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3-[2-((2*S*)-2-Cyano-pyrrolidin-1-yl)-2-oxo-ethylamino]-3-methyl-butyramide analogues as selective DPP-IV inhibitors for the treatment of type-II diabetes

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Abstract—Based on the structures of NVP-DPP728 (**1**) and NVP-LAF237 (Vildagliptin, **2**), three series of DPP-IV inhibitors were synthesized by linking substituted anilines, benzylamines, and phenylethylamines to (2*S*)-cyanopyrrolidine through a linker. More than 20 compounds were evaluated for their in vitro DPP-IV inhibition and selectivity profile over DPP-II, DPP8, and FAP enzymes. Selected compounds **5f** and **7i** showed in vivo plasma DPP-IV inhibition and inhibited glucose excursion in OGTT after oral administration in Wistar rats. Compound **5f** (DPP-IV IC_{50} = 116 nM) has the potential for development as antidiabetic agent.

One of the emerging new drug treatments for type-II diabetes therapy is dipeptidyl peptidase IV (DPP-IV; CD26; E.C. 3.4.14.5) inhibition with small molecules.[1–3] Several DPP-IV inhibitors have entered clinical development, including NVP-DPP728 (**1**),[4,5] NVP-LAF237 (Vildagliptin, **2**),[6–8] MK-0431 (Sitagliptin, **3**),[9–11] and BMS-477118 (Saxagliptin, **4**)[12] (Fig. 1). Vildagliptin and Sitagliptin are under review by US FDA as new treatment option for type-II diabetes.

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by intestinal L-cells in response to food intake.[13] The active form of GLP-1 is a 30-amino acid peptide, which stimulates insulin release, inhibits glucagons release, and slows gastric emptying, each a benefit in the control of glucose homeostasis in patients with type II diabetes. The active form of GLP-1 is rapidly

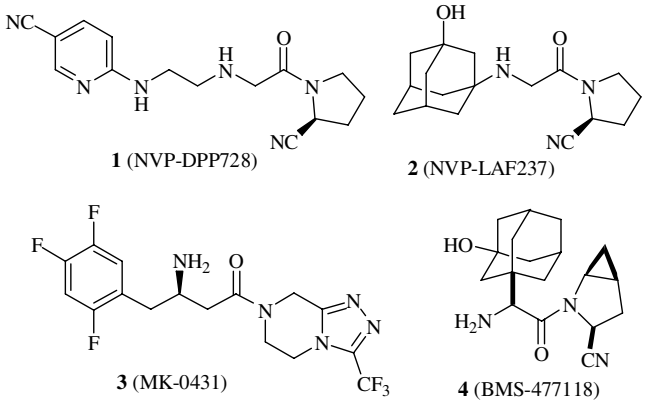

Figure 1. DPP-IV inhibitors.

inactivated ($t_{1/2} \sim 1$ min) by the plasma DPP-IV, which cleaves a dipeptide from the N-terminus.[14,15] Thus inhibition of DPP-IV extends the half-life of endogenously secreted GLP-1, which in turn enhances insulin secretion and improves the glucose tolerance. DPP-IV inhibitors offer several potential advantages over existing therapies including decreased risk of hypoglycemia, potential for

