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(2*S*,4*S*)-1-[2-(1,1-Dimethyl-3-oxo-3-pyrrolidin-1-yl-propylamino)acetyl]-4-fluoro-pyrrolidine-2-carbonitrile: A potent, selective, and orally bioavailable dipeptide-derived inhibitor of dipeptidyl peptidase IV

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ABSTRACT

A series of 2-[3-[2-[(2*S*)-2-cyano-1-pyrrolidinyl]-2-oxoethylamino]-3-methyl-1-oxobutyl]-based DPP-IV inhibitors with various monocyclic amines were synthesized. The structure–activity relationships (SAR) led to the discovery of potent DPP-IV inhibitors, having IC_{50} values of <100 nM with excellent selectivity over the closely related enzymes, DPP-II, DPP8, DPP9 and FAP (IC_{50} > 20 μM). Of these compounds, the analogues **12a**, **12h** and **12i** exhibited a long-lasting ex vivo DPP-IV inhibition in rats.

Dipeptidyl peptidase IV (DPP-IV, also known as CD26) (EC 3.4.14.5) 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] Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by intestinal L-cells in response to food intake.[3] 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.[4–7] 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 weight loss, and the potential for regeneration and differentiation of pancreatic β-cells.[8] Therefore, DPP-IV has become a validated target for the treatment of type II diabetes, and several inhibitors of DPP-IV are currently undergoing late-stage clinical trials; the first DPP4 inhibitor, sitagliptin **1** (Januvia, Merck) was approved by FDA in October 2006 (Fig. 1),[9,10] vildagliptin **2** (Glavus, Novartis) was approved for use in Europe in September 2007.[11] Recently, The FDA approved

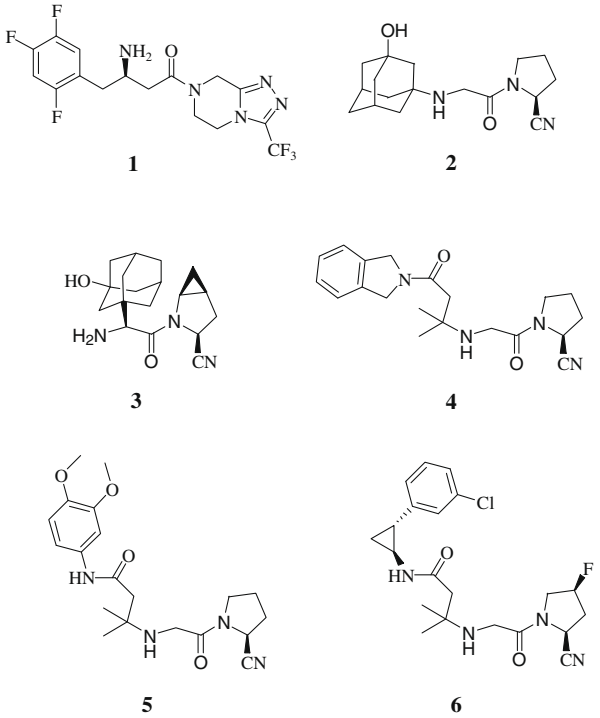

Figure 1. Inhibitors of DPP-IV.

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saxagliptin **3** (Onglyza, Bristol–Myers Squibb), a once-daily treatment for type 2 diabetes to be taken in combination with diet and exercise.[12]

With few exceptions, most DPP-IV inhibitors resemble the P2–P1 dipeptidyl substrate cleavage product. As shown in Figure 1, compounds **2** and **3** are covalent inhibitors. Often this electrophilic cyanopyrrolidine is able to bind covalently with the serine 630 in the S1 pocket of DPP-IV, whereas the non-covalent inhibitor **1** depends on non-covalent protein–ligand interactions and the substituted phenyl group occupies very well the hydrophobic S1 pocket of the enzyme.[9] Previous report from our laboratories described three related series of 2-[3-[[2-[(2*S*)-2-cyano-1-pyrrolidinyl]-2-oxoethyl]amino]-3-methyl-1-oxobutyl]-based (structure **I**) DPP-IV inhibitors (Fig. 2), compounds **4**–**6** are the representative inhibitors in these three series (Fig. 1). Compound **4** is a potent, selective and orally available inhibitor of DPP-IV. In the mice ex vivo plasma DPP-IV inhibition assay, **4** provided >80% inhibition and lasted for at least 4 h after oral administration of 18 mg/kg.[13] Compound **5** is less potent than compounds **4** and **6** (Table 1), and furthermore the abilities of **5** in decreasing the glucose excursion and inhibiting ex vivo plasma DPP-IV activity are not comparable to compound **6**.[14] Novel *trans*-2-arylcyclopropylamine analogue **6** is equipotent with **4** in DPP-IV inhibition and exhibits good selectivity profile, but this analogue does not exhibit a long-lasting ex vivo DPP-IV inhibition profile at a dose of 3 mg/kg in rats (30% inhibition at 8 h after an oral dosing).[15]

