2-[3-[2-[(2*S*)-2-Cyano-1-pyrrolidinyl]-2-oxoethylamino]-3-methyl-1-oxobutyl]-1,2,3,4-tetrahydroisoquinoline: A Potent, Selective, and Orally Bioavailable Dipeptide-Derived Inhibitor of Dipeptidyl Peptidase IV

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Dipeptidyl peptidase IV (DPP-IV) inhibitors are expected to become a new type of antidiabetic drugs. Most known DPP-IV inhibitors often resemble the dipeptide cleavage products, with a proline mimic at the P1 site. As off-target inhibitions of DPP8 and/or DPP9 have shown profound toxicities in the in vivo studies, it is important to develop selective DPP-IV inhibitors for clinical usage. To achieve this, a new class of 2-[3-[[2-[(2*S*)-2-cyano-1-pyrrolidinyl]-2-oxoethyl]amino]-1-oxopropyl]-based DPP-IV inhibitors was synthesized. SAR studies resulted in a number of DPP-IV inhibitors, having IC_{50} values of <50 nM with excellent selectivity over both DPP8 (IC_{50} > 100 μM) and DPP-II (IC_{50} > 30 μM). Compound **21a** suppressed the blood glucose elevation after an oral glucose challenge in Wistar rats and also inhibited plasma DPP-IV activity for up to 4 h in BALB/c mice. The results show that compound **21a** possesses in vitro and in vivo activities comparable to those of NVP-LAF237 (**4**), which is in clinical development.

Introduction

Glucagon-like peptide-1 (GLP-1), the most potent insulinotropic hormone known,[1] is a 30 amino acid hormone produced by L-cells of the intestinal mucosa that results from tissue-specific processing of the proglucagon gene.[2] The active form of GLP-1 stimulates insulin secretion, inhibits glucagons release,[3] and slows gastric emptying,[4] each a benefit in the control of glucose homeostasis in patients with type 2 diabetes.[5] However, therapeutic use of GLP-1 itself is severely compromised by its lack of oral activity and rapid degradation by plasma DPP-IV. Thus, a small molecule inhibitor of DPP-IV could extend the duration of action of GLP-1 and prolong the beneficial effects of this incretin hormone. Indeed, inhibition of DPP-IV has been shown to be effective in improving glucose tolerance in diabetic patients and healthy volunteers in human clinical trials and therefore offers a new strategy for treating type 2 diabetes.[6]

Dipeptidyl peptidase IV (DPP-IV) is a serine protease cleaving the N-terminal dipeptide with a preference for L-proline or L-alanine at the penultimate position.[7] Many DPP-IV inhibitors resemble the P2−P1 dipeptidyl substrate cleavage product. The simplest inhibitors of DPP-IV are product-like compounds lacking the carbonyl function of the proline residue, such as aminoacyl pyrrolidines and thiazolidines, which bind and inhibit DPP-IV with moderate ability. One of these compounds, *N*-isoleucylthiazolidine **1** (Figure 1, also called P32/98 by Probiodrug),[8] has moderate potency toward DPP-IV. In NIDDM patients, 60 mg of **1** improved insulin release by 70% and, in combination with acarbose or glibenclamide, suppressed glucose excursions by 20 and 30%, respectively.[9] In some cases,

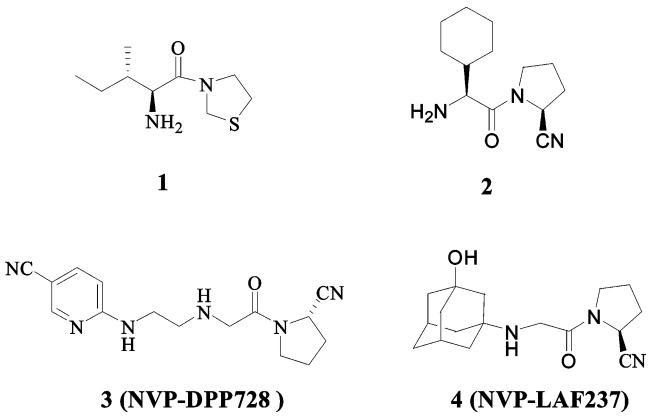

Figure 1. Reported DPP-IV inhibitors.

replacing hydrogen with an electrophilic nitrile group at the 2-position of the pyrrolidine resulted in a 1000-fold increase in potency compared to the unsubstituted pyrrolidines.[10] A series of 2-cyanopyrrolidine derivatives was prepared for optimizing the N-terminal residue, and the result showed that bulky and lipophilic α-substituted amino acid gave more potent compounds. Cyclohexylglycine-(2*S*)-cyanopyrrolidine (**2**) is one of the potent and stable representatives of this nitrile class. This compound has a K_i value of 1.4 nM and an excellent chemical stability $t_{1/2} \sim 48$ h at pH 7.4.[11] Another class of proline-mimetic inhibitors was synthesized by Novartis with diverse N-substituted glycines in the P2 site.[6a] The side chain in this class was moved from the α-carbon to the terminal nitrogen. Two potent derivatives in this series were **3** and **4**, both of which showed great efficacy in clinical trials. A monotherapy for 12 weeks with **4** showed sustained reductions in HbA1c (from a baseline value of 8 to 7.4% by the end of the study),[12] and when given in combination with metformin, no weight gain was reported over the study period.[13]

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Table 1. Inhibition of DPP-IV, DPP8, and DPP-II by Compounds **1**−**4**

	IC_{50}[a] (nM)		
compd	DPP-IV	DPP8	DPP-II
1	1660 ± 230	2283 ± 201	68985 ± 598
2	12 ± 4	27 ± 4	39873 ± 1670
3	53 ± 8	4573 ± 220	26520 ± 764
4	51 ± 10	14219 ± 579	>100000

[a] Values are expressed as the mean ± SD of three independent determinations.

In addition to DPP-IV, the prolyl peptidase family contains DPP-II, DPP8, DPP9, and FAP (fibroblast activation protein), which also cleave the peptide bond at the penultimate proline residue.[14] The selectivity of the DPP-IV inhibitors against other proline-specific dipeptidyl peptidases is one of the key issues in drug development, since the inhibition of DPP-II[15] has been shown to result in the apoptosis of quiescent T-cells.[15b] Recent in vivo studies indicate that selective inhibition of DPP8/9[16] may be associated with profound toxicities (but it is not known if both DPP8 and DPP9 inhibition are required and how much inhibition of each is required for producing the toxicity), whereas selective inhibition of DPP-IV is not.[17] We first assessed the selectivity of existing compounds **1**−**4** for DPP-IV, DPP-II, and DPP8 inhibition. As shown in Table 1,[18] α-carbon-substituted glycine in the P2 site (**1** and **2**) exhibited DPP8 inhibition with IC_{50} values of 2283 and 27 nM, respectively, with no selectivity for DPP-IV over DPP8. In comparison, *N*-substituted glycines in the P2 site combined with a (2*S*)-cyanopyrrolidine in the P1 site (**3** and **4**) were weak DPP8 inhibitors with IC_{50} values of 4.6 and 14.2 μM, respectively, with high selectivity for DPP-IV over DPP8. Thiazolidine (**1**) showed equipotent inhibition against DPP8 and DPP-IV. In addition, compounds **1**−**3** showed weak inhibitory activities against DPP-II with IC_{50} values ranging from 27 to 69 μM, while there is no inhibitory activity for compound **4** against DPP-II (Table 1). Compound **4** exhibits excellent selectivity over other compounds shown in Table 1. Clinical results show that **4** does not have serious side effects. In fact, MK-0431 [(2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)-butan-2-amine] developed by Merck is a highly potent and selective inhibitor of DPP-IV. It does not inhibit DPP-II or DPP8/9 among other proteases tested and currently it has successfully gone into phase III clinical trials.[6c,e]

