolidine-2,4-dicarboxylic acid amides as potent dipeptidyl peptidase IV inhibitors

Ting-Yueh Tsai,† Mohane Selvaraj Coumar,† Tsu Hsu,† Hsing-Pang Hsieh, Chia-Hui Chien, Chiung-Tong Chen, Chung-Nien Chang, Yu-Kang Lo, Ssu-Hui Wu, Chung-Yu Huang, Yu-Wen Huang, Min-Hsien Wang, Hsin-Yi Wu, Hong-Jen Lee, Xin Chen, Yu-Sheng Chao and Weir-Torn Jiaang*

Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, No. 35, Keyan Road, Zhunan Town, Miaoli County 350, Taiwan, ROC

Received 12 January 2006; revised 1 March 2006; accepted 13 March 2006
Available online 11 April 2006

Abstract—A series of substituted pyrrolidine-2,4-dicarboxylic acid amides were synthesized as potential antidiabetic agents, and many of them showed good in vitro DPP-IV inhibition (IC_{50} = 2–250 nM) with selectivity over DPP-II, DPP8, and FAP enzymes. Selected compounds **8c** and **11a** showed in vivo plasma DPP-IV inhibition after oral administration in Wistar rats.

Dipeptidyl peptidase IV (DPP-IV; CD26; E.C. 3.4.14.5) inhibition is a new and promising approach for the treatment of type-II diabetes.[1,2] Inhibition of DPP-IV results in elevated circulating levels of endogenously secreted glucagon-like peptide-1 (GLP-1),[3] which is produced by L-cells of the small intestine in response to food.[4] GLP-1 stimulates the secretion of insulin in a glucose dependent fashion, inhibits glucagon release, slows gastric emptying, and induces satiety, each a benefit in the control of glucose homeostasis in patients with type-II diabetes. But the active form of GLP-1 is rapidly inactivated ($t_{1/2} \sim 1$ min) by the plasma DPP-IV, through the cleavage of the dipeptide from the *N*-terminus, thereby limiting its duration of action.[5,6] Thus, inhibition of DPP-IV could lead to longer-lasting GLP-1 levels, which in turn enhance insulin secretion and improve the glucose tolerance. In fact human clinical trials proved unequivocally the benefits of DPP-IV inhibition in type-II diabetes patients.[7] Several DPP-IV inhibitors are under late-stage clinical development, including NVP-LAF237 (**1**),[8] MK-0431 (**2**),[9] and BMS-477118 (**3**)[10] (Fig. 1).

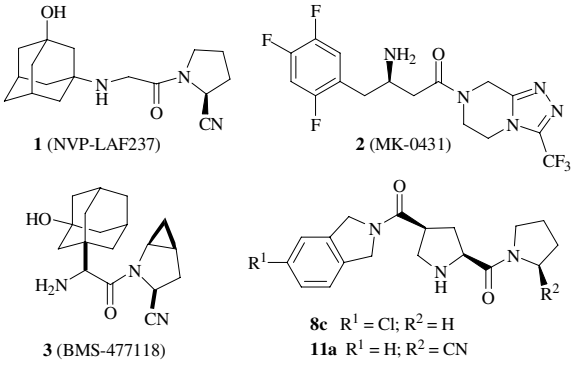


Figure 1. DPP-IV inhibitors.

DPP-IV is a serine protease that preferentially hydrolyzes *N*-terminal dipeptide from proteins having proline or alanine in the penultimate position. Design of small molecule inhibitors of DPP-IV has mainly revolved around investigation of compounds which resemble the P2-P1 dipeptide substrate cleavage product with the P1 site occupied by a proline mimic.[11–13] Based on this strategy, various groups have successfully reported the use of (2*S*)-cyanopyrrolidine as the P1 portion to mimic the proline in the development of potent DPP-IV inhibitors. Some of these workers emphasized the development of selective

Keywords: DPP-IV; Diabetes; Pyrrolidine.
* Corresponding author. Tel.: +886 37 246166x35712; fax: +886 37 586456; e-mail: wtjiaang@nhri.org.tw
† These authors contributed equally to this work.

