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Discovery of potent dipeptidyl peptidase IV inhibitors derived from β-aminoamides bearing substituted [1,2,3]-triazolopiperidines for the treatment of type 2 diabetes

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ABSTRACT

A series of novel [1,2,3]-triazolopiperidine derivatives **5a–5y** were synthesized and evaluated as inhibitors of dipeptidyl peptidase IV (DPP-4) for the treatment of type 2 diabetes, most of the compounds exhibited excellent in vitro potency (IC_{50} <50 nM) against DPP-4. Among these, compound **5d** with potent in vitro activity against DPP-4 and good pharmacokinetic profiles exhibited pronounced in vivo efficacy in an oral glucose tolerance test (OGTT) in ICR mice. On the base of these properties, compound **5d** was selected as a potential new candidate for the treatment of type 2 diabetes.

Type 2 diabetes is a chronic disease characterized by elevated plasma glucose in the presence of high endogenous insulin levels, causing serious debilitating cardiovascular, significant morbidity, nerve damage and mortality.[1] In recent years, inhibition of dipeptidyl peptidase IV (DPP-4), a serine protease, has emerged as an attractive new approach to the treatment of type 2 diabetes. It is generally accepted that DPP-4 inhibitors exert their beneficial effects by increasing the levels of active glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), both of which enhance insulin secretion in a glucose-dependent manner.[2] Additionally, GLP-1 stimulates insulin biosynthesis, inhibits glucagon secretion, slows gastric emptying, reduces appetite, and stimulates the regeneration and differentiation of islet β-cells. DPP-4 inhibitors increase circulating GLP-1 and GIP levels in humans, which leads to decreased blood glucose levels, hemoglobin A1C levels and glucagon levels.[3]

To date, several DPP-IV inhibitors have obtained approval as a class of novel antidiabetic agents, the first marketed DPP-4 inhibitor, Sitagliptin **1** was approved by FDA in October 2006 (Fig. 1); Vildagliptin **2** was approved by the European Medicines Agency in September 2007; Saxagliptin **3** was approved by FDA as a once-daily treatment for type 2 diabetes to be taken in combination with diet and exercise.[4] Recently, Alogliptin **4** had been launched in Japan as a potent (IC_{50} <10 nM) and highly selective DPP-4 inhibitor

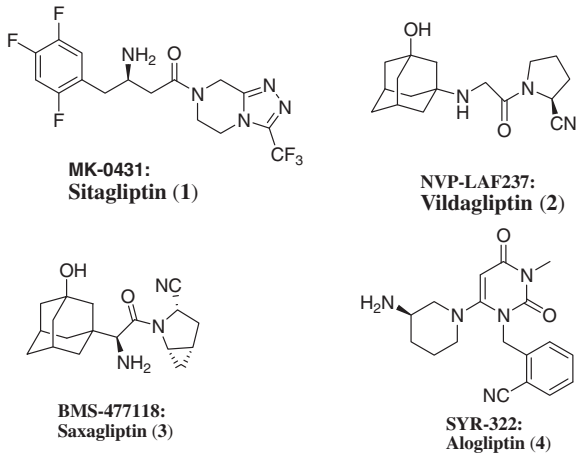

Figure 1. Selected DPP-4 inhibitors.

(selectivity >10,000 over DPP-8 and -9).[5] DPP-4 inhibitors possess advantages over alternative diabetes therapies including a lowered risk of hypoglycemia, a potential for weight loss, and the potential for the regeneration and differentiation of pancreatic β-cells.[6]

Since Sitagliptin was identified as a potent, selective and orally active antidiabetic agent for the treatment of type 2 diabetes, development of novel antidiabetic agents based on the β-aminoamide backbone of Sitagliptin has attracted the attention of

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researchers from both academic and industrial fields.[7] According to some reported structure–activity relationships (SARs) studies of DPP-4 inhibitors, we knew that substitution of alkyl around β-aminoamide backbone was found to be detrimental to potency, alkyl substitutions along with other modifications such as lengthening, shortening, or tethering were also proved to be ineffective. We therefore became interested in modifying the 3-(trifluoromethyl)-[1,2,4]-triazolopiperazine group of Sitagliptin **1** to various C-4 substituted [1,2,3]-triazolopipera-zine group (Fig. 2). Herein, we report our efforts towards the synthesis, SARs and biological properties of a series of potent DPP-4 inhibitors derived from β-aminoamides bearing substituted [1,2,3]-triazolopiperdine groups.