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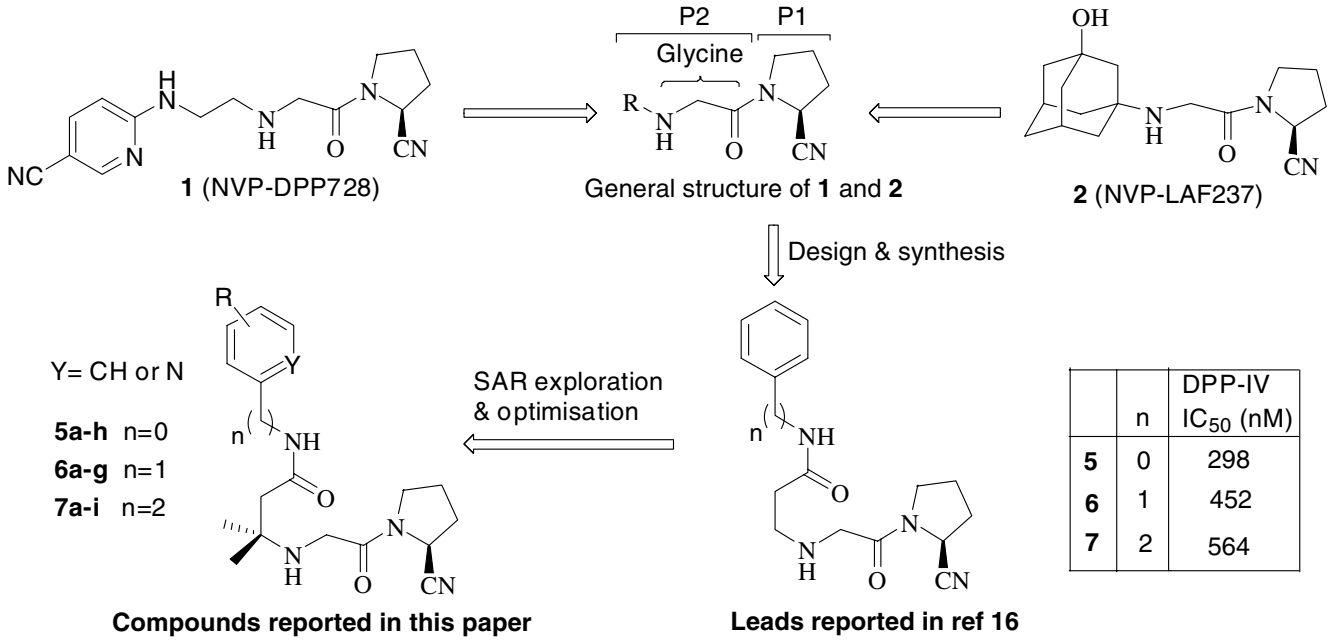
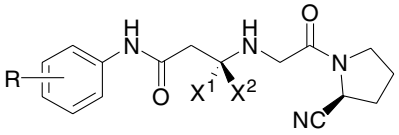


Figure 2. Design of DPP-IV inhibitors based on NVP-DPP728 (**1**) and NVP-LAF237 (**2**).

weight loss, and the potential for regeneration and differentiation of pancreatic β-cells.[1]

We are involved in the design and synthesis of DPP-IV inhibitors and had reported potent and selective DPP-IV inhibitors.[16–18] Design of DPP-IV inhibitors in one of our previous publication[16] was based on the general structure of NVP-DPP728 (**1**) and NVP-LAF237 (**2**) as shown in Figure 2, by retaining the (2*S*)-cyanopyrrolidine in the P1 site and modifying the *N*-substitution of glycine in the P2 site. Aniline, benzylamine and phenylethylamine were attached to the *N*-terminus of glycine through a linker to give leads **5**, **6**, and **7**, respectively. All the three compounds showed moderate DPP-IV inhibition (IC_{50} = 298–564 nM) in in vitro assay, but poor selectivity over related enzymes DPP8 and DPP-II (see Tables 1–3).[16] Selectivity of the DPP-IV inhibitors for closely related prolyl peptidase such as DPP8/9, DPP-II, and FAP is an important criteria for their further development as antidiabetic agents, since inhibition of DPP-II results in the apoptosis of quiescent T-cells[19] and also recent in vivo studies indicate that selective inhibition of DPP8/9 may be associated with profound toxicities.[20] Reports suggest that fibroblast activation protein (FAP) is involved in tumor growth and invasion.[21] Based on these facts we embarked on lead optimization of compounds **5**–**7** to improve the overall potency and selectivity. Herein we report the

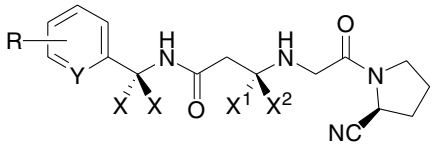
Table 1. Inhibition of DPP-IV, DPP8, DPP-II, and FAP by aniline derivatives



Compound	R	$X^1 = X^2$	IC_{50}[a] (μM)			
			DPP-IV[b]	DPP8	DPP-II	FAP
5	H	H	0.298[c]	0.855[c]	5.7176[c]	NA
5a	H	CH_3	0.207	>20	>20	>20
5b	4-F	CH_3	0.197	>20	>20	>20
5c	4-OCH_3	CH_3	0.253	>20	>20	>20
5d	3,4-di-F	CH_3	0.225	>20	>20	>20
5e	3,4-di-CH_3	CH_3	0.174	>20	>20	>20
5f	3,4-di-OCH_3	CH_3	0.116	>20	>20	>20
5g	2,3,4-tri-OCH_3	CH_3	0.127	>20	>20	>20
5h	4-Ph	CH_3	0.269	>20	0.800	>20

NA, not available.
[a] See Ref. 23.
[b] Values are expressed as the mean of three independent determinations.
[c] Data from reference 16.