From the selectivity data of compounds **1**−**4**, it can be concluded that (2*S*)-cyanopyrrolidine derivatives with *N*-substituted glycine in the P2 site (**3** and **4**) are more selective for DPP-IV than α-carbon-substituted glycine (**1** and **2**). On the basis of the results, we endeavored to develop a new pharmacophore in the P-2 site with N-substituted glycine. We first designed P-2 site amine extensions using β-alanine as building block and coupled the C-terminal with various substituted amines to generate a novel pharmacophore in the P2 site. Then, the N-terminal of the β-alanine derivative was combined with the P1 site α-bromoacetyl (2*S*)-cyanopyrrolidide to construct 2-[3-[[2-[(2*S*)-2-cyano-1-pyrrolidinyl]-2-oxoethyl]amino]-1-oxopropyl]-based (structure **I**) DPP-IV inhibitors (Figure 2). The structure−activity relationships of several series of structure **I**-based DPP-IV inhibitors were investigated. This study has led to the discovery of the potent and selective DPP-IV inhibitor **21a** and its analogues. This selective DPP-IV inhibitor **21a** exhibits in vivo potency comparable to that of DPP-IV inhibitor **4**.

Chemistry. (2*S*)-Cyanopyrrolidine analogues **17**−**21** were prepared as described in Scheme 1 and are listed in Table 2.

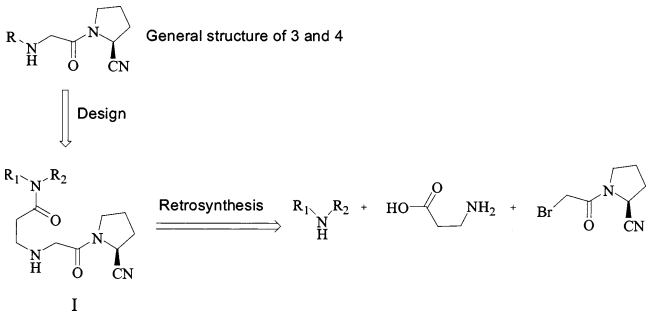
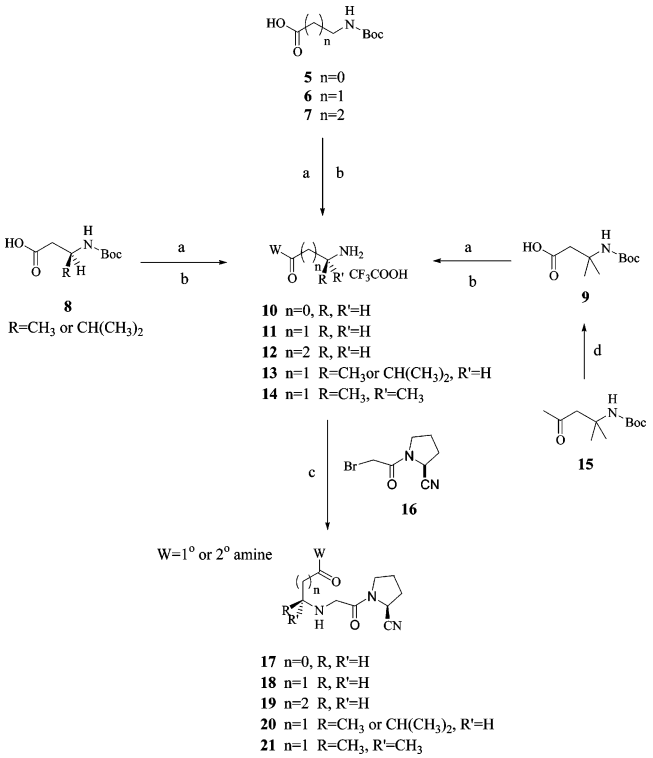


Figure 2. Design and retrosynthesis of 2-[3-[[2-[(2*S*)]-2-cyano-1-pyrrolidinyl]-2-oxoethyl]amino]-1-oxopropyl]-based DPP-IV inhibitors.

Scheme 1[a]



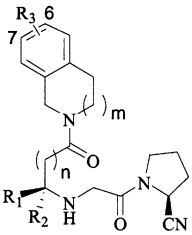
[a] Reagents: (a) DCC, HOSu 1,4-dioxane/CH_2Cl_2, various amines; (b) CF_3COOH; (c) K_2CO_3, THF; (d) KOCl, 1,4-dioxane/H_2O.

Starting materials Boc-glycine **5**, Boc-β-alanine **6**, 4-Boc-amino-butyric acid **7**, Boc-L-β-alanine or Boc-L-β-leucine **8**, and 3-Boc-amino-3-methylbutyric acid **9** were DCC-coupled with various amines followed by Boc-deprotection by TFA to provide TFA salts **10**, **11**, **12**, **13**, and **14**, respectively. Acid **9** was prepared through oxidation of *N*-Boc-diacetonamine **15** using potassium hypochlorite. 1-Bromoacetyl-2-cyano-(*S*)-pyrrolidine **16** was coupled with free base amines **10**−**14** to provide the desired 2-cyanopyrrolidine analogues **17**−**21**. The synthesis of bromo compound **16** was carried out according to the literature procedure.[19]

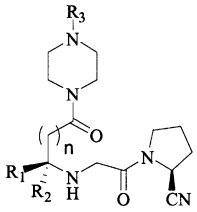
Results and Discussion

Structure **I**-based inhibitors with various substituted amines were explored, including the bicyclic ring system (series I), monocyclic piperazine ring (series II) and phenylalkyl groups (series III). These N-substituted glycine derivatives **17**−**21** described above were tested for inhibition of DPP-IV, DPP8, and DPP-II, and the data is presented in Table 2. Since significant inhibition of DPP-II (IC_{50} < 20 μM) was not observed in all the series of inhibitors shown in Table 2 except

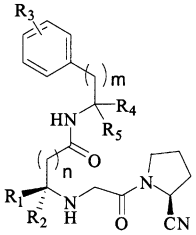
Table 2. Inhibitory Properties of Structure **I**-Based DPP-IV Inhibitors