The synthesis of substituted [1,2,3]-triazolopiperdine intermediates began by converting azide **6**[8] to **7a–7c** through intramolecular cycloaddition reaction (Scheme 1). Treatment of **7a** with NBS in CH_3CN afforded **8a**, while **7b** underwent standard hydrolysis reaction to give **8b**. Compound **8a** was treated with corresponding Boronates via Suzuki–Miyaura couplings[9] to generate **9a–9l** or with corresponding Stannane agents via Stille couplings[10] to afford **9m–9p**. Compound **8b** readily underwent Swern oxidation[11] to give **10**, which was reacted with DAST to obtain difluoromethy **9q** or with TosMIC to afford fused oxazol-5-yl **9r**.[12] Meanwhile, **8b** was treated with iodine in 2,2,2-trifluoroethanol to form **11**,[13] which was substituted by methanol, ammonium hydroxide and dimethylamine to afford **9s–9u**, respectively.

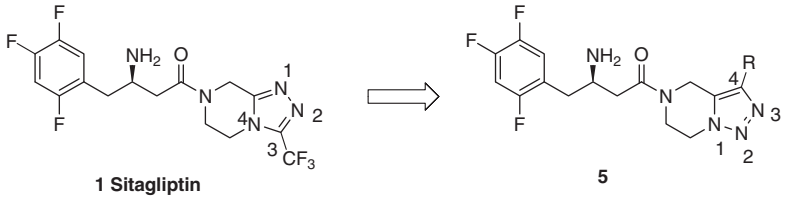
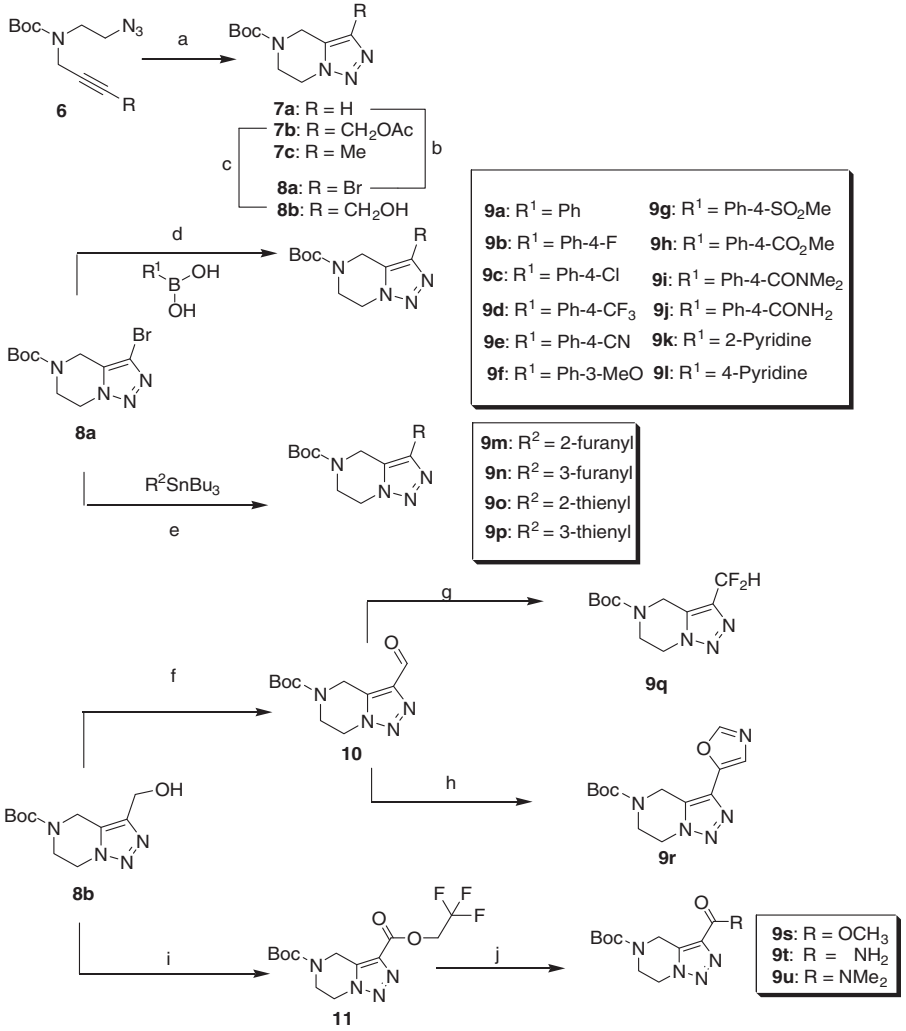
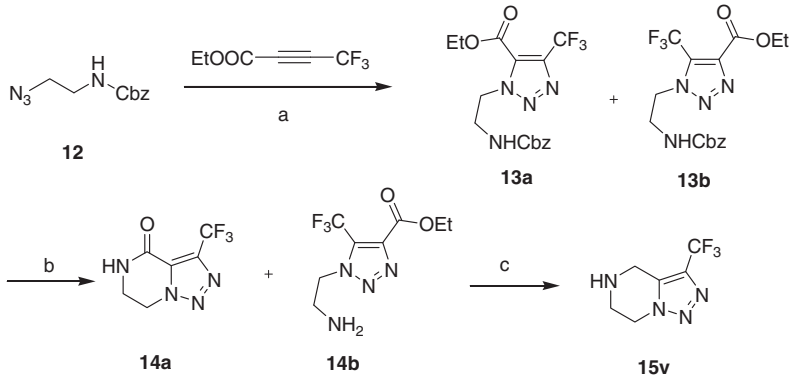


