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Letter

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Design, Synthesis, and Pharmacological Evaluation of Fused β-Homophenylalanine Derivatives as Potent DPP-4 Inhibitors

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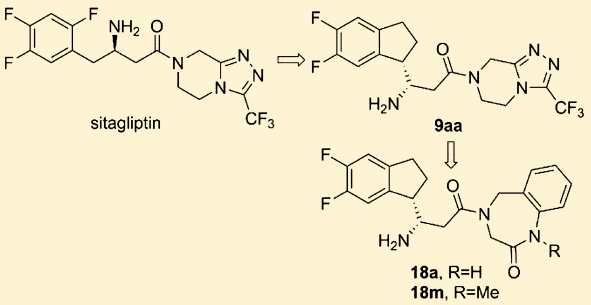
Supporting Information

ABSTRACT: Dipeptidyl peptidase-4 (DPP-4) inhibitors are accepted as a favorable class of agents for the treatment of type 2 diabetes. Herein, a series of fused β-homophenylalanine derivatives as novel DPP-4 inhibitors were designed, synthesized, and evaluated for their inhibitory activities against DPP-4. Most of them displayed excellent DPP-4 inhibitory activities and good selectivity. Among them, **9aa**, **18a**, and **18m** also showed good efficacy in an oral glucose tolerance test (OGTT) in ICR mice. Moreover, when dosed 8 h prior to glucose challenge, **18m** showed significantly greater potency than sitagliptin. It thus provides potential candidates for the further development into potent drugs targeting DPP-4.



KEYWORDS: *Diabetes, DPP-4 inhibitor, β-homophenylalanine derivatives, sitagliptin*

Type 2 diabetes mellitus (T2DM) is a chronic, progressive disease that affects millions of people worldwide. The death toll and economic costs associated with diabetes and its complications are enormously high.[1] The incretin hormone glucagon-like peptide 1 (GLP-1) has been the subject of intense research efforts related to the treatment of type 2 diabetes.[2] Secreted from the L cells of the small intestine,[3] GLP-1 stimulates glucose-induced insulin secretion, inhibits glucagon secretion,[4] and delays the gastric emptying, all of which are beneficial in controlling the blood glucose.[5] GLP-1 is one of the substrates of dipeptidyl peptidase-4 (DPP-4, also known as CD26).[3] By cleaving a dipeptide from the N terminus, DPP-4 efficiently inactivates the active form of GLP-1.[6] Therefore, inhibitors of DPP-4 would increase the half-life of active GLP-1 and prolong the beneficial effects of this incretin hormone on balancing the blood glucose level, leading to their potential to be antidiabetic agents.

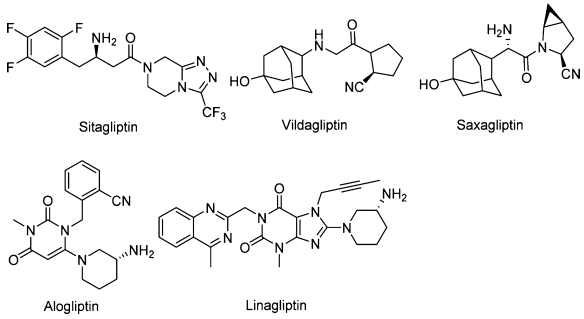


Figure 1. Approved DPP-4 inhibitors.

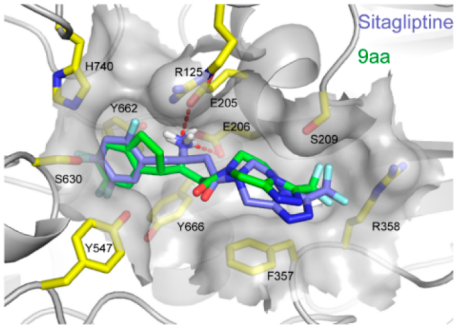


Figure 2. Three-dimensional structural modes of inhibitors **9aa** (green) and sitagliptin (blue) to DPP-4 derived from the docking simulations.