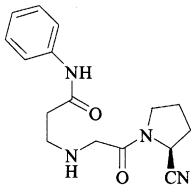
Series I



Series II



Series III



18p

									IC_{50}[a] (nM)		
compd	series	*n*	*m*	R_1	R_2	R_3	R_4	R_5	DPP-IV	DPP8	DPP-II
17	I	0	1	H	H	H			3236 ± 599	4169 ± 736	>100000
18a	I	1	1	H	H	H			116 ± 11	3583 ± 281	>100000
18b	I	1	1	H	H	6,7-diOMe			651 ± 21	3340 ± 599	>100000
18c	I	1	1	H	H	6-F			83 ± 3	1700 ± 761	>100000
18d	I	1	0	H	H	H			132 ± 14	2121 ± 483	>100000
18e	II	1		H	H	$CO(3,5\text{-}F_2C_6H_3)$			676 ± 75	202 ± 33	>100000
18f	II	1		H	H	$SO_2C_6H_4$-4-$NHCOCH_3$			1418 ± 5	3416 ± 3928	>10000
18g	II	1		H	H	nicotinonitrile			629 ± 78	2000 ± 141	18100 ± 987
18h	II	1		H	H	benzothiazole			527 ± 46	2117 ± 294	6452 ± 756
18i	III	1	0	H	H	H	H	H	452 ± 20	10744 ± 917	17400 ± 3865
18j	III	1	0	H	H	4-NO_2	H	H	317 ± 39	2387 ± 151	36003 ± 2573
18k	III	1	0	H	H	H	H	ethyl[b]	447 ± 45	21961 ± 1638	>100000
18l	III	1	0	H	H	3,5-F_2	H	H	369 ± 23	5532 ± 941	34444 ± 1683
18m	III	1	0	H	H	H	H	i-Pr[b]	784 ± 54	12847 ± 3251	29315 ± 1749
18n	III	1	0	H	H	H	CH_3	CH_3	119 ± 40	8338 ± 262	>100000
18o	III	1	1	H	H	H	H	H	564 ± 60	2592 ± 891	20720 ± 424
18p									298 ± 11	855 ± 288	57176 ± 3548
19	I	2	1	H	H	H			428 ± 27	1407 ± 300	31248 ± 1027
20a	I	1	1	CH_3	H	H			54 ± 3	5346 ± 291	18869 ± 3193
20b	I	1	1	i-Pr	H	H			811 ± 60	41859 ± 942	>100000
21a	I	1	1	CH_3	CH_3	H			49 ± 5	>100000	34201 ± 1661
21b	I	1	1	CH_3	CH_3	6-F			30 ± 2	>100000	50146 ± 3858
21c	I	1	1	CH_3	CH_3	6,8-F_2			22 ± 3	>100000	31165 ± 2355
21d	I	1	0	CH_3	CH_3	H			15 ± 2	>100000	>100000
21e	III	1	0	CH_3	CH_3	H	CH_3	CH_3	1108 ± 82	>100000	>100000

[a] Values are expressed as the mean ± SD of three independent determinations. [b] The stereochemistry at the benzylic carbon is not defined (mixture of diastereomers).

18h (IC_{50} = 6452 nM), discussion of SAR studies is focused on inhibition of DPP-IV and DPP8. We started initially with the bicyclic system (series I) using the isoquinoline ring. Compound **18a** inhibited DPP-IV with an IC_{50} value of 116 nM, had 31-fold selectivity over DPP8, and was inactive toward DPP-II. A focused structure−activity profiling effort was initiated by modifying the aromatic ring of isoquinoline. Introduction of 3,4-dimethoxy substituents in **18b** had a moderate decrease in DPP-IV potency by 4-fold of magnitude relative to **18a**, but compound **18b** was approximately equipotent to **18a** as DPP8 inhibitor. On the other hand, introduction of an electron-withdrawing fluorine atom at the 6-position of the isoquinoline ring[20] in **18c** led to some increase in potency (IC_{50} = 83 nM) with a slight drop in selectivity over DPP8 (20-fold). When the isoquinoline ring system was replaced by isoindoline in compound **18d**, it resulted in a similar level of DPP-IV inhibition, as seen for **18a**, but selectivity was decreased by half (16-fold). Replacement of the bicyclic ring system (series I) with monocyclic piperazine derivatives (series II), such as benzoyl **18e**, sulfonamide **18f**, nicotinonitrile **18g**, and benzothiazole **18h**, led to at least a 4.5-fold decrease in DPP-IV inhibitory potency compared to lead compound **18a**. In contrast, all the four compounds exhibited better inhibition of DPP8 than **18a**. The benzoyl derivative **18e** even showed 3-fold higher inhibitory activity for DPP8 (IC_{50} = 202 nM) over DPP-IV (IC_{50} = 676 nM). This compound is the most potent DPP8 inhibitor among the compounds in our investigation.

Next, we brought the nitrogen out of the ring system and developed the acyclic benzylamine and phenylethylamine derivatives (series III). Benzylamine **18i** had DPP-IV potency similar to the phenylethylamine **18o**, but benzylamine **18i** showed good selectivity over DPP8 (24-fold) compared to phenylethylamine analogue **18o** (5-fold). Therefore, we focused our efforts on studying the benzylamine series of compounds (**18j**−**n**). Introduction of an electron-withdrawing group in the phenyl ring, such as the 4-nitro substituent in **18j** and 3,5-difluoro substituents in **18l**, gave compounds with a similar range of activities against DPP-IV, but a decreased selectivity (<15-fold) for inhibition of DPP-IV over DPP8 relative to **18i**. Introduction of ethyl group at the carbon 1 of benzylamine gave the diastereomer **18k**, which provided no improvement in DPP-IV inhibition (IC_{50} ∼ 447 nM). However, there was a significant increase in selectivity over DPP8 (49-fold). Replacement of the ethyl group with isopropyl gave diastereomer **18m**, which showed reduced potency (IC_{50} ∼ 784 nM) and selectivity over DPP8 (16-fold). A significant improvement in DPP-IV inhibition was obtained with 1,1-dimethylbenzylamine **18n**, which was more potent as a DPP-IV inhibitor (IC_{50} ∼ 119 nM) compared to the unsubstituted **18i** and also showed improved selectivity (70-fold). Moving the aromatic ring close to the nitrogen yielded aniline derivative **18p**, which was only twice less potent (IC_{50} ∼ 298 nM) compared to **18a** at DPP-IV inhibition. However, it showed a drastic reduction in selectivity for DPP-IV over DPP8 (3-fold).

Having failed to improve both the potency and selectivity of the lead compound **18a** significantly by the above manipulations of the N-substituents, we set out to investigate the effect of varying the length of alkyl chain between the P2 site amine and carboxylic position. In comparison with **18a**, shortening by one carbon (**17**) or lengthening by one carbon (**19**) decreased the inhibitory potency of DPP-IV by 28- and 4-fold, respectively. The study of the P2 site N-substituted glycines was continued with the evaluation of acyclic branching α to the terminal amine. Introduction of methyl substitution into the β-alanine chain of **18a** to give the diastereomeric pair **20a** was well-tolerated, providing both significantly improved potency ($IC_{50} \sim 54$ nM) and selectivity (99-fold) over DPP8. Increasing the bulk of the branching by introduction of an isopropyl group gave diastereomers **20b**, leading to a 7-fold reduction in DPP-IV potency ($IC_{50} \sim 811$ nM) but still maintaining good selectivity (51-fold). Apparently, the alkyl group adjacent to the P2 site amine has some effect on the selectivity over DPP8. In view of this, we introduced a *gem*-dimethyl group adjacent to the P2 site amine. Compound **21a** exhibited an IC_{50} value of 49 nM and selectivity index of more than 2000 over DPP8; this compound can be considered as an equipotent but more selective DPP-IV inhibitor than **20a**. Compound **21a** also showed weak inhibitory activity against DPP-II ($IC_{50} \sim 34$ μM). Having arrived at the optimized structure in the β-alanine portion of the P2 site for good potency and selectivity, we went back to optimize the isoquinoline portion of the inhibitor. As expected, the *gem*-dimethyl series of derivatives with the introduction of 6-fluoro (**21b**) or 6,8-difluoro (**21c**) substituents[20] in the phenyl ring of **21a**, gave slight improvement in the inhibitory potency of DPP-IV, while both still maintained excellent selectivity for DPP-IV over DPP8 and DPP-II. Replacement of the isoquinoline ring of **21a** with isoindoline gave a very potent DPP-IV inhibitor **21d**, with an IC_{50} value of 15 nM and >6700-fold selectivity over DPP8 and DPP-II. Interestingly, the effect of the *gem*-dimethyl substituent was not observed in the acyclic (series III) compound **21e**, which showed 10-fold reduced potency compared to **18n**. Nevertheless, **21e** was still inactive toward DPP8. Beginning with our lead compound **18a** in bicyclic series I and carrying out a focused structure−activity optimization, we have demonstrated that structure **I**-based DPP-IV inhibitors prefer a bicyclic ring system (series I) with a *gem*-dimethyl group adjacent to the P-2 site amine. Inhibitors with these structure entities, **21a**−**d**, showed better potencies and selectivities than the other series of compounds investigated.