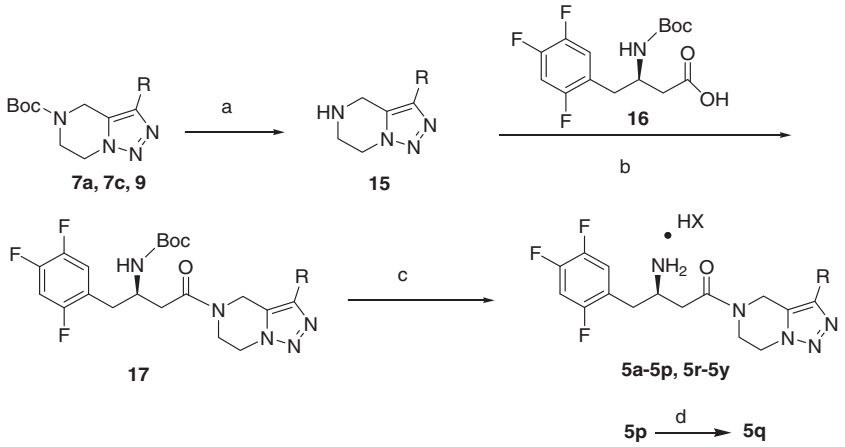
Figure 2. Design of substituted [1,2,3]-triazolopiperdine derivatives as DPP-4 inhibitors.