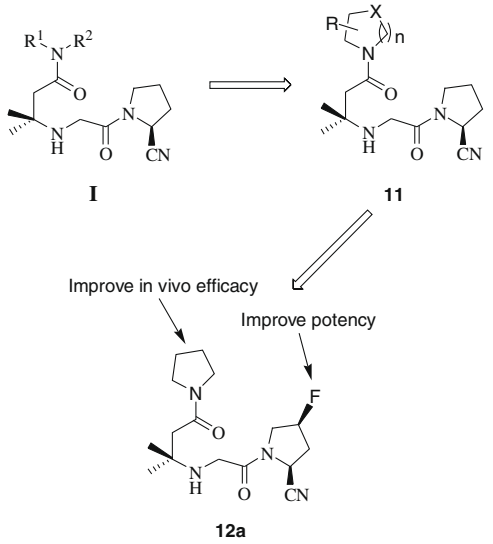
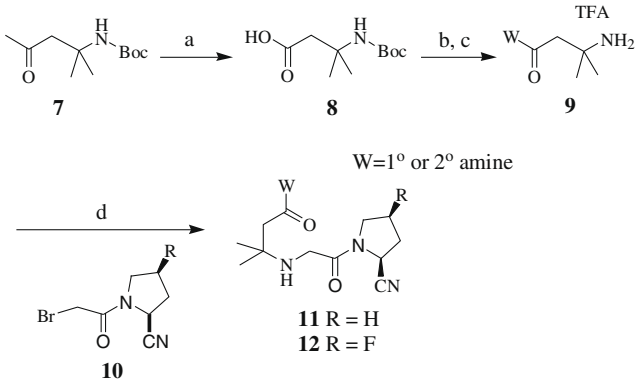


Figure 2. Design of monocyclic amine derivatives as DPP-IV inhibitors.

Table 1
Inhibitory profiles of compounds **1**–**6**

Compound	IC_{50} (μM)[a]				
	DPP-IV	DPP8	DPP9	DPP-II	FAP
1	0.030	>20	>100	>100	>100
2	0.051	14.2	1.2	>50	>100
3[b]	0.0034	0.24	0.10	ND[c]	ND
4	0.015	>100	>100	>100	>100
5	0.116	>50	>20	>20	>50
6	0.015	>20	72%[d]	>100	>20

[a] Means of at least three experiments; standard derivatives are ±20%.
[b] Matsuyama-Yokono et al. Ref. 16.
[c] ND, no data.
[d] Inhibition at 20 μM.



Scheme 1. Reagents: (a) KOCl, 1,4-dioxane/H_2O; (b) DCC, HOSu, 1,4-dioxane/CH_2Cl_2, various amines; (c) CF_3COOH; and (d) K_2CO_3, THF.

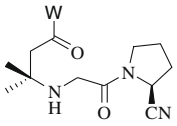
The data shown in Table 1 compare the DPP-IV potency and selectivity of compounds **4**–**6** to known DPP-IV inhibitors **1**–**3**.[16]

Since the purpose of the project was to identify a drug candidate, we continued to search for other series of structure **I**-based inhibitors that increased the inhibitory activity and duration of in vivo DPP-IV inhibition. In this letter, we describe our extensive SAR studies regarding structure **I**-based derivatives to develop long-acting DPP-IV inhibitors, we here report on the discovery of a novel series of monocyclic amine derivatives **11** as DPP-IV inhibitors (Fig. 2). To further improve the potency of this series of compounds, introduction of fluoro substitution at the 4-position of 2-cyanopyrrolidine led to the discovery of compound **12a** (Fig. 2).[17] This approach resulted in not only increased potency, but also improved in vivo efficacy. Compound **12a** is a potent DPP-IV inhibitor (IC_{50} = 15 nM) with excellent selectivity over DPP-II, DPP8, DPP9 and FAP (>50 μM), and **12a** demonstrated in vivo efficacy better than that of inhibitors **5** and **6**.