After the discovery that compounds **21a**−**d** were potent and selective inhibitors of DPP-IV in vitro, the aqueous solubility and stability of these analogues need to be investigated before testing the in vivo efficacy of these compounds. The representative analogue **21a** was found to have good solubility (>1000 μg/mL) in distilled water. The aqueous solution stability of **21a** was determined by monitoring the disappearance of **21a** on reverse-phase HPLC in aqueous solution at room temperature. HPLC mass spectral analysis revealed that the amino group of **21a** did not intramolecularly cyclize with the nitrile to form a cyclic amidine or diketopiperazine[19,21] during the time frame of the stability experiment (72 h). Because of the steric size imposed by the fully branched carbon adjacent to the P2 site amine, compound **21a** is much more stable than less branched **3** in aqueous solution.[19]

Compound **21a**, with its potent, selective in vitro DPP-IV inhibition, excellent stability, and water solubility, was chosen for further evaluation of its ability to improve glucose tolerance in Wistar rats and DPP-IV inhibition in mice. The fasting rats were orally dosed with water or with 10 mg/kg inhibitors **21a** and **4**, followed by the administration of 1.5 g/kg glucose 0.5 h after the dosing. Glucose levels were then monitored over a 2 h period. Figure 3 shows the glucose excursion in response to an oral glucose challenge. Vehicle-treated controls were markedly glucose intolerant with an average peak glucose level of 160 mg/dL at 30 min after glucose challenge, whereas the drug-treated animals exhibited a 36% decrease in glucose levels compared to controls (**21a**-treated animals, 102 mg/dL; **4**-treated animals, 103 mg/dL). In addition, the inhibitory effects of orally administered compounds **4**, **21a**, and **21d** at a single dose of 18 mg/kg on plasma DPP-IV activity were measured in mice (Figure 4). A maximum inhibition of plasma DPP-IV activity was observed approximately 30 min after the oral dosing of compound **4**, **21a**, or **21d** and the inhibition of >80% lasted for at least 4 h. The most potent compound **21d** showed similar inhibition profile of plasma DPP-IV activity as **21a**, even though **21d** was 3-fold more potent than **21a** in the in vitro assay. Interestingly, potent fluoro analogues **21b** and **21c** inhibited plasma DPP-IV activity (80−90%) within 30 min, but the duration of DPP-IV inhibition of fluoro analogues was shorter than that of **21a** when compared at the same dose (data not shown). The in vivo effects shown in Figures 3 and 4 demonstrate the ability of **21a** to both significantly decrease the glucose excursion and inhibit plasma DPP-IV activity, and

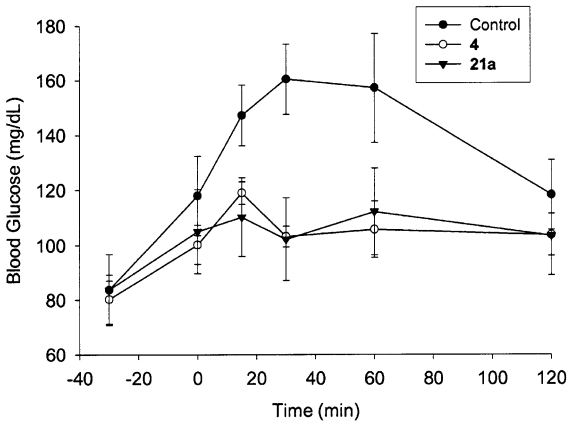


Figure 3. Effects of **21a** and **4** on the glucose excursion levels of adult male Wistar rats during oral glucose tolerance test. All rats received 1.5 g/kg glucose orally at 0 min. Each compound was orally administered at a 10 mg/kg dose to rats at −30 min. Data are represented as mean ± SEM (n = 4/group).

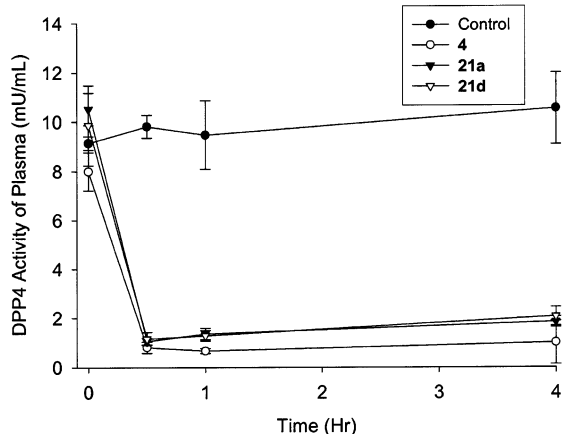


Figure 4. Effects of **21a**, **21d**, and **4** on the plasma DPP-IV activity in mice. Each compound was orally administered at a single dose of 18 mg/kg to mice at 0 h. Data are expressed as mean ± SEM (n = 4/group).

these pharmacological profiles of **21a** are comparable to those of compound **4**, currently in late-stage clinical development.

In summary, we have discovered a novel series of potent and selective DPP-IV inhibitors.

Notable among these is series I inhibitors with a *gem*-dimethyl group adjacent to the P2 site amine (**21a**−**d**), which are IC_{50} < 50 nM DPP-IV inhibitors with an excellent selectivity profile over DPP8 (IC_{50} > 100 μM) and DPP-II (IC_{50} > 30 μM). This series of inhibitors also exhibited excellent aqueous solubility and stability. The in vivo effects of compound **21a**, including inhibition of plasma DPP-IV activity and suppression of blood glucose elevation, were also demonstrated. The result of these pharmacological studies indicates that **21a** is a potent, selective, and orally available inhibitor of DPP-IV.

Experimental Section

All commercial chemicals and solvents are reagent grade and were used without further treatment unless otherwise noted. ^{1}H NMR spectra were obtained with a Varian Mercury-300 spectrometer operating at 300 MHz. Chemical shifts were recorded in parts per million (ppm, δ) and were reported relative to the solvent peak of TMS. High-resolution mass spectra (HRMS) were measured with a Finnigan (MAT-95XL) electron impact (EI) mass spectrometer. LC/MS data were measured on an Agilent MSD-1100 ESI-MS/MS system. Flash column chromatography was done using silica gel (Merck Kieselgel 60, No. 9385, 230−400 mesh ASTM). Reactions were monitored by TLC using Merck 60 F_{254} silica gel glass-backed plates (5 × 10 cm); zones were detected visually under ultraviolet irradiation (254 nm) or by spraying with phosphomolybdic acid reagent (Aldrich) followed by heating at 80 °C. All starting materials and amines were commercially available unless otherwise indicated. The purity of compounds was determined by an Agilent 1100 series HPLC system. HPLC retention times were reported using a Chromolith performance RP-18e 4.6 mm × 100 mm column with two elution conditions. Condition A was 40:60 MeOH−H_2O and condition B was 70:30 MeOH−H_2O. The flow rate was 0.2 mL/min and the injection volume was 5 μL. The system operated at 25 °C. Peaks were detected at 210 nm.