Scheme 1. Reagents and conditions: (a) DMSO, 150 °C; (b) NBS, CH_3CN (51% yield); (c) LiOH, $MeOH/H_2O$ (80% yield); (d) $Pd(PPh_3)_4$, K_2CO_3, dioxane/H_2O; (e) $Pd(PPh_3)_4$, toluene; (f) $(COCl)_2$, DMSO, DIPEA, CH_2Cl_2, −78 °C (78% yield); (g) DAST, CH_2Cl_2 (51% yield); (h) TosMIC, MeOH, K_2CO_3, reflux (59% yield); (i) CF_3CH_2OH, I_2, K_2CO_3 (35% yield); (j) CH_3OH, reflux (for **9s**, 45% yield); NH_3H_2O, rt (for **9t**, 86% yield); $NH(CH_3)_2$, K_2CO_3 (for **9u**, 98% yield).



Scheme 2. A novel synthetic method for **15v**. Reagents and conditions: (a) toluene, rt, overnight (97% yield); (b) 10% Pd/C, H_2, rt; reflux (35% yield, 2 steps); (c) $LiAlH_4$, THF (43% yield).



Scheme 3. Reagents and conditions: (a) TFA, CH_2Cl_2; (b) HOBT, EDC, TEA, CH_2Cl_2, rt; (c) CF_3COOH, CH_2Cl_2; HX, rt; (d) LiOH, TEA, CH_2Cl_2.

As shown in Scheme 2, trifluoromethyl intermediate **15v** was readily synthesized from azide **12** in three steps. Cycloaddition of azide **12** with ethyl 4,4,4-trifluorobut-2-ynoate in toluene to give a mixture of isomers (**13a** and **13b**). Without further purification, the mixture of **13a** and **13b** directly underwent deprotection of Cbz group by catalytic hydrogenation, followed by filtration and refluxing overnight to form **14a** and **14b**, which were treated with 1 M HCl to afford the pure **14a** in organic phase, finally, the desired intermediate **14a** reacted with LAH to yield trifluoro-methyl **15v**.

After removal of N-protected Boc group of previously prepared intermediate amines **7a**, **7c**, **9** (shown in Scheme 1), the resulting amino **15** were treated with β-amino acid **16** under the typical HOBT/EDC condensation conditions[14] followed by deprotection of Boc group to afford the target compounds **5a–5p** and **5r–5y** (Scheme 3), while compound **5q** was obtained from compound **5p** by hydrolysis reaction. All the target compounds were confirmed by ^{1}H NMR and MS (ESI) data.[15]

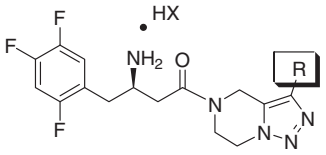
Inhibitory potency of the synthesized compounds was measured by monitoring the hydrolytic reaction of Ala-Pro-amidomethyl-coumarin (Ala-Pro-AMC) by human DPP-4 with Sitagliptin as positive control, and the results are reported as concentrations for 50% inhibition (IC_{50}). As shown in Table 1, most of the compounds exhibited excellent in vitro potency (IC_{50} <50 nM) against DPP-4. The C4-unsubstituted compound **5a** exhibited the weakest activity with IC_{50} of 89.5 nM; while substituted **5a** with simple alkyl group (compounds **5b–5d)** resulted in superior activity to compound **5a**, especially compound **5c** was similar in potency to Sitagliptin with IC_{50} of 22.53 nM. Introduction of methoxycarbonyl or amide substitutents to compound **5a** afforded compounds **5e–g**, which showed improved potency against DPP-4 than simple alkyl analogues **5a–d**, electron-withdrawing groups may be favorable to potency, especially compound **5e** (IC_{50} = 15.09 nM) displayed more potent activity than Sitagliptin. Unfortunately, compound **5e** did not have the required solution stability to warrant further evaluation.