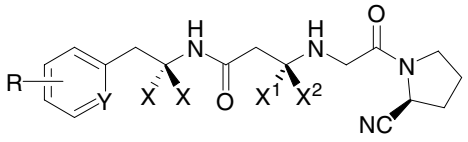
Table 2. Inhibition of DPP-IV, DPP8, DPP-II, and FAP by benzylamine derivatives



Compound	R	X	X^1	X^2	Y	IC_{50}[a] (μM)			
						DPP-IV[b]	DPP8	DPP-II	FAP
6	H	H	H	H	CH	0.452[c]	10.744[c]	17.400[c]	>20
6a	H	H	CH_3	CH_3	CH	0.239	>20	>20	>20
6b	3-CF_3	H	CH_3	CH_3	CH	0.190	>20	>20	>20
6c	H	H	CH_3	CH_3	N	0.433	>20	>20	>20
6d	H	CH_3	H	H	CH	0.119[c]	8.338[c]	>20[c]	>20
6e	H	CH_3	CH_3	CH_3	CH	1.108[c]	>20[c]	>20[c]	>20
6f	H	CH_3	CH_3	H	CH	0.911	>20	>20	>20
6g	H	CH_3	H	CH_3	CH	0.042	1.473	>20	NA

NA, not available.
[a] See Ref. 23.
[b] Values are expressed as the mean of three independent determinations.
[c] Data from reference 16.

Table 3. Inhibition of DPP-IV, DPP8, DPP-II, and FAP by phenylethylamine derivatives



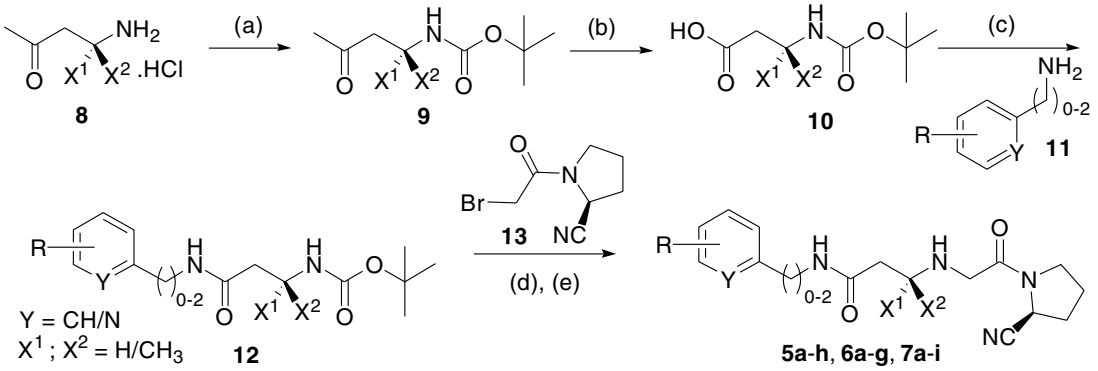
Compound	R	X	$X^1 = X^2$	Y	IC_{50}[a] (μM)			
					DPP-IV[b]	DPP8	DPP-II	FAP
7	H	H	H	CH	0.564[c]	2.592[c]	>20[c]	>20
7a	H	H	CH_3	CH	0.144	>20	>20	>20
7b	H	CH_3	CH_3	CH	1.300	>20	>20	>20
7c	4-CH_3	H	CH_3	CH	0.252	>20	>20	>20
7d	4-CF_3	H	CH_3	CH	0.236	>20	>20	>20
7e	3-CF_3	H	CH_3	CH	0.247	>20	>20	>20
7f	2-CF_3	H	CH_3	CH	0.320	>20	>20	>20
7g	3,4-di-OCH_3	H	CH_3	CH	0.232	>20	>20	>20
7h	3,4-di-Cl	H	CH_3	CH	0.181	>20	>20	>20
7i	H	H	CH_3	N	0.118	>20	>20	>20

[a] See Ref. 23.
[b] Values are expressed as the mean of three independent determinations.
[c] Data from reference 16.

structural optimization of the three lead compounds **5**–**7** for DPP-IV activity and selectivity, also in vivo efficacy of selected compounds is reported.