General Procedure for DCC Coupling Reaction for Compounds 10−14. A solution of BOC-protected carboxyl compounds **5**−**9** (1.0 equiv) and HOSu (1.0 equiv) in 20 mL of CH_2Cl_2/1,4-dioxane (2/1) was cooled in an ice−water bath. To this solution was added DCC (1.1 equiv) and the mixture stirred at room temperature for 1 h. To the resulting solution was added the appropriate amine (1,2,3,4-tetrahydroisoquinoline derivatives, benzylamine derivatives, phenylethylamine derivatives, piperazine derivatives, 1.5 equiv). After 4 h of stirring at room temperature, the precipitate was removed by filtration and washed by ethyl acetate (EA). The filtrate and washings were combined and washed sequentially with 10% aqueous citric acid and saturated aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification by flash column chromatography (eluted with hexane/CH_2Cl_2/EA = 4/5/1) yielded the desired compounds **10**−**14** (>70%) as a foam.

General Procedure for the Synthesis of Compounds 17−21. A solution of compound **10**−**14** (3 equiv) in cool TFA was stirred at room temperature for 10 min and concentrated in vacuo for 3 h. The resultant yellowish oil was dissolved in THF, K_2CO_3 (9 equiv) was added, and the mixture stirred for a period of 3 h to liberate free amine. The solution was diluted with EA and filtered to remove potassium salts. The filtrate was concentrated in vacuo so as to obtain about 0.5 M free amine solution. To this solution was added bromide compound **16** (0.4 equiv), and the reaction mixture was stirred overnight at room temperature. The mixture was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo. Purification by flash column chromatography (eluted with CH_2Cl_2/MeOH = 97/3−95/5) yielded desired compounds **17**−**21** (30−45%) as light yellow gums.

2-[2-[2-[2-Cyano-(*S*)-pyrrolidin-1-yl]-2-oxoethylamino]-1-oxoethyl]-1,2,3,4-tetrahydroisoquinoline (17): ^{1}H NMR ($CDCl_3$) (mixture of trans/cis amide rotomers) δ 2.09−2.37 (m, 4H), 2.84−2.93 (m, 2H), 3.42−3.73 (m, 7H), 3.83 (t, 1H, J = 6.0 Hz), 4.57 (s, 0.9H, ArCH_2N), 4.73−4.78 (m, 1.9H, overlapped singlet at 4.73, 1.1H ArCH_2N and 0.8H C*H*CN), 4.95 (d, 0.2H, J = 7.5 Hz, C*H*CN), 7.09−7.27 (m, 4H); HRMS (EI) *m*/*z* calcd for $C_{18}H_{22}N_4O_2$ 326.1743, found 326.1743.

2-[3-[2-[2-Cyano-(*S*)-pyrrolidin-1-yl]-2-oxoethylamino]-1-oxopropyl]-1,2,3,4-tetrahydroisoquinoline (18a): ^{1}H NMR (CD_3OD) δ 2.16−2.28 (m, 4H), 2.87 (t, 1H, J = 6.0 Hz), 2.94−2.98 (m, 3H), 3.41 (t, 2H, J = 5.9 Hz), 3.46−3.51 (m, 2H), 3.63−3.68 (m, 1H), 3.73 (t, 1h, J = 6.0 Hz), 3.81 (t, 1H, J = 6.0 Hz), 4.11−4.13 (m, 2H), 4.70 (d, 2H, J = 9.9 Hz, ArCH_2N), 4.81 (dd, 1H, J = 9.9 and 5.1 Hz, C*H*CN), 7.18−7.19 (m, 4H); HRMS (EI) *m*/*z* calcd for $C_{19}H_{24}N_4O_2$ 340.1899, found 340.1899; HPLC (condition A) t_R = 9.85 min, 97.4%, (condition B) t_R = 6.57 min, 97.4%.

2-[3-[2-[2-Cyano-(*S*)-pyrrolidin-1-yl]-2-oxoethylamino]-1-oxopropyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (18b): ^{1}H NMR ($CDCl_3$) (9/1 mixture of trans/cis amide rotomers) δ 2.16−2.28 (m, 4H), 2.68 (t, 2H, J = 6.0 Hz), 2.77 (t, 1H, J = 6.0 Hz), 2.83 (t, 1H, J = 6.0 Hz), 3.04 (t, 2H, J = 6.3 Hz), 3.41−3.62 (m, 4H, overlapped singlet at 3.51), 3.67 (t, 1H, J = 6.0 Hz), 3.79−3.87 (m, 7H, overlapped singlet of OCH_3 at 3.87), 4.56 (s, 0.9H, ArCH_2N), 4.65 (s, 1.1H, ArCH_2N), 4.73−4.74 (m, 0.9H, C*H*CN), 4.83 (d, 0.1H, J = 6.0 Hz, C*H*CN), 6.60−6.63 (m, 2H); HRMS (EI) *m*/*z* calcd for $C_{21}H_{28}N_4O_4$ 400.2111, found 400.2112.

2-[3-[2-[2-Cyano-(*S*)-pyrrolidin-1-yl]-2-oxoethylamino]-1-oxopropyl]-6-fluoro-1,2,3,4-tetrahydroisoquinoline (18c): ^{1}H NMR ($CDCl_3$) (4/1 mixture of trans/cis amide rotomers) δ 1.94−2.37 (m, 4H), 2.55−2.70 (m, 2H), 2.83 (t, 1H, J = 6.0 Hz), 2.90 (t, 1H, J = 6.0 Hz), 2.93−3.10 (m, 2H), 3.30−3.72 (m, 5H, overlapped triplet at 3.68, J = 6.0 Hz and singlet at 3.47), 3.80 (t, 1H, J = 6.0 Hz), 4.50−4.80 (m, 2.8H, two overlapped singlet at 4.58 and 4.68, 2H ArCH_2N and 0.8H C*H*CN), 4.85 (d, 0.2H, J = 7.5 Hz, C*H*CN), 6.75−6.92 (m, 2H), 7.01−7.11 (m, 1H); MS (ES$^+$) *m*/*z* 359.2 (M + H)$^+$.

2-[3-[2-[2-Cyano-(*S*)-pyrrolidin-1-yl]-2-oxoethylamino]-1-oxopropyl]-1,3-dihydroisoindole (18d): ^{1}H NMR ($CDCl_3$) δ 2.05−2.31 (m, 4H), 2.62−2.66 (m, 2H), 3.04−3.08 (m, 2H), 3.43−3.50 (m, 3H, overlapped singlet at 3.50, ArCH_2NCH_2, C*H*CN), 3.59−3.65 (m, 1H), 4.75−4.83 (m, 5H, two overlapped singlet at 4.79 and 4.83), 7.28−7.30 (m, 4H); HRMS (EI) *m*/*z* calcd for $C_{18}H_{22}N_4O_2$ 326.1743, found 326.1744.