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.03.037

DPP-IV inhibitors over other related prolyl peptidase such as DPP-II, DPP8, DPP9, and FAP (fibroblast activation protein).[12,14] Achieving selective inhibition of DPP-IV becomes very important, as inhibition of DPP-II results in the apoptosis of quiescent T-cells and DPP8/9 inhibition causes toxicities in animal studies.[15,16]

In continuation to our efforts to develop selective DPP-IV inhibitors,[17] we have reported compound **4**, a potent DPP-IV inhibitor (IC_{50} = 15 nM) with high selectivity over DPP8 (IC_{50} >100 μM) and DPP-II (IC_{50} >100 μM).[18] To further explore the structure–activity relationships (SAR), and to develop potent and selective DPP-IV inhibitors, introduction of ring-constraint in the P2 portion of lead compound (**4**) as depicted in Figure 2 was contemplated. Sakashita et al. improved DPP-IV potency by conformationally constraining NVP-DPP728.[19] Thus, by conformationally constraining the P2 portion of **4**, a better binding with DPP-IV was envisaged. Herein, we report the synthesis, DPP-IV inhibition, selectivity profile, and in vivo efficacies of substituted pyrrolidine-2,4-dicarboxylic acid amides.

The general synthetic route for the preparation of various substituted pyrrolidine-2,4-dicarboxylic acid amides is shown in Scheme 1. Compound **5** was prepared according to the literature procedure[20,21] and was coupled with the required amine (R^1H) using DCC and HOBt in 1,4-dioxane to give **6**. Saponification, followed by coupling with the required amine (R^2H) and Cbz deprotection with HBr/AcOH mixture, gave the desired compounds **8a**–**m** in 36–55% overall yields. For the synthesis of compounds with (2*S*)-cyanopyrrolidine as the P1 portion, the Cbz protection of **6** was changed to Boc protection in **9**. DCC coupling of **9** with L-prolinamide yielded carboxamide **10**. Dehydration of compound **10** using $POCl_3$ gave the corresponding nitrile,[10] and then removal of Boc protection by treatment with trifluoroacetic acid gave the desired compounds **11a**,**b** in 24–32% yields. Substituted isoindolines were synthesized starting from corresponding phthalic anhydride by reaction with formamide to obtain phthalimide derivatives, followed by reduction with borane–THF according to the literature procedure.[22,23]

For the SAR explorations of the ring-constrained analogues, pyrrolidine ring without the dimethyl

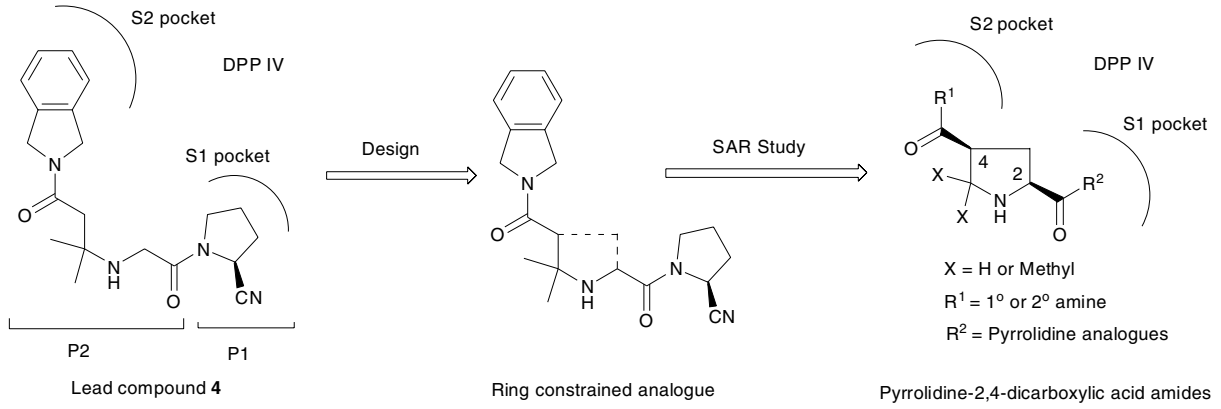
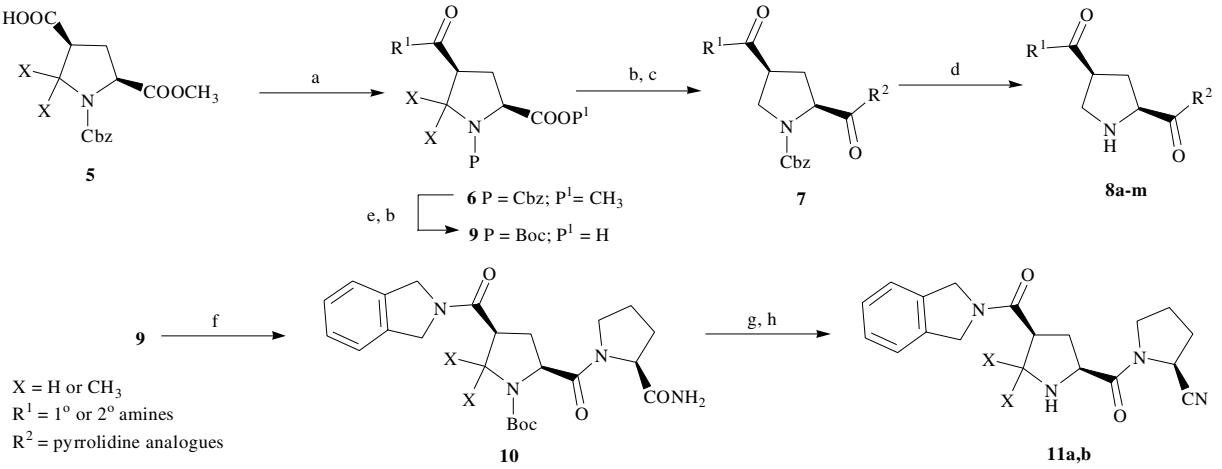