Recently, a number of small molecule DPP-4 inhibitors, including sitagliptin, vildagliptin, saxagliptin, alogliptin, and linagliptin (Figure 1) have emerged as a favorable class of agents for the treatment of type 2 diabetes. The glucose-lowering effect of DPP-4 inhibitors is glucose-dependent, thus these agents could provide a lower risk of hypoglycemia.[7]

Previous study reported the structure−activity relationship (SAR) of the DPP-4 inhibitors derived from sitagliptin, which showed that the trifluorophenyl subunit occupied the S1 hydrophobic pocket and the β-amino group, which forms hydrogen bonding interactions with the side chains of a

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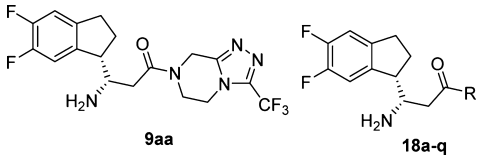
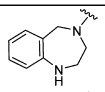
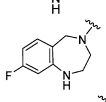
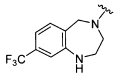
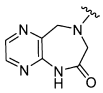
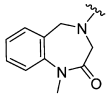
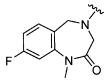
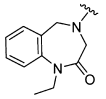
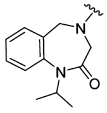
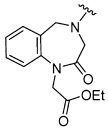
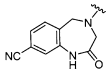
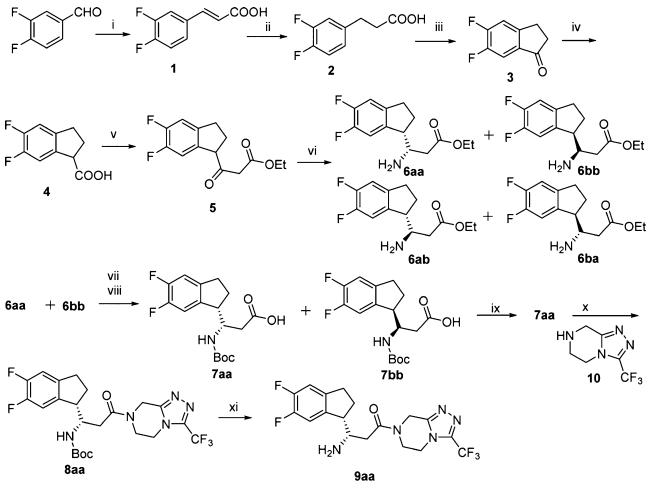
Table 1. DPP-4 Inhibitory Activity



Cpd	R	IC_{50} (nM)	Cpd	R	IC_{50} (nM)
9aa	-	10.8	**18j**		299.0
18a		4.9	**18k**		346.0
18b		148	**18l**		2290
18c		89.6	**18m**		3.0
18d		3.8	**18n**		3.4
18e		20.0	**18o**		2.9
18f		19.2	**18p**		6.4
18g		15.4	**18q**		3.4
18h		18.6	sitagliptin	-	19.0
18i		19.8			

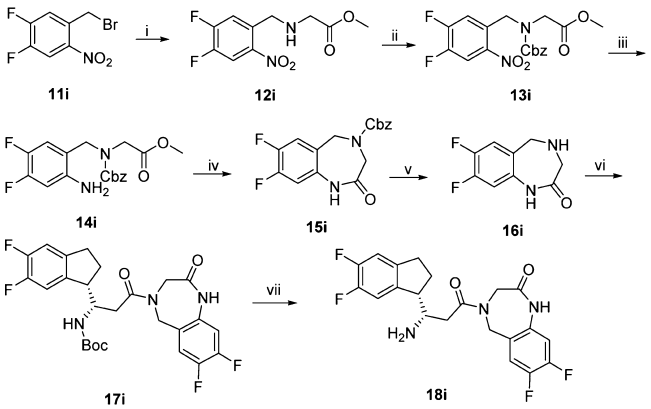
tyrosine (Tyr662) and two glutamate residues (Glu205 and Glu206), are essential for their inhibitory activity against DPP-4 (Figure 2). Recently, most of the reported modifications focus on the P2 binding moiety (the fused heterocyclic ring) and little effort has been made to improve the trifluorophenyl moiety. We present our hypothesis and validation that fusing the aromatic S1-binding moiety could increase the molecular rigidity, lower the entropy loss of binding, and eventually lead to higher potency. To fuse the fluorine-substituted phenyl ring, we first proposed potential strategies using five- and six-membered saturated carboatomics. Among them, compound

Scheme 1. Synthesis of Compound 9aa[a]