1-[3-[2-[2-Cyano-(*S*)-pyrrolidin-1-yl]-2-oxoethylamino]-1-oxopropyl]-4-[3,5-difluorobenzoyl]piperazine (18e): ^{1}H NMR ($CDCl_3$) δ 2.10−2.20 (m, 2H), 2.21−2.30 (m, 2H), 2.60 (bs, 2H), 2.85−2.95 (bm, 2H), 3.36−3.55 (m, 3H), 3.56−3.88 (m, 9H), 4.77−4.80 (m, 1H, C*H*CN), 6.88−6.96 (m, 3H); HRMS (EI) *m*/*z* calcd for $C_{21}H_{25}F_2N_5O_3$ 433.1925, found 433.1922.

1-[3-[2-[2-Cyano-(*S*)-pyrrolidin-1-yl]-2-oxoethylamino]-1-oxopropyl]-4-[4-[1-oxoethylamino]phenylsulfonyl]piperazine (18f): ^{1}H NMR (CD_3OD) δ 2.11−2.23 (m, 7H, overlapped singlet of NHC(O)CH_3 at 2.13), 2.57 (t, 2H, J = 6.6 Hz), 2.88 (t, 2H, J = 6.6 Hz), 2.96 (bt, 2H, J = 4.8 Hz), 3.01 (bt, 2H, J = 4.8 Hz), 3.41−3.67 (m, 8H, overlapped doublet at 3.49, J = 6.0 Hz), 4.71 (t, 1H, J = 5.4 Hz), 7.69−7.73 (m, 2H), 7.79−7.83 (m, 2H); MS (ES$^+$) *m*/*z* 491.4 (M + H)$^+$, 513.3 (M + Na)$^+$.

1-[3-[2-[2-Cyano-(*S*)-pyrrolidin-1-yl]-2-oxoethylamino]-1-oxopropyl]-4-[benzothiazol-2-yl]piperazine (18h): ^{1}H NMR ($CDCl_3$) δ 2.09−2.36 (m, 4H), 2.62 (t, 2H, J = 6.3 Hz), 2.97−3.04 (m, 2H), 3.37−3.52 (m, 3H, overlapped singlet at 3.47), 3.53−3.81 (m, 9H), 4.74−4.78 (m, 1H), 7.09−7.14 (m, 1H), 7.29−7.35 (m, 1H), 7.56−7.64 (m, 2H); HRMS (EI) *m*/*z* calcd for $C_{21}H_{26}N_6O_2S$ 426.1838, found 426.1841.

***N*-Benzyl-3-[2-(2-cyano-(*S*)-pyrrolidin-1-yl)-2-oxoethylamino]-propionamide (18i):** ^{1}H NMR ($CDCl_3$) (4/1 mixture of trans/cis amide rotomers) δ 2.05−2.32 (m, 4H), 2.45 (t, 2H, J = 6.0 Hz), 2.95 (t, 2H, J = 6.0 Hz), 3.31−3.57 (m, 4H, overlapped doublet at 3.38, J = 3.3 Hz), 4.45 (d, 2H, J = 5.7 Hz), 4.64 (dd, 1/5H, J = 7.5 and 1.5 Hz, C*H*CN), 4.70−4.73 (m, 4/5H, C*H*CN), 7.22−7.36 (m, 5H), 7.56 (bs, 1/5H, ArCH$_2$N*H*), 7.74 (bs, 4/5H, ArCH$_2$N*H*); HRMS (EI) *m*/*z* calcd for $C_{17}H_{22}N_4O_2$ 314.1743, found 314.1741.

3-[2-(2-Cyano-(*S*)-pyrrolidin-1-yl)-2-oxoethylamino]-*N*-(4-nitrobenzyl)propionamide (18j): ^{1}H NMR ($CDCl_3$) (4/1 mixture of trans/cis amide rotomers) δ 2.05−2.35 (m, 4H), 2.50 (t, 2H, J = 6.0 Hz), 3.00 (t, 2H, J = 6.0 Hz), 3.30−3.70 (m, 4H, overlapped doublet at 3.44, J = 7.5 Hz), 4.44−4.60 (m, 2.2H, overlapped doublet at 4.54, J = 7.5 Hz, 2H ArCH_2N and 0.2H C*H*CN), 4.68−4.76 (m, 0.8H, C*H*CN), 7.50 (d, 2H, J = 12.0 Hz), 8.16 (d, 2H, J = 12.0 Hz), 8.28 (bt, 1/5H, ArCH$_2$N*H*), 8.40 (bt, 4/5H, ArCH$_2$N*H*); HRMS (EI) *m*/*z* calcd for $C_{17}H_{21}N_5O_4$ 359.1594, found 359.1594.

3-[2-(2-Cyano-(*S*)-pyrrolidin-1-yl)-2-oxoethylamino]-*N*-(1-phenylpropyl)propionamide (18k): ^{1}H NMR ($CDCl_3$) (9/1 mixture of trans/cis amide rotomers) δ 0.88 (t, 3H, J = 7.5 Hz), 1.74−1.90 (m, 2H), 2.02−2.31 (bm, 4H), 2.37−2.49 (m, 2H), 2.86−3.01 (m, 2H), 3.32−3.62 (m, 4H), 4.62 (d, 1/10H, J = 7.5 Hz, C*H*CN) 4.73−4.77 (m, 9/10H, C*H*CN), 4.89 (q, 1H, J = 7.5 Hz), 7.22−7.40 (m, 5H), 7.81 (bt, 1H); HRMS (EI) *m*/*z* calcd for $C_{19}H_{26}N_4O_2$ 342.2056, found 342.2060.

3-[2-(2-Cyano-(*S*)-pyrrolidin-1-yl)-2-oxoethylamino]-*N*-(3,5-difluorobenzyl)propionamide (18l): ^{1}H NMR ($CDCl_3$) (9/1 mixture of trans/cis amide rotomers) δ 2.14−2.35 (m, 4H), 2.49 (t, 2H, J = 5.7 Hz), 2.98 (t, 2H, J = 6.0 Hz), 3.35−3.66 (m, 4H, overlapped doublet at 3.44, J = 4.2 Hz), 4.37−4.51 (m, 2H), 4.64 (d, 1/10H, J = 7.5 Hz, C*H*CN) 4.73−4.76 (m, 9/10H, C*H*CN), 6.64−6.71 (m, 1H), 6.81−6.87 (m, 2H), 8.08 (bs, 1/10H, ArCH$_2$N*H*), 8.19 (bt, 9/10H, ArCH$_2$N*H*); HRMS (EI) *m*/*z* calcd for $C_{17}H_{20}F_2N_4O_2$ 350.1554, found 350.1550.