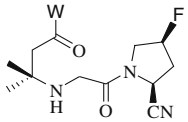
(2*S*)-2-Cyanopyrrolidine analogues **11**–**12** were prepared as described in Scheme 1 and are listed in Table 2. 3-Boc-amino-3-methylbutyric acid **8** was prepared through oxidation of commercially available *N*-Boc-diacetonamine **7** using potassium hypochlorite. Acid **8** was DCC-coupled with various amines followed by Boc-deprotection by TFA to provide TFA salt **9**. 1-Bromoacetyl-2-cyano-(*S*)-pyrrolidine **10** was coupled with free base amine **9** to provide the desired 2-cyanopyrrolidine analogues **11**–**12**. The synthesis of bromoacetylated compound **10** was carried out according to the literature procedure.[13,17a]

Generally, this class of inhibitors shown in Table 2 exhibited potent DPP-IV inhibition (IC_{50} < 100 nM), therefore improvement of selectivity against the off-target enzymes, in particular DPP8 and DPP9 was of great concern since inhibition of DPP8 and/or DPP9 is associated with significant toxicity in preclinical species.[18,19] These inhibitors were also tested against DPP-II[20] and FAP (Fibroblast activation protein),[21] significant inhibition (IC_{50} < 20 μM) was not observed, thus only DPP-IV, DPP8 and DPP9 data are reported. As shown in Table 2, the lead compound, the unsubstituted pyrrolidine **11a**, is a potent DPP-IV inhibitor with an IC_{50} value of 71 nM. When the polar hydroxyl substituent was introduced at the 3-position of pyrrolidine, (3*R*)-pyrrolidin-3-ol **11b** led to a 2.5-fold decrease in DPP-IV inhibitory potency (IC_{50} = 180 nM). To further improve the potency of compounds **11a** and **11b**, introduction of fluoro substituent at the 4-position of 2-cyanopyrrolidine (*S* form) at the P1 site provided compounds **12a** and **12b**, and evaluated fluoro effects on both potency and selectivity. As expected, 4-fluoro-2-cyanopyrrolidine derivatives **12a** and **12b** exhibited 5- and 6-fold better DPP-IV inhibitory activity than **12a** and **12b**, respectively. In view of this fact, we

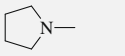
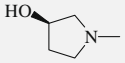
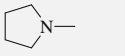
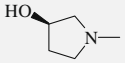
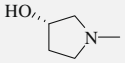
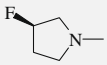
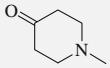
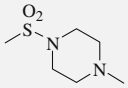
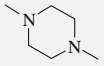
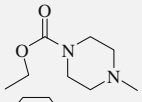
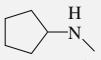
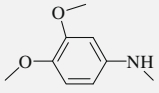
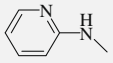
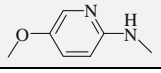
Table 2
Inhibition of DPP-IV, DPP8 and DPP9 by compounds **11**–**12**



11



12

Compound	W	IC_{50}[a] (μM)		
		DPP-IV	DPP8	DPP9
11a		0.071	>20	>20
11b		0.18	>20	>20
12a		0.015	>100	>50
12b		0.030	>20	>20
12c		0.050	>20	>20
12d		0.039	>20	>20
12e		0.024	>20	>20
12f		0.032	>20	>20
12g		0.027	>20	>20
12h		0.027	>20	>20
12i		0.018	>20	50%[b]
12j		0.026	>20	>20
12k		0.020	>20	>20
12l		0.024	>20	>20
12m		0.093	>20	86%[b]
12n		0.063	>20	>20
12o		0.16	>20	>20
12p		0.066	>20	>20
12q		0.029	>20	>20
12r		0.027	>20	73%[b]
12s		0.019	>20	>20
12t		0.019	>20	61%[b]

[a] Means of at least three experiments; standard derivatives are ±20%.
[b] Inhibition at 20 μM.

focused our efforts on studying the diverse monocyclic amines with (4*S*)-4-fluoro-2-cyanopyrrolidine at the P1 site.