[a]Reagents and conditions: (i) malonic acid, piperidine, pyridine, reflux; (ii) Pd/C, H_2, EtOH, rt; (iii) oxalyl chloride, CH_2Cl_2, rt, then $AlCl_3$, CH_2Cl_2, reflux; (iv) ZnI_2, cyanotrimethylsilane, CH_2Cl_2, rt, then $SnCl_2$, HCl(conc.), AcOH, reflux, then KOH, 1,4-dioxane/H_2O, reflux; (v) carbonyl diimidazole, potassium methylmalonate, $MgCl_2$, CH_3CN, rt; (vi) $HCOONH_4$, CH_3OH, 60 °C, then $NaBH_3CN$, rt; (vii) Boc_2O, CH_2Cl_2, rt; (viii) LiOH, CH_3OH/H_2O, rt; (ix) Chiralpak AD-H 250 mm × 5μm × 20 mm; hexane/ethanol (85:15); flow rate 8.0 mL/min; (x) HATU, NEt_3, DMF, rt; (xi) CF_3COOH, CH_2Cl, rt.

Scheme 2. Synthesis of Compound 18i[a]



[a]Reagents and conditions: (i) glycine methyl ester HCl salt, DMF, rt; (ii) benzyl chloroformate, $NaHCO_3$, 1,4-dioxane/H_2O, 0 °C; (iii) Fe, NH_4Cl, CH_3OH/H_2O, 70 °C; (iv) LiOH, THF/H_2O and then HOBT, EDCI, DIPEA, CH_2Cl_2, rt; (v) H_2, Pd/C, rt; (vi) **7aa**, HATU, **10**, NEt_3, DMF; (vii) CF_3COOH, CH_2Cl.

9aa is able to adopt very similar binding conformation compared to sitagliptin (Figure 2).

Encouraged by these docking studies,[8,9] we selected compound **9aa** to verify our hypothesis. To our delight, compound **9aa** exhibited higher DPP-4 inhibitory activity than

Table 2. Pharmacokinetic Properties of Compound 9aa (Mean) in Male SD Rats[a]

cpd	route	dose (mg/kg)	C_{max} (μg/L)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{0-\infty}$ (μg/L·h)	CL_p (L/h/kg)	V_{ss} (L/kg)	F (%)
9aa	p.o.	10	466	0.55	5.11	2848	3.545	25.792	61
	i.v.	5	1493		4.39	2266	2.223	14.182	

[a]Abbreviations: C_{max}, peak plasma concentration of a drug after administration; T_{max}, time to reach C_{max}; $T_{1/2}$, elimination half-life; AUC, area under the concentration−time curve; CL_p, plasma clearance; V_{ss}, volume of distribution at steady state; F, bioavailability; p.o., per oral; i.v., intravenous.

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Table 3. Inhibitory Activities of Selected Compounds against DPP-8, DPP-9, and hERG and Rat Liver Microsome Stability

	IC_{50} (μM)			
cpd	DPP-8	DPP-9	hERG	$T_{1/2}$ (min)[b]
sitagliptin	32	88		
9aa	>10	>10	>20	353
18a	>10	>10	>20	84
18d				111
18i	>10	>10	NT[a]	
18m	>10	>10	NT[a]	
18n	>10	>10	NT[a]	

[a]NT means "not tested". [b]Elimination half-life toward rat liver microsomes.

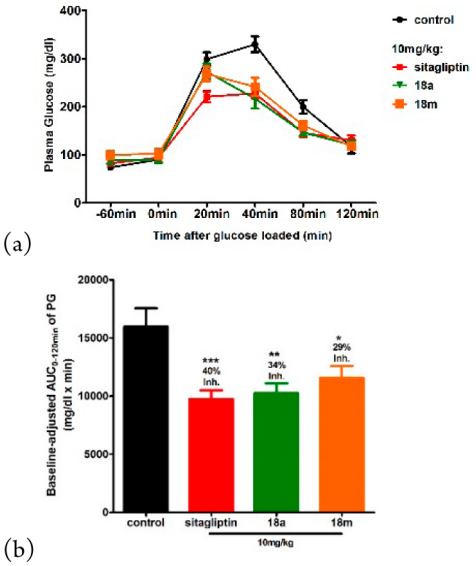

Figure 3. Effect of compounds on plasma glucose (a) and baseline (0 min)-adjusted $AUC_{0-120\ min}$ of plasma glucose (b) after an oral glucose load in glucose tolerance test for ICR mice. Data are represented as mean ± SEM (n = 6−8); *p = 0.05, **p = 0.01, ***$p \leq 0.001$, Student's t test.