3-[2-(2-Cyano-(*S*)-pyrrolidin-1-yl)-2-oxoethylamino]-*N*-(2-methyl-1-phenylpropyl)propionamide (18m). A solution of isopropyl phenyl ketone (296 mg, 2 mmol), ammoniumacetate (1.54 g, 20 mmol), and $NaBH_3CN$ (251 mg, 4 mmol) in MeOH (30 mL) was refluxed for 1 h. The resulting solution was cooled to room temperature and ice-cold 6 N HCl was added until pH ∼ 2. The methanol was evaporated in vacuo and the remaining aqueous solution washed with CH_2Cl_2. Solid KOH was added to the aqueous solution until pH ∼ 12, and the aqueous solution was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to yield crude amine. The crude 2-methyl-1-phenyl-1-propylamine was used without further purification in the next step. DCC coupling with BOC-protected carboxyl acid, followed by TFA deprotection and then amine coupling with bromide compound **16**, was done according to the described general procedure to give **18m**: ^{1}H NMR ($CDCl_3$) (4/1 mixture of trans/cis amide rotomers) δ 0.83 (d, 3H, J = 6.6 Hz), 0.94 (d, 3H, J = 6.6 Hz), 1.99−2.32 (m, 5H), 2.34−2.41 (m, 2H), 2.89−2.96 (m, 2H), 3.32−3.59 (m, 4H), 4.50 (d, 0.2H, J = 7.5 Hz, C*H*CN) 4.70−4.80 (m, 1.8H, 1H ArCH_2N and 0.8H C*H*CN), 7.22−7.34 (m, 5H), 8.14 (bs, 1H); HRMS (EI) *m*/*z* calcd for $C_{20}H_{28}N_4O_2$ 356.2212, found 356.2210.

3-[2-(2-Cyano-(*S*)-pyrrolidin-1-yl)-2-oxoethylamino]-*N*-(1-methyl-1-phenylethyl)propionamide (18n): ^{1}H NMR ($CDCl_3$) (9/1 mixture of trans/cis amide rotomers) δ 1.68 (s, 6H), 2.05−2.31 (m, 4H), 2.38−2.46 (m, 2H), 2.92−2.98 (m, 2H), 3.31−3.44 (m, 1H), 3.45−3.65 (m, 3H, overlapped singlet at 3.48), 4.67 (dd, 1/10H, J = 7.5 and 2.1 Hz, C*H*CN) 4.73−4.75 (m, 9/10H, C*H*CN), 7.17−7.45 (m, 6H); HRMS (EI) *m*/*z* calcd for $C_{19}H_{26}N_4O_2$ 342.2056, found 342.2057; HPLC (condition A) t_R = 10.32 min, 98.6%, (condition B) t_R = 6.67 min, 96.7%.

3-[2-(2-Cyano-(*S*)-pyrrolidin-1-yl)-2-oxoethylamino]-*N*-phenethylpropionamide (18o): ^{1}H NMR ($CDCl_3$) (4/1 mixture of trans/cis amide rotomers) δ 2.05−2.35 (m, 6H, overlapped triplet at 2.31, J = 6.0 Hz), 2.75−2.83 (m, 4H), 3.17−3.35 (m, 3H, overlapped doublet at 3.23, J = 3.0 Hz), 3.42−3.51 (m, 3H), 4.60 (dd, 1/5H, J = 7.5 and 1.8 Hz, C*H*CN), 4.69−4.73 (m, 4/5H, C*H*CN), 7.16−7.27 (m, 5H), 7.29 (bs, 1/5H, ArCH$_2$CH$_2$N*H*), 7.52 (bs, 4/5H, ArCH$_2$CH$_2$N*H*); HRMS (EI) *m*/*z* calcd for $C_{18}H_{24}N_4O_2$ 328.1899, found 328.1904.

3-[2-(2-Cyano-(*S*)-pyrrolidin-1-yl)-2-oxoethylamino]-*N*-phenylpropionamide (18p): ^{1}H NMR ($CDCl_3$) (9/1 mixture of trans/cis amide rotomers) δ 2.03−2.28 (m, 4H), 2.56 (t, 2H, J = 6.0 Hz), 3.04 (t, 2H, J = 6.0 Hz), 3.34−3.75 (m, 4H, overlapped doublet at 3.48, J = 4.2 Hz), 4.63 (d, 1/10H, J = 5.4 Hz, C*H*CN), 4.74−4.77 (m, 9/10H, C*H*CN), 7.07 (t, 1H, J = 7.4 Hz), 7.30 (t, 2H, J = 8.1 Hz), 7.61 (d, 2H, J = 7.8 Hz), 9.86 (s, 1/10H, ArN*H*), 9.99 (s, 9/10H, ArN*H*); HRMS (EI) *m*/*z* calcd for $C_{16}H_{20}N_4O_2$ 300.1586, found 300.1586.

2-[4-[2-[2-Cyano-(*S*)-pyrrolidin-1-yl]-2-oxoethylamino]-1-oxobutyl]-1,2,3,4-tetrahydroisoquinoline (19): ^{1}H NMR ($CDCl_3$) δ 1.86−1.91 (m, 2H), 2.05−2.32 (m, 4H), 2.48−2.55 (m, 2H), 2.69−2.75 (m, 2H), 2.85 (t, 1H, J = 6.0 Hz), 2.91 (t, 1H, J = 6.0 Hz), 3.37−3.61 (m, 4H, overlapped doublet at 3.39, J = 11.1 Hz), 3.70 (t, 1H, J = 6.0 Hz), 3.83 (t, 1H, J = 6.0 Hz), 4.66−4.77 (m, 3H, two overlapped singlets at 4.66 and 4.73), 7.10−7.22 (m, 4H); HRMS (EI) *m*/*z* calcd for $C_{20}H_{26}N_4O_2$ 354.2056, found 354.2059.

2-[3-[2-[2-Cyano-(*S*)-pyrrolidin-1-yl]-2-oxoethylamino]-1-oxobutyl]-1,2,3,4-tetrahydroisoquinoline (20a): ^{1}H NMR ($CDCl_3$) δ 1.20 (d, 3H, J = 8.4 Hz), 2.07−2.32 (m, 4H), 2.41−2.49 (m, 1H), 2.59−2.68 (m, 1H), 2.85 (t, 1H, J = 6.0 Hz), 2.92 (t, 1H, J = 6.0 Hz), 3.24−3.31 (m, 1H), 3.36−3.58 (m, 4H), 3.61−3.73 (m, 1H), 3.75−3.86 (m, 1H), 4.65−4.77 (m, 3H, overlapped doublet at 4.65, J = 3.9 Hz and a singlet at 4.74, ArCH_2N and C*H*CN), 7.10−7.23 (m, 4H); HRMS (EI) *m*/*z* calcd for $C_{20}H_{26}N_4O_2$ 354.2056, found 354.2057; HPLC (condition A) t_R = 10.07 min, 99.2%, (condition B) t_R = 6.68 min, 98.6%.

2-[3-[2-[2-Cyano-(*S*)-pyrrolidin-1-yl]-2-oxoethylamino]-4-methyl-1-oxopentyl]-1,2,3,4-tetrahydroisoquinoline (20b): ^{1}H NMR ($CDCl_3$) δ 0.93−0.99 (m, 5H), 1.90−2.00 (m, 1H), 2.01−2.27 (m, 4H), 2.40−2.52 (m, 2H), 2.86−3.07 (m, 3H, three overlapped multiplets), 3.25−3.89 (m, 6H), 4.49−4.86 (m, 3H), 7.14−7.18 (m, 4H); HRMS (EI) *m*/*z* calcd for $C_{22}H_{30}N_4O_2$ 382.2369, found 382.2369.