The general synthetic route for the preparation of three series of DPP-IV inhibitors (**5a**–**h**, **6a**–**g**, and **7a-i**) is shown in Scheme 1. Commercially available anilines, phenylethylamines or benzylamines (**11**) were condensed with *N*-Boc protected substituted β-alanine **10** using DCC. Deprotection of **12** using TFA, followed by reaction of the free amine with 1-(2-bromo acetyl)-(2*S*)-cyanopyrrolidine **13** gave the final products. The β-alanine derivative **10** could be synthesized starting from commercially available keto amines (**8**) in two steps, first by masking the amino functionality with Boc group, followed by oxidation of the keto function to the carboxylic acid group using freshly generated KOCl.[22] Compound **13** was synthesized in 2 steps starting from L-prolinamide as reported in literature.[4] All the synthesized compounds were analyzed by LC–MS and ^{1}H NMR to confirm their structures.

Final compounds (**5a**–**h**, **6a**–**g**, and **7a**–**i**) were assayed for their ability to inhibit DPP-IV in in vitro and their results are reported in Tables 1–3 along with their DPP-II, DPP8, and FAP inhibitory potentials.[23] The lead aniline derivative **5** (Table 1) had a moderate DPP-IV inhibition (IC_{50} = 298 nM) and a poor selectivity index of 3-fold over DPP8 and 19-fold over DPP-II. Introduction of *gem*-dimethyl substituent adjacent to the P2 site amine in **5** gave **5a**, which resulted in a slight enhancement in DPP-IV activity and more than 97-fold



Scheme 1. Reagents: (a) Et_3N, $(BOC)_2O$, 1,4-dioxane; (b) KOCl, 1,4-dioxane/H_2O; (c) HOSu, DCC, 1,4-dioxane; (d) CF_3COOH; (e) K_2CO_3, **13**, THF.

selectivity over DPP8, DPP-II, and FAP. The improvement in the selectivity of **5a** by the introduction of *gem*-dimethyl group was consistent with our previous study.[16]

Having attained one of our objectives of improving the selectivity of **5**, we turned our attention to the effect of aromatic ring substitution. The ring-substituted compounds **5b–5g** retained the high degree of selectivity (>100-fold) toward DPP-IV over DPP8, DPP-II, and FAP similar to that of **5a**. Monosubstituted compounds with 4-F (**5b**) and 4-OCH_3 (**5c**) groups showed very similar range of DPP-IV inhibition to that of **5a**. The disubstituted compounds with 3,4-di-F (**5d**) and 3,4-di-CH_3 (**5e**) also showed similar level of DPP-IV inhibition. But the 3,4-di-OCH_3 compound **5f** showed almost 2-fold improvement in DPP-IV activity (IC_{50} = 116 nM) compared to **5a**. Three other dimethoxy substituted compounds (2,4-di-OCH_3; 2,5-di-OCH_3; 3,5-di-OCH_3) were synthesized and tested for DPP-IV inhibitory activity (data not shown) and found to be less potent than **5f**. As the dimethoxy compound **5f** was twice as potent as the monomethoxy compound **5c**, we synthesized 3,4,5-tri-OCH_3 substituted **5g**, but the introduction of the third methoxy group did not show any further improvement in potency. Having found the electronic effects of substituents, we looked at the steric effect of aromatic substitution by introducing a bulky phenyl ring at the 4-position. Compound **5h** showed a decrease in DPP-IV potency and also loss of selectivity over DPP-II compared to the unsubstituted **5a**. By chemical modification of the lead **5**, we obtained **5f** with an improved DPP-IV potency (3-fold) and better selectivity (57-fold over DPP8) in comparison to **5**.