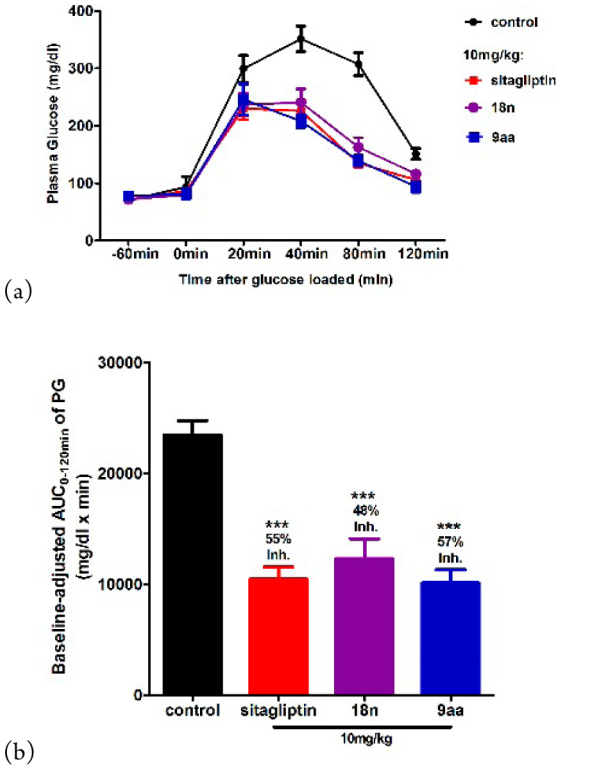

Figure 4. Effect of compounds on plasma glucose (a) and baseline (0 min)-adjusted $AUC_{0-120\ min}$ of plasma glucose (b) after an oral glucose load in glucose tolerance test for ICR mice. Data are represented as mean ± SEM (n = 6−8); *p = 0.05, **p = 0.01, ***$p \leq 0.001$, Student's t test.

sitagliptin with an IC_{50} value of 10.8 nM (Table 1). Of particular interest, compound **9aa** also demonstrated an excellent PK profile (Table 2).

Compound **9aa** was synthesized starting from commercially available 3,4-difluorobenzaldehyde, which was converted to compound **3** via Knoevenagel condensation, reduction, and Friedel−Crafts reaction. Compound **4** was obtained in high yields by converting compound **3** to the cyanohydrin intermediate and subsequent acidic hydrolysis. Compound **4** was then converted to compound **5** by activation with carbonyl diimidazole, followed by treatment with potassium ethyl-malonate. Reductive amination of intermediate **5** gives compound **6**, the mixture of four optical isomers. Compound **6aa** with its enantiomer **6bb** was separated from the mixture by flash column chromatography. After the protection of amino with *t*-butyloxycarbonyl, hydrolysis, and following resolution on a chiral column chromatography, intermediates **7aa** (ee: 97.9%) and **7bb** (ee: 99.9%) were obtained. The key intermediate **7aa** was then condensed with **10** in the presence of HATU to produce compound **8aa**, which was then deprotected with TFA in dichloromethane to produce the target compound **9aa** (Scheme 1). The absolute configuration of **7bb** was confirmed by X-ray single crystallography of compound (3*R*)(12*S*)(29*R*)-**19** (see Supporting Information).

Further modification on the P2-binding moiety (the fused heterocyclic ring) was also done, we expected that modification on these parts could increase the potency of the compounds. With this rationale, a combinatorial approach was then taken to prepare different amides by using a wide variety of differentiated amines coupled with the intermediate **7aa**, which led to the discovery of inhibitors **18a** with an IC_{50} value of 4.9 nM. Then a number of analogues were synthesized and evaluated. Among them, replacement of phenyl moiety with a heteroaromatic ring resulted in a significant decrease in potency. Analogues having pyridine or pyridazine ring, **18b** and **18c**, showed a 30- and 18-fold decrease in the DPP-4 potency over **18a**, respectively; when the metabolic stability of **18a** toward rat liver microsomes was determined, we found a 50% turnover of **18a** after 84 min, which indicated metabolic instability. We reasoned that the unsubstituted phenyl ring and the lactam may be the potential sites for metabolism. Aiming for DPP-4 inhibitors that exhibit a long plasma half-life, various substituents on the phenyl ring were introduced to explore the substitution effects in the region. Our initial SAR development began with monosubstitution on the phenyl ring. Addition of a fluorine at the 8-position of the phenyl ring had a slight effect in increasing potency (**18d**). However, when other substitutions with electron-rich (OMe) and electron-poor (CF_3, CN, Br) residues were introduced on the phenyl moiety, the potency for both was decreased compared to that of compound **18a**. From the above results, it appears that substitution except fluorine on