2-[3-[2-[2-Cyano-(*S*)-pyrrolidin-1-yl]-2-oxoethylamino]-3-methyl-1-oxobutyl]-1,2,3,4-tetrahydroisoquinoline (21a): ^{1}H NMR ($CDCl_3$) (4/1 mixture of trans/cis amide rotomers) δ 1.23 (s, 3H), 1.25 (s, 3H), 2.05−2.33 (m, 4H), 2.53 (s, 2H), 2.85 (t, 1H, J = 6.0 Hz), 2.91 (t, 1H, J = 6.0 Hz), 3.38−3.48 (m, 3H, overlapped doublet at 3.39, J = 7.5 Hz), 3.53−3.64 (m, 1H) 3.73 (t, 1H, J = 6.0 Hz), 3.83 (t, 1H, J = 6.0 Hz), 4.66−4.76 (m, 2.8H, two overlapped singlets at 4.67 and 4.73, 2H ArCH_2N and 0.8H C*H*CN), 5.10 (d, 0.2H, J = 7.5 Hz, C*H*CN), 7.09−7.22 (m, 4H); HRMS (EI) *m*/*z* calcd for $C_{21}H_{28}N_4O_2$ 368.2212, found 368.2215; HPLC (condition A) t_R = 9.69 min, 99.8%, (condition B) t_R = 6.74 min, 98.2%.

2-[3-[2-[2-Cyano-(*S*)-pyrrolidin-1-yl]-2-oxoethylamino]-3-methyl-1-oxobutyl]-6-fluoro-1,2,3,4-tetrahydroisoquinoline (21b). The synthesis of 6-fluoro-1,2,3,4-tetrahydroisoquinoline was carried out as described in ref 20: ^{1}H NMR ($CDCl_3$) (4/1 mixture of trans/cis amide rotomers) δ 1.22 (s, 3H), 1.25 (s, 3H), 2.02−2.28 (m, 4H), 2.53 (s, 2H), 2.83 (t, 1H, J = 6.0 Hz), 2.89 (t, 1H, J = 6.0 Hz), 3.40−3.53 (m, 3H, overlapped doublet at 3.41, J = 5.4 Hz), 3.58−3.67 (m, 1H), 3.72 (t, 1H, J = 6.0 Hz), 3.80 (t, 1H, J = 6.0 Hz), 4.64−4.76 (m, 2.8H, two overlapped singlets at 4.64 and 4.68, 2H ArCH_2N and 0.8H C*H*CN), 5.07 (d, 0.2H, J = 7.5 Hz, C*H*CN), 6.84−6.93 (m, 2H), 7.06−7.12 (m, 1H); HRMS (EI) *m*/*z* calcd for $C_{21}H_{27}FN_4O_2$ 386.2118, found 386.2116; HPLC (condition A) t_R = 10.40 min, 100.0%, (condition B) t_R = 6.97 min, 99.5%.

2-[3-[2-[2-Cyano-(*S*)-pyrrolidin-1-yl]-2-oxoethylamino]-3-methyl-1-oxobutyl]-6,8-difluoro-1,2,3,4-tetrahydroisoquinoline (21c). ^{1}H NMR ($CDCl_3$) (4/1 mixture of trans/cis amide rotomers) δ 1.23 (s, 3H), 1.26 (s, 3H), 2.05−2.37 (m, 4H), 2.54 (s, 2H), 2.83 (t, 1H, J = 6.0 Hz), 2.89 (t, 1H, J = 5.7 Hz), 3.43−3.55 (m, 3H, overlapped doublet at 3.43, J = 3.3 Hz), 3.60−3.64 (m, 1H), 3.73 (t, 1H, J = 6.0 Hz), 3.83 (t, 1H, J = 6.0 Hz), 4.61 (s, 1H), 4.70 (s, 1H), 4.74−4.77 (m, 4/5H, C*H*CN), 5.04−5.09 (m, 1/5H, C*H*CN), 6.65−6.72 (m, 2H); HRMS (EI) *m*/*z* calcd for $C_{21}H_{26}F_2N_4O_2$ 404.2024, found 404.2025; HPLC (condition A) t_R = 11.16 min, 99.4%, (condition B) t_R = 6.67 min, 99.1%.

2-[3-[2-[2-Cyano-(*S*)-pyrrolidin-1-yl]-2-oxoethylamino]-3-methyl-1-oxobutyl]-1,3-dihydroisoindole (21d): ^{1}H NMR ($CDCl_3$) (7/1 mixture of trans/cis amide rotomers) δ 1.28 (s, 6H), 2.05−2.35 (m, 4H), 2.51 (s, 2H), 3.44−3.57 (m, 3H, overlapped singlet at 3.45), 3.62−3.68 (m, 1H), 4.76−4.80 (m, 2.7H, overlapped singlet at 4.80, 2H ArCH_2N and 0.7H C*H*CN), 4.85 (s, 2H, ArCH_2N), 5.13

(dd, 0.3H, $J = 7.8$ and 1.5 Hz, C*H*CN), 7.24−7.31 (m, 4H); HRMS (EI) *m*/*z* calcd for $C_{20}H_{26}N_4O_2$ 354.2056, found 354.2058; HPLC (condition A) $t_R = 9.67$ min, 100.0%, (condition B) $t_R = 6.75$ min, 97.9%.

3-[2-(2-Cyanopyrrolidin-1-yl)-2-oxo-ethylamino]-3-methyl-*N*-(1-methyl-1-phenylethyl)butyramide (21e): ^{1}H NMR ($CDCl_3$) (10/1 mixture of trans/cis amide rotomers) δ 1.19 (s, 6H), 1.67 (s, 6H), 2.11−2.31 (m, 6H, overlapped singlet at 2.25), 3.37−3.43 (m, 3H, overlapped singlet at 3.37), 3.54−3.60 (m, 1H), 4.72−4.75 (m, 1H), 7.16−7.21 (m, 1H), 7.26−7.32 (m, 2H), 7.36−7.40 (m, 2H), 8.12 (bs, 1/10H, ArC$(CH_3)_2$N*H*), 8.50 (bs, 9/10H, ArC$(CH_3)_2$N*H*); HRMS (EI) *m*/*z* calcd for $C_{21}H_{30}N_4O_2$ 370.2369, found 370.2370.

Inhibition of DPP-IV, DPP8, and DPP-II in Vitro.[15a,22] IC_{50} determination was done as described in the literature with the modifications described below.[15a] A sigma plot was used to obtain the IC_{50} values. DPP-IV and DPP8 were purified as described in our previously published method.[22] The purification of DPP-II was carried out as described in ref 15a with modifications. The buffer for DPP-IV and DPP8 assays are 2 mM Tris-HCl (pH 8.0) and PBS buffer, respectively. The substrate used is Gly-Pro-pNA from Bachem at the concentrations of 500 and 2500 μM for DPP-IV and DPP8 assays, respectively. DPP-II activity was assayed by 1.5 mM Gly-Pro-pNA in 50 mM potassium phosphate buffer, pH 5.5. The concentration of DPP-II is 10 nM. Reaction developed at 37 °C and OD_{405} was monitored.

Oral Glucose Tolerance Test in Wistar Rats. Adult male Wistar rats were fasted overnight. Blood samples were obtained from the tail veins with 27G needles, and the blood glucose was measured with the Accu-Chek Compact System from Roche (Basel, Switzerland). Animals were grouped according to the glucose levels and then orally gavaged with the test compounds dissolved in distilled water at a dose indicated in Figure 3. 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.

DPP-IV Inhibition in Mice.[23] Adult male BALB/c mice were orally gavaged with the test compounds dissolved in 0.5% methyl cellulose at a single dose of 18 mg/kg. Blood samples of 25−50 μL were collected from the tail veins at the time points indicated in Figure 4, 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.

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Supporting Information Available: ^{1}H NMR spectra and HPLC purity analysis for **18a**, **18n**, **20a**, **21a**, **21b**, **21c**, and **21d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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