Figure 2. Design of substituted pyrrolidine-2,4-dicarboxylic acid amides as DPP-IV inhibitors.

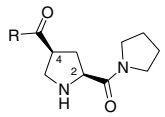


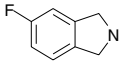
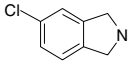
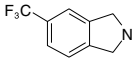
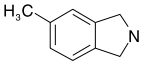
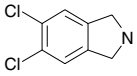
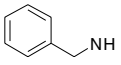
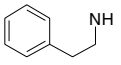
Scheme 1. Reagents: (a) R^1H, HOBt, DCC, 1,4-dioxane; (b) NaOH, THF/H_2O 1:1; (c) R^2H, HOBt, DCC (and 4-DMAP for R^2H = thiazolidine); (d) HBr/AcOH; (e) i—H_2, Pd/C, CH_3OH; ii—$(Boc)_2O$, CH_2Cl_2; (f) L-prolinamide, HOBt, 4-DMAP, DCC; (g) imidazole, $POCl_3$, pyridine; (h) CF_3COOH.

substitution was selected, as the synthesis was easy compared to that of the 5,5-dimethyl pyrrolidine core. First, the requirements of S2 pocket were explored, by keeping pyrrolidine as the 2-position substituent and varying the 4-position substituent. The data shown in Table 1 compare DPP-IV, DPP-II, DPP8, and FAP inhibitory properties of these compounds.[24] Use of isoindoline (bicyclic system) as the 4-position substituent (**8a**) led to a potent DPP-IV inhibition (IC_{50} = 109 nM), with a high selectivity over other enzymes assayed. Introduction of a monosubstitution at the 5-position of isoindoline ring (**8b–e**) only slightly increased the inhibitory activity at DPP-IV, and most of the compounds retained their selectivity over other enzymes. Maximum of 2-fold increase in DPP-IV inhibition was observed for **8c** analogue with a chlorine substituent (IC_{50} = 50 nM), which also showed a weak FAP inhibition. However, 4,5-dichloro substituted compound **8f** showed a drop in activity. This compound is less selective compared to **8a** and mono-substituted compounds **8b–e**. As monocyclic system, aniline **8g**, benzylamine **8h**, and phenylethylamine **8i** were tested. All these modifications led to a minimum of 20-fold decrease in DPP-IV potency. The most potent compound **8c** in this series showed similar range of activity to that of LAF237 (**1**) and MK-0431 (**2**).