As shown in Table 2, the unsubstituted pyrrolidine **12a** is a potent DPP-IV inhibitor with an IC_{50} value of 15 nM. However, substitution at the pyrrolidine ring had little effect on DPP-IV activity and selectivity; compounds **12b**–**g** exhibited high potency (IC_{50} < 50 nM) against DPP-IV, with excellent selectivity versus DPP8 and DPP9 (IC_{50} > 20 μM). Next, we investigated the effects of six-membered ring derivatives, such as piperidine **12h**–**j**, morpholine **12k** and piperazine **12l**–**n**. In comparison with five-membered ring **12a**, six-membered ring **12h**–**l** exhibited a similar potency and selectivity profile (DPP-IV IC_{50} = 18–27 nM, DPP8/9 IC_{50} > 20 μM). As for 1-methylpiperazine **12m** (IC_{50} = 63 nM) and ethyl 1-piperazinecarboxylate **12n** (IC_{50} = 93 nM), both of them showed a significant reduction in DPP-IV inhibition compared to **12a** (IC_{50} = 15 nM). The relatively lipophilic azepane analogue **12o** and 7-azabicyclo[2.2.1]heptane analogue **12p** were 10- and 4-fold, respectively, less potent than **12a** as DPP-IV inhibitors. Further modification was carried out at bringing the nitrogen out of the ring system and developing the cyclopentylamine derivative **12q**, aniline derivative **12r** and pyridine derivatives **12s**–**t**. Cyclopentylamine **12q** is still a potent inhibitor of DPP-IV (IC_{50} = 29 nM). Aniline **12r** (4-fluoro-2-cyanopyrrolidine) is 4-fold more potent than its closely relative analogue 5 (2-cyanopyrrolidine), but **12r** had weak inhibition against DPP9. When the phenyl ring of **12q** was replaced with a more polar heterocyclic ring, pyridin-2-ylamine **12s** and 5-methoxypyridin-2-ylamine **12t** are equipotent against DPP-IV (IC_{50} = 19 nM), but **12t** had weak inhibition against DPP9.

A further evaluation of potency in 50% human (rat) serum and ex vivo DPP-IV inhibition[22] was carried out to provide a finer discrimination between these potent compounds within this structurally related series of inhibitors with respect to pharmacodynamic effects (serum potency shift) and duration of action.[12] DPP-IV inhibitory activities in 50% human (rat) serum and ex vivo DPP-IV inhibition for selected structure **I**-based inhibitors are listed in Table 3. For comparison, first-generation DPP-IV inhibitors **1** and **2** are also included in the table. *trans*-2-Arylcyclopropylamine **6** and pyrrolidine **12a** were all comparable to **1** and **2** in terms of intrinsic potency, but **6** suffered from large serum potency shift (IC_{50} = 847 and 210 nM in human and rat plasma, respectively), resulting in only 66% inhibition of plasma DPP-IV activity at 30 min after oral administration of 3 mg/kg to rats. High non-covalent plasma protein binding of **1** might be one of reasons for its unexpectedly less potent plasma DPP-IV inhibition (30% at 30 min). Compounds **2** and **12a** maintained excellent DPP-IV potency in both human and rat plasma, afforded potent plasma DPP-IV inhibition (>80% at 30 min, 70% at 8 h). Other pyrrolidine analogues **12b**, **12f** and **12g** exhibited a shorter duration of action than **12a**. (3*R*)-pyrrolidin-3-ol **12b** showed less potent ex vivo DPP-IV inhibition (63% at 30 min) most likely due to the decreased oral absorption for the increase in hydrophilicity. As for six-membered ring derivatives, the slightly more lipophilic piperidine analogues **12h**–**i** exhibited ex vivo potency (>85% at 30 min) and duration of action (>70% at 8 h) similar to that of pyrrolidine **12a**, the more hydrophilic morpholine analogue **12k** exhibited 89% plasma DPP-IV inhibition with a modest duration of action, however another hydrophilic analogue, 1-methanesulfonylpiperazine **12l**, showed weaker inhibition of plasma DPP-IV and its efficacy diminished within 8 h. When the nitrogen was brought out of ring system, cyclopentylamine **12q** showed no improvement in ex vivo potency and duration compared to that of **12a**. Aromatic analogue **12r** and heteroaromatic analogues **12s**–**t** reached at least 85% inhibition at 30 min, but none of them are long-lasting DPP-IV inhibitors in rats.

Table 3
Potency in the presence of 50% human and rat serum and ex vivo plasma DPP-IV inhibition in rats

Compound	50% HS[a] IC_{50} (nM)	% plasma DPP-IV inhibition at 3 mg/kg oral dose, normal rats	
		30 min	8 h
1	18 (46)[b]	30	20
2	17 (16)	84	73
6	847 (210)	66	30
12a	5 (10)	82	70
12b	21 (23)	63	15
12f	ND[c]	75	48
12g	ND	77	56
12h	14	86	74
12i	ND	85	70
12k	13 (14)	89	59
12l	15	64	7
12q	ND	81	60
12r	ND	86	18
12s	19 (24)	87	31
12t	18	88	41

[a] Assay done in the presence of 50% human serum.
[b] Values in parentheses are the IC_{50} values in the presence of 50% rat serum.
[c] ND, not determined.