Next, we took up lead **6** for optimization, which showed moderate DPP-IV inhibition (IC_{50} = 452 nM) and 24-fold selectivity over DPP8 and 38-fold selectivity over DPP-II (Table 2). Introduction of *gem*-dimethyl substituent adjacent to P2 site amine similar to the aniline series resulted in compound **6a**, which showed almost 2-fold improvement in DPP-IV inhibition and an enhancement in selectivity (>84-fold over DPP8 and DPP-II). Modification in the aromatic portion of the molecule by the introduction of 3-CF_3 group resulted in **6b** with a similar level of DPP-IV inhibition and selectivity profile as **6a**. But the replacement of the phenyl ring with a 2-pyridyl ring resulted in 2-fold loss of DPP-IV potency in **6c** compared to **6a**. Thus, we focused our attention back to the modification of the linker. Moving the *gem*-dimethyl substituent of **6a** to a position adjacent to the aromatic ring gave compound **6d** with 2-fold improved DPP-IV potency, but with a slight loss of selectivity (70-fold) over DPP8 compared to **6a** (>84-fold). This prompted us to introduce another set of *gem*-dimethyl group, however compound **6e** with two sets of *gem*-dimethyl group showed a 4.5-fold loss of DPP-IV potency compared to **6a**. Since steric crowding in **6e** may be the reason for the loss of DPP-IV potency, we thought to introduce a single methyl group adjacent to P2 site amine instead of the *gem*-dimethyl group. A differential effect on activity was obtained depending on the stereochemistry of the methyl group introduced. When a *S*-methyl group was introduced, a highly potent DPP-IV inhibitor **6g** (IC_{50} = 42 nM) was obtained, but it also possessed DPP8 inhibition (IC_{50} = 1.47 μM). While, a *R*-methyl group introduction (**6f**) led to a 4-fold loss of DPP-IV potency compared to **6a**.

The last series of compounds were developed from the lead **7** (Table 3), which showed a moderate DPP-IV inhibition (IC_{50} = 564 nM) and poor selectivity (4.5-fold) over DPP8. Introduction of *gem*-dimethyl substituent adjacent to P2 site amine similar to that in the previous two series of compounds resulted in compound **7a**, which showed a 4-fold improvement in DPP-IV inhibition and an enhancement in selectivity (>140-fold over DPP8) in comparison to **7**. Introduction of additional set of *gem*-dimethyl group in the linker portion however led to a 9-fold loss of DPP-IV potency in **7b** in comparison to **7a**. As two sets of *gem*-dimethyl group in the linker seems to be deleterious for the DPP-IV activity, we retained one set of *gem*-dimethyl group adjacent to P2 site amine and modified the aromatic portion to optimize the activity and selectivity. Introduction of mono substituents such as 4-CH_3 (**7c**), 4-CF_3 (**7d**), 3-CF_3 (**7e**) or 2-CF_3 (**7f**) only led to loss of potency in comparison to the unsubstituted compound **7a**. Even a 3,4-di-OCH_3 (**7g**) and 3,4-di-Cl (**7h**) substituents led to a loss of DPP-IV potency. However when we replaced the phenyl ring (**7a**) with 2-pyridyl ring system (**7i**), we were able to improve the DPP-IV potency (IC_{50} = 118 nM) slightly

with retention of high selectivity (>167-fold) over other enzymes.

Potent and selective compound **5f** (Table 1) from the aniline series and **7i** (Table 3) from phenylethylamine series were selected for further evaluation based on their in vitro DPP-IV potency and selectivity profile. As one of the limiting factor for the development of DPP-IV inhibitors containing 2(*S*)-cyanopyrrolidine portion is their inherent chemical instability due to the formation of inactive diketopiperazine,[6,12] we analyzed an aqueous solution of **5f** and **7i** by LC–MS. Both the compounds were stable over extended periods of time in aqueous solutions and no formation of cyclized diketopiperazine was observed even after 2 days.

To access the in vivo efficacy of **5f** and **7i**, ability to inhibit glucose excursion after oral glucose load in oral glucose tolerance test[24] (OGTT, Fig. 3) and in vivo plasma DPP-IV inhibition assay[25] (Fig. 4) were carried out. For the OGTT, compounds were administered by oral route at 10 mpk to Wistar rats 30 min before glucose administration (1.5 g/kg), and then blood samples drawn and analyzed for glucose levels. Both compounds **5f** and **7i** significantly suppressed the glucose excursion observed after glucose load. For the plasma DPP-IV inhibition assay, the compounds were administered to Wistar rats by oral route at 10 mpk, blood samples were collected and analyzed for plasma DPP-IV activity. Both compounds showed strong plasma DPP-IV inhibition after oral administration with similar onset of action, but **5f** is more potent at inhibiting the plasma DPP-IV level than **7i**. Compound **5f** inhibited plasma DPP-IV activity (>80%) within 30 min, and more than 50% inhibition lasted upto 8 h after drug treatment. The DPP-IV activity returned back to original values after around 24 h in both **5f**- and **7i**-treated rats. Suppression of glucose elevation in OGTT and inhibition of DPP-IV activity in the plasma of drug treated rats