After optimizing the P2 portion, we turned our attention to the P1 portion of the molecule. The requirements of the S1 pocket for DPP-IV inhibition were explored by keeping isoindoline as the 4-position substituent and varying the 2-position substituent. Their inhibitory properties are shown in Table 2. Changing the 2-position substituent from pyrrolidine **8a** to thiazolidine **8j** showed a slight improvement in potency. Introduction of electron-withdrawing fluorine atom into the pyrrolidine ring (**8k–m**) did not improve the activity. When (2*S*)-cyano group was introduced in the pyrrolidine ring (**11a**), more than 50-fold increase in DPP-IV inhibitory activity was observed compared to that of unsubstituted pyrrolidine **8a**. Even though **11a** showed a potent DPP-IV inhibition (IC_{50} = 1.7 nM), it also exhibited strong inhibitory activity against FAP (IC_{50} = 175 nM) and moderate inhibition against DPP8 (IC_{50} = 2.7 μM). Next, the effect of introduction of gem-dimethyl substituent adjacent to the P2-site amine was investigated. Compound **11b** showed a 500-fold loss of DPP-IV inhibitory potency in comparison to its unsubstituted ana-

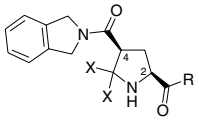
Table 1. Inhibition of DPP-IV, DPP8, DPP-II, and FAP by substituted 2-(pyrrolidine-1-carbonyl)-pyrrolidine-4-carboxylic acid amides



Compound	R	IC_{50}[a] (μM)			
		DPP-IV[b]	DPP8	DPP-II	FAP
8a		0.109	>20	>20	16.985
8b		0.073	>20	>20	13.609
8c		0.050	>20	>20	7.951
8d		0.073	>20	>20	>20
8e		0.072	>20	>20	8.803
8f		0.144	12.210	>20	5.129
8g		4.130	>20	>20	>20
8h		1.957	>20	>20	>20
8i		2.587	>20	>20	>20
4		0.015	>20	>20	>20
1 (LAF237)		0.051	14.219	>20	>20
2 (MK-0431)		0.030	>20	>20	>20

[a] See Ref. 24.
[b] Values are expressed as means of three independent determinations.

Table 2. Inhibition of DPP-IV, DPP8, DPP-II, and FAP by substituted 4-(isoindolin-2-carbonyl)-pyrrolidine-2-carboxylic acid amides



Compound	X	R	IC_{50}[a] (μM)			
			DPP-IV[b]	DPP8	DPP-II	FAP
8a	H		0.109	>20	>20	16.985
8j	H		0.084	>20	>20	10.352
8k	H		0.245	>20	>20	>20
8l	H		0.228	>20	>20	>20
8m	H		0.129	>20	19.250	19.478
11a	H		0.0017	2.727	>20	0.175
11b	CH_3		0.984	>20	>20	>20
4			0.015	>20	>20	>20

[a] See Ref. 24.
[b] Values are expressed as means of three independent determinations.

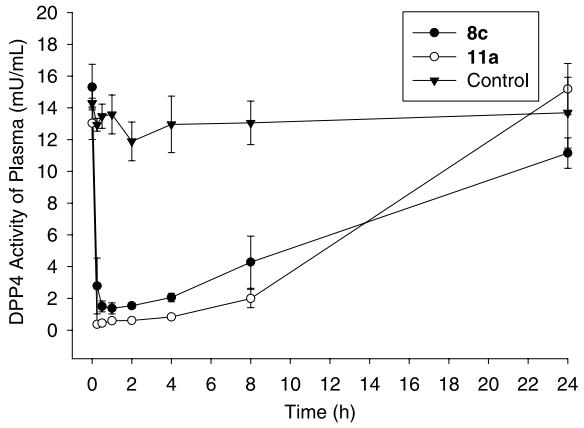


Figure 3. Effects of **8c** and **11a** on the plasma DPP-IV activity in Wistar rat. Each compound was orally administered at a single dose of 10 mg/kg to rat at 0 h. Data are expressed as means ± SEM ($n = 4$/group).

logue **11a**. The results show that gem-dimethyl substituent adjacent to the P2-site amine is not essential for activity in this series of compounds, but rather detrimental to the activity. In the lead compound (**4**) series, gem-dimethyl substituent is essential for potent and selective DPP-IV activity.[18] The difference could be due to unfavorable steric interactions by the introduction of gem-dimethyl substituent in the P2-site pyrrolidine ring. Thus, by introducing ring-constraint in lead compound **4** and carrying out SAR studies in a series of substituted pyrrolidine-2,4-dicarboxylic acid amides, we obtained **11a** with a 10-fold improvement in the in vitro DPP-IV inhibition.