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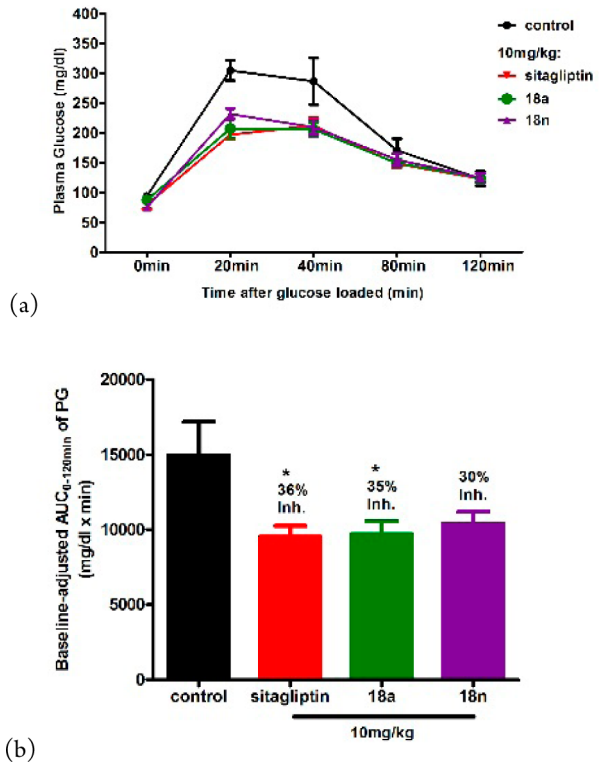

Figure 5. Effect of compounds on plasma glucose (a) and baseline (0 min)-adjusted $AUC_{0-120\ min}$ of plasma glucose (b) after an oral glucose load in glucose tolerance test for ICR mice. Data are represented as mean ± SEM (n = 6−8); *p = 0.05, **p = 0.01, ***$p \leq 0.001$, Student's t test.

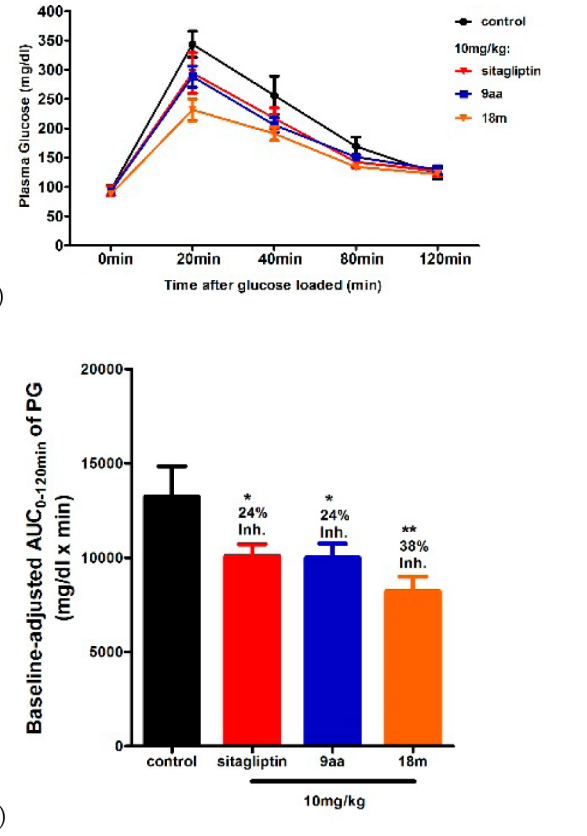

Figure 6. Effect of compounds on plasma glucose (a) and baseline (0 min)-adjusted $AUC_{0-120\ min}$ of plasma glucose (b) after an oral glucose load in glucose tolerance test for ICR mice. Data are represented as mean ± SEM (n = 6−8); *p = 0.05, **p = 0.01, ***$p \leq 0.001$, Student's t test.