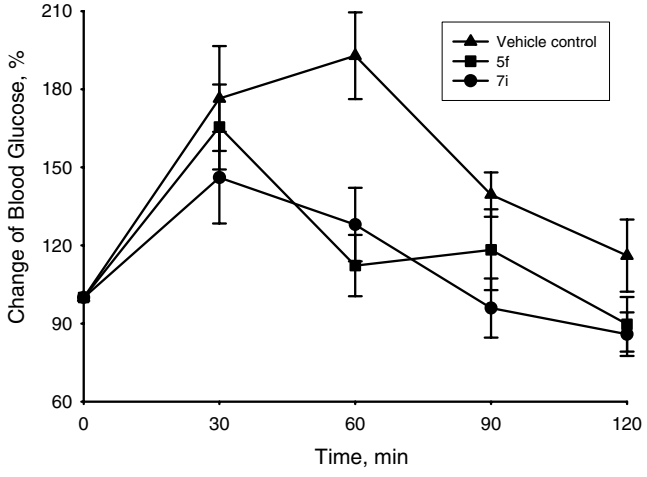


Figure 3. Effects of **5f** and **7i** on the glucose excursion levels of adult male Wistar rats during oral glucose tolerance test. All rats received 1.5 g/kg glucose orally at 0 min. Each compound was orally administered at a 10 mg/kg dose to rats at −30 min. Data are represented as means ± SEM (n = 4/group).

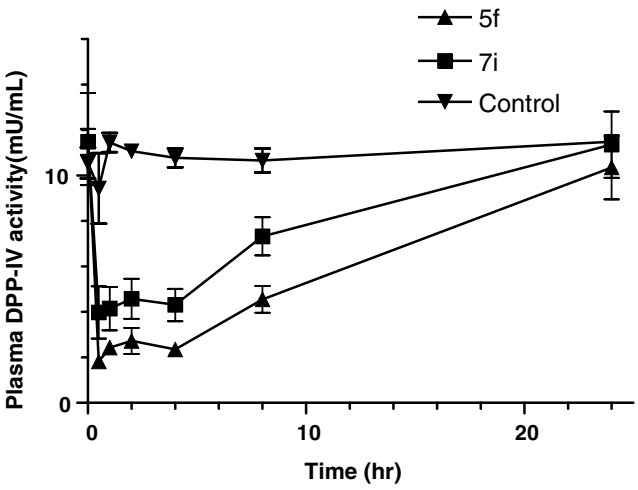


Figure 4. Effects of **5f** and **7i** on the plasma DPP-IV activity in Wistar rats. Each compound was orally administered at a single dose of 10 mg/kg to rats at 0 h. Data are expressed as means ± SEM (n = 4/group).

are a proof of the oral absorption and downstream antidiabetic activity of the compounds. Further pharmacological evaluation is required to appreciate the antidiabetic effect of the compounds.

In summary, three series of compounds (>20 analogs) were synthesized by structural modification of the lead compounds **5**–**7** with improved DPP-IV inhibition potency and selectivity profile. Selected compounds **5f** and **7i** showed in vivo efficacy in two animal models after oral administration. Compound **5f** has the potential for further development as antidiabetic agent.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.12.019.

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23. DPPII, DPP-IV, and DPP8 IC_{50} determinations were performed as discussed in ref. 16. 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). The expression and purification of FAP was carried out essentially as described in ref Chen et al., *Protein Expr. Purif.* **2004**, *35*, 142. The substrates used for FAP assay was Gly-Pro-pNA at 6 mM concentration.
24. OGTT was performed on overnight fasted adult male Wistar rats and the blood glucose was measured with the Accu-Chek Compact System from Roche (Basel, Switzerland). The animals were orally gavaged with the test compounds dissolved in distilled water at a 10 mpk dose. Thirty minutes after the oral dosing of test compounds, the animals were orally gavaged with freshly prepared glucose solution of 400 mg/ml in distilled water at 1.5 g glucose/kg. Blood glucose levels of these dosed animals were monitored at 0, 15, 30, 60, and 120 min after the oral glucose challenge.
25. The in vivo DPP-IV inhibition assay was performed as discussed in ref. 16. 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-methylcoumarin; BACHEM).