Compound **12a** was chosen for more extensive study in acute efficacy model measurement due to its excellent human plasma DPP-IV inhibition (IC_{50} = 5 nM) and pharmacodynamic duration of action in this preliminary ex vivo assay in rats. In an oral glucose tolerance test (OGTT), when compound **12a** was administered by oral route at 3 mg/kg to C57BL/6j mice 30 min before glucose administration (3 g/kg), and then blood samples drawn and analyzed for glucose levels. The glucose AUC was determined from 0 to 120 min, and OGTT data on **12a** were summarized in Figure 3. Compound **12a** significantly suppressed the glucose excursion observed after glucose challenge (67% reduction in the area under the glucose levels vs. time curve) and provided a comparable efficacy to both the known DPP-IV inhibitors **1** and **2**.

Compound **12a** is a potent, selective and orally active inhibitor of DPP-IV, and **12a** showed modest and good oral bioavailability in rats and dogs (F = 19% and 68%, respectively). Besides, **12a** also possesses an excellent safety pharmacology profile: additional in vitro profiling of **12a** in an extensive panel of 8 enzyme assays showed no significant inhibition at a test concentration of 10 μM,[23] and it did not block the Potassium Channel hERG, Calcium Channel L-type and Sodium Channel Site II at a test concentration of 10 μM (−6%, −9% and 1% inhibition, respectively). Based on these results presented above, compound **12a** was chosen for further evaluation in preclinical studies. Preliminary acute toxicity studies in rats and monkeys demonstrated the compound to be well tolerated.

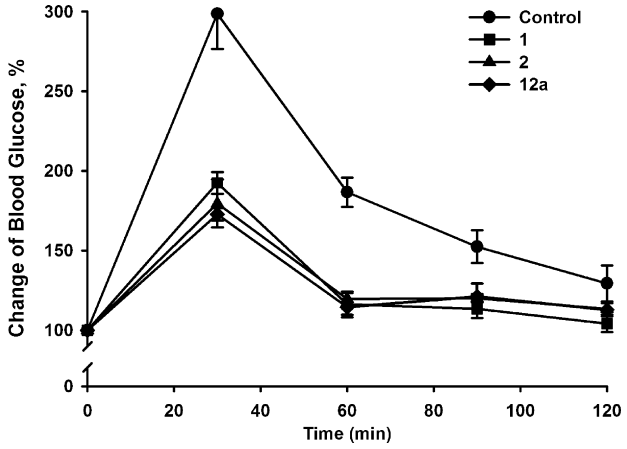


Figure 3. Effects of **1**, **2** and **12a** on the glucose levels after an oral glucose tolerance test in C57BL/6j mice (3 mg/kg, po).

In summary, we have identified a novel series of 2-[3-[[2-[(2*S*)-2-cyano-1-pyrrolidinyl]-2-oxoethyl]amino]-3-methyl-1-oxobutyl]-based analogues as potent and selective DPP-IV inhibitors. Notable among these is compound **12a** having monocyclic pyrrolidine ring in the P2 site. This compound is an IC_{50} = 15 nM DPP-IV inhibitors and displays a more than 3000-fold selectivity over DPP8, DPP9, FAP and DPP-II. The in vivo effects of compound **12a**, including inhibition of plasma DPP-IV activity and suppression of blood glucose elevation, were also demonstrated. The results of these studies indicate that **12a** is a potent, selective, long-acting and safe DPP-IV inhibitor as a potential treatment of type 2 diabetes mellitus.

Acknowledgment

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22. *DPP-IV inhibition in rats:* Adult male Wistar rats were orally gavaged with the test compounds dissolved in 0.5% methyl cellulose at a single dose of 3.0 mg/kg. Blood samples of 25–50 μL were collected from the tail veins at the time points indicated in Table 3, and the plasma fraction was kept frozen until DPP-IV activity measurement. The plasma DPP-IV activity was determined by the cleavage rate of Gly-Pro-AMC (H-glycyl-prolyl-7-amino-4-methylcoumarin; BACHEM). Plasma (10 μL) was mixed with 140 μL of 150 μM Gly-Pro-AMC in assay buffer that was composed of 25 mM tris(hydroxymethyl)aminomethane HCl (pH 7.4), 140 mM NaCl, 10 mM KCl, and 0.1% bovine serum albumin. The fluorescence was determined by using a Fluoroskan Ascent FL (excitation at 390 nm and emission at 460 nm) (Thermo LabSystems; Thermo Electron Corporation). DPP-IV activity in plasma was described as units per milliliter (U/mL). One unit of activity is defined as the amount of enzyme that produces 1 μM products per minute.
23. Compound **12a** (10 μM) inhibition of selected proteases: β-Secretase (3%), Caspase 1 (23%), Caspase 10 (8%), Cathepsin B (−10%), Cathepsin H (0%), Factor Xa (2%), Thrombin (−7%), Trypsin (0%).