Two of the most potent compounds **8c** and **11a** were tested for in vivo DPP-IV inhibition. The compounds were orally administered to Wistar rats at 10 mpk and the plasma DPP-IV inhibition was assessed ex vivo, and shown in Figure 3.[25] Both compound showed maximum plasma DPP-IV inhibition around 30 min after oral dosing, with similar inhibition onset and duration of action. For both compounds, more than 50% of inhibition lasts up to 12 h after oral dosing. Plasma DPP-IV inhibition by the compounds could be considered as an indicator of down stream antihyperglycemic activity and oral bioavailability. Compound **8c** is a better candidate for further evaluation, since **11a** exhibits FAP and DPP8 inhibitory activity. Moreover, in **11a**, the amino function could undergo intramolecular cyclization with the nitrile group resulting in inactive cyclic amidine or diketopiperazine product as reported for other DPP-IV inhibitors containing 2(*S*)-cyanopyrrolidine portion.[8,26] An aqueous solution of **11a** analyzed by LC–MS revealed the formation of the diketopiperazine, but **8c** analogue was stable due to the absence of (2*S*)-cyano function.

In summary, by introducing ring-constraint in the lead compound **4** and carrying out SAR studies in a series of substituted pyrrolidine-2,4-dicarboxylic acid amides, compound **11a** with 10-fold improvement in the in vitro DPP-IV inhibition was synthesized. The SAR suggests that incorporation of the gem-dimethyl substituent in the pyrrolidine ring is detrimental to potency relative to the unsubstituted analogue. Compounds **8c** (DPP-IV $IC_{50} = 50$ nM) and **11a** (DPP-IV $IC_{50} = 1.7$ nM) were orally bioavailable and showed in vivo plasma DPP-IV inhibition in Wistar rats. Further work is in progress to improve the selectivity and stability of **11a.**

Acknowledgment

The study was financially supported by National Health Research Institutes, Taiwan.

Supplementary data

LC–MS of **8a**–**m** and **11a**,**b** available as supplementary data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.03.037.

References and notes

1. McIntosh, C. H.; Demuth, H. U.; Pospisilik, J. A.; Pederson, R. *Regul. Pept.* **2005**, *128*, 159.
2. Deacon, C. F.; Holst, J. J. *Int. J. Biochem. Cell Biol.* **2006**, *38*, 831.
3. Deacon, C. F.; Hughes, T. E.; Holst, J. J. *Diabetes* **1998**, *47*, 764.
4. Holst, J. J.; Orskov, C.; Nielsen, O. V.; Schwartz, T. W. *FEBS Lett.* **1987**, *211*, 169.
5. Mentlein, R.; Gallwitz, B.; Schmidt, W. E. *Eur. J. Biochem.* **1993**, *214*, 829.
6. Kieffer, T. J.; McIntosh, C. H.; Pederson, R. A. *Endocrinology* **1995**, *136*, 3585.
7. Ahren, B.; Simonsson, E.; Larsson, H.; Landin-Olsson, M.; Torgeirsson, H.; Jansson, P. A.; Sandqvist, M.; Bavenholm, P.; Efendic, S.; Eriksson, J. W.; Dickinson, S.; Holmes, D. *Diabetes Care* **2002**, *25*, 869.
8. 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.
9. 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.
10. 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.
11. Weber, A. E. *J. Med. Chem.* **2004**, *47*, 4135.
12. Augustyns, K.; Van der Veken, P.; Senten, K.; Haemers, A. *Curr. Med. Chem.* **2005**, *12*, 971.
13. Hunziker, D.; Hennig, M.; Peters, J. U. *Curr. Top. Med. Chem.* **2005**, *5*, 1623.
14. Rosenblum, J. S.; Kozarich, J. W. *Curr. Opin. Chem. Biol.* **2003**, *7*, 496.