the phenyl ring was somewhat detrimental to activity. Once this was established, we focused to study the SAR of the diazepin-2-one moiety. In order to improve the metabolic stability of compounds, the lactam was reduced. Unfortunately, compounds **18j**, **18k**, and **18l** showed a significantly decrease in potency, which indicated that the lactam was essential to their inhibitory activity. When the lactam was alkylated, the inhibitory activity showed a slight change: The methylated reaction product of **18a** (**18m**) showed a slight increase in DPP-4 potency. Such increase was also seen when the lactam of compound **18a** was introduced to an ethyl or a 2-ethoxy-2-oxoethyl, while isopropylation of **18a** slightly decreased inhibitory activity.

The benzo[e][1,4]diazepine-2-one derivatives[10] (represented as example **18i**) were synthesized starting from 1-(bromomethyl)-4,5-difluoro-2-nitrobenzene (**11i**), which was converted to compound **12i** via replacement reaction with glycine methyl ester, and the later was protected by benzyl chloroformate to furnish **13i**. Compound **13i** was then reduced to give **14i**, which was converted to **15i** via hydrolysis and subsequent intramolecular cyclization. Deprotection of **15i** produced **16i**, which was condensed with **7aa** in the present of HATU to produce compound **17i**. Deprotection of **17i** with TFA in dichloromethane produced the target compound **18i** (Scheme 2).

As a result of their high potency against DPP-4, **9aa**, **18a**, **18i**, **18m**, and **18n** were selected to investigate their selectivity versus other related serine proteases. Differing from their excellent DPP-4 inhibitory potency, all test compounds showed weak inhibitory activity against DPP-8, DPP-9, and hERG. Compared to compound **18a**, **18d** in which a fluorine was introduced showed a slight increase in metabolic stability toward rat liver microsomes (Table 3).

Based on *in vitro* potency and selectivity analysis, compounds **9aa**, **18a**, **18m**, and **18n** were selected for acute efficacy evaluation by oral glucose tolerance test (OGTT) in ICR mice. Compounds dissolved in water were orally administrated at a dose of 10 mg/kg 1 h prior to glucose challenge (2.5 g/kg). The blood glucose was monitored at different time intervals from 0 to 2 h.

Compared to sitagliptin, compounds **9aa**, **18a**, and **18n** showed comparable glucose lowering effect at 10 mg/kg dose: **18a** (34%), **18m** (29%) vs sitagliptin (40%); **9aa** (57%), **18n** (48%) vs sitagliptin (55%), respectively (Figures 3 and 4).

To determine whether these compounds have long effective duration *in vivo*, the most potent compounds **9aa**, **18a**, and **18n** were selected for assessment of their efficacy at 8 h after administration. At a dose of 10 mg/kg, compounds **9aa** and **18a** were as effective as sitagliptin (24% vs 24%, 35% vs 36%), whereas **18n** was less potent (30% vs 36%, Figures 5 and 6). Considering that **18m** might be metabolized into **18a** *in vivo*, the long duration efficacy of **18m** was also investigated. It was found that, after 8 h administration, **18m** also had greater potency than sitagliptin in glucose challenge (Figure 6).

In summary, based on the analysis of a large volume of crystal structure data available in the protein data bank (PDB), we designed, synthesized, and evaluated a series of novel fused β-homophenylalanine derivatives as potent and selective DPP-4 inhibitors. Compounds **9aa**, **18a**, and **18m** possessed an

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excellent DPP-4 inhibitory activity, high selectivity, and good *in vivo* efficacy in an OGTT in ICR mice. Moreover, **18m** had significantly greater potency than sitagliptin when dosed 8 h prior to a glucose challenge, thus providing potential candidates for the further development into potent drugs targeting DPP-4.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and spectroscopic data for the compounds described in this Letter. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

†These authors contributed equally to this work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

ICR, Institute of Cancer Research; HbA1c, glycated hemoglobin; SAR, structure−activity relationship; PDB, protein data bank; Cbz, benzyloxycarbonyl; rt, room temperature; THF, tetrahydrofuran; DMAP, 4-(*N*,*N*-dimethylamino) pyridine; HOBT, hydroxybenzotriazole; EDC, 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide; DIPEA, *N*,*N*-diisopropylethylamine; TFA, trifluoroacetic acid; HATU, *o*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*,*N*′-tetramethyluroniumhex-afluorophosphate

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