15. Leiting, B.; Pryor, K. D.; Wu, J. K.; Marsilio, F.; Patel, R. A.; Craik, C. S.; Ellman, J. A.; Cummings, R. T.; Thornberry, N. A. *Biochem. J.* **2003**, *371*, 525.
16. Lankas, G. R.; Leiting, B.; Roy, R. S.; Eiermann, G. J.; Beconi, M. G.; Biftu, T.; Chan, C. C.; Edmondson, S.; Feeney, W. P.; He, H.; Ippolito, D. E.; Kim, D.; Lyons, K. A.; Ok, H. O.; Patel, R. A.; Petrov, A. N.; Pryor, K. A.; Qian, X.; Reigle, L.; Woods, A.; Wu, J. K.; Zaller, D.; Zhang, X.; Zhu, L.; Weber, A. E.; Thornberry, N. A. *Diabetes* **2005**, *54*, 2988.
17. Lu, I. L.; Lee, S. J.; Tsu, H.; Wu, S. Y.; Kao, K. H.; Chien, C. H.; Chang, Y. Y.; Chen, Y. S.; Cheng, J. H.; Chang, C. N.; Chen, T. W.; Chang, S. P.; Chen, X.; Jiaang, W. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.
18. Tsu, H.; Chen, X.; Chen, C. T.; Lee, S. J.; Chang, C. N.; Kao, K. H.; Coumar, M. S.; Yeh, Y. T.; Chien, C. H.; Wang, H. S.; Lin, K. T.; Chang, Y. Y.; Wu, S. H.; Chen, Y. S.; Lu, I. L.; Wu, S. Y.; Tsai, T. Y.; Chen, W. C.; Hsieh, H. P.; Chao, Y. S.; Jiaang, W. T. *J. Med. Chem.* **2006**, *49*, 373.
19. Sakashita, H.; Kitajima, H.; Nakamura, M.; Akahoshi, F.; Hayashi, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2441.
20. Paradisi, M. P.; Mollica, A.; Cacciatore, I.; Di Stefano, A.; Pinnen, F.; Caccuri, A. M.; Ricci, G.; Dupre, S.; Spirito, A.; Lucente, G. *Bioorg. Med. Chem.* **2003**, *11*, 1677.
21. Aldous, D. J.; Drew, M. G. B.; Hamelin, E. M. N.; Harwood, L. M.; Jahans, A. B.; Thurairatnam, S. *Synlett* **2001**, 1836.
22. Peng, Y.; Song, G.; Qian, X. *Synth. Commun.* **2001**, *31*, 1927.
23. Gawley, R. E.; Chemburkar, S. R.; Smith, A. L.; Anklekar, T. V. *J. Org. Chem.* **1988**, *53*, 5381.
24. DPPII, DPP-IV, and DPP8 IC_{50} determinations were performed as discussed in Ref. 18. The detailed protocol for the cloning and expression of FAP will be described elsewhere. Briefly, cDNA of FAP was cloned into pBac-PAC8-CD5 vector (Ref. Chien et al. *J. Biol. Chem.* **2004**, *279*, 52338–52345). The expression and purification of FAP was carried out essentialy as described in Ref. Chen et al. *Protein Expr. Purif.* **2004**, *35*, 142–146. The substrate used for FAP assay was Gly-Pro-*p*-NA at 6 mM concentration.
25. The in vivo DPP-IV inhibition assay was performed as discussed in ref. 18. Adult male rats were orally gavaged with the test compounds at a single dose of 10 mg/kg. The plasma DPP-IV activity was determined by cleavage rate of Gly-Pro-AMC (*H*-glycyl-prolyl-7-amino-4-methyl-coumarin; BACHEM).
26. Magnin, D. R.; Robl, J. A.; Sulsky, R. B.; Augeri, D. J.; Huang, Y.; Simpkins, L. M.; Taunk, P. C.; Betebenner, D. A.; Robertson, J. G.; Abboa-Offei, B. E.; Wang, A.; Cap, M.; Xin, L.; Tao, L.; Sitkoff, D. F.; Malley, M. F.; Gougoutas, J. Z.; Khanna, A.; Huang, Q.; Han, S. P.; Parker, R. A.; Hamann, L. G. *J. Med. Chem.* **2004**, *47*, 2587.