To further improve the potency of this series of compounds, (un)substituted phenyl analogues **5h–5r** were synthesized, all compounds except compound **5k** exhibited potent activity with IC_{50} <50 nM, methyl benzoate derivative **5p** showed the most potent activity with IC_{50} of 12.4 nM. To further study, compounds **5s–5y** with different heteraryl substitutents were prepared. Among these, 2-furyl derivative **5u** showed more potent activity than the other heteraryl derivatives, but there is not significant difference between them, compounds **5u** (IC_{50} = 15.8 nM) and **5y** (IC_{50} = 17.83 nM) showed more potent activity than Sitagliptin (IC_{50} = 19.4 nM).

Selected compounds (**5c**, **5d**, **5f**, **5q**, **5t**, **5u** and **5y**) were further subjected to pharmacokinetic properties study in healthy male rats (200–220 g weight, four rats in each group), the test compounds were administered orally at the dose of 20 mg/kg. The results seen in Table 2. Trifluoromethyl derivative **5d** exhibited favorable pharmacokinetic profiles with moderate half-life (2.53 h) and excellent AUC (4559 ng/mL h). Compounds **5c** and **5t** exhibited accepted pharmacokinetic properties, while compounds **5f** and **5u** were unfavorable.

Furthermore, compounds **5c**, **5d** and **5t**, exhibited excellent in vitro potency and favorable pharmacodynamic profiles, were chosen for the assessment of their ability to improve oral glucose

Table 1
Inhibitory properties of substituted [1,2,3]-triazolopiperazine analogues **5a–5y**



Compd	R	HX	DPP-IV IC_{50}[a] (nM)
5a	H	Fumaric acid	89.50
5b	CH_3	HCl	77. 06
5c	CF_2H	HCl	22.53
5d	CF_3	Fumaric acid	50.66
5e	CO_2Me	HCl	15.09
5f	CO_2NH_2	HCl	29.92
5g	CO_2NMe_2	HCl	52.34
5h	Ph	H_3PO_4	37.54
5i	Ph-4-F	H_3PO_4	45.20
5j	Ph-4-Cl	HCl	46.89
5k	Ph-4-CF_3	HCl	75.35
5l	Ph-4-CN	HCl	30.03
5m	Ph-3- CO_2Me	HCl	46.61
5n	Ph-4-$CONH_2$	HCl	36.43
5o	Ph-4-$CONMe_2$	HCl	26.64
5p	Ph-4-CO_2Me	H_3PO_4	12.40
5q	Ph-4-COOH	HCl	33.78
5r	Ph-4-SO_2Me	HCl	33.17
5s	4-Pyr	HCl	28.60
5t	2-Pyr	Fumaric acid	33.25
5u		HCl	15.80
5v		Fumaric acid	26.17
5w		HCl	22.76
5x		Fumaric acid	47.27
5y		Fumaric acid	17.83
1			19.40

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

Table 2
Pharmacokinetic properties of the selected compounds[a]

Entry	Compd	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (ng/mL h)
1	**5c**	695	1.00	2.27	2524
2	**5d**	965	0.88	2.53	4559
3	**5f**	231	0.63	2.23	871
4	**5q**	600	0.38	2.18	1128
5	**5t**	484	1.38	2.67	2509
6	**5u**	255	1.00	1.87	730
7	**5y**	475	1.88	1.35	1643

[a] All PK was measured in rats ($n = 4$) at 20 mg/kg dose. C_{max}, maximal concentration when dosed orally; T_{max}, time of maximum concentration; $t_{1/2}$, terminal half-life when dose orally; AUC, area under curve. For structures, see Table 1.

tolerance in ICR mice. Results on an oral glucose tolerance test (OGTT) and corresponding pharmacodynamic (PD) studies in ICR mice are shown in Figure 3, Each of compounds or water (vehicle) was orally administered 60 min prior to an oral dextrose challenge (5.0 mg/kg), compound **5d** significantly reduced peak blood glucose excursion.

In summary, we described the synthesis, SARs and biological evaluations of a novel series of [1,2,3]-triazolopiperidine derivatives as highly potent DPP-IV inhibitors. Among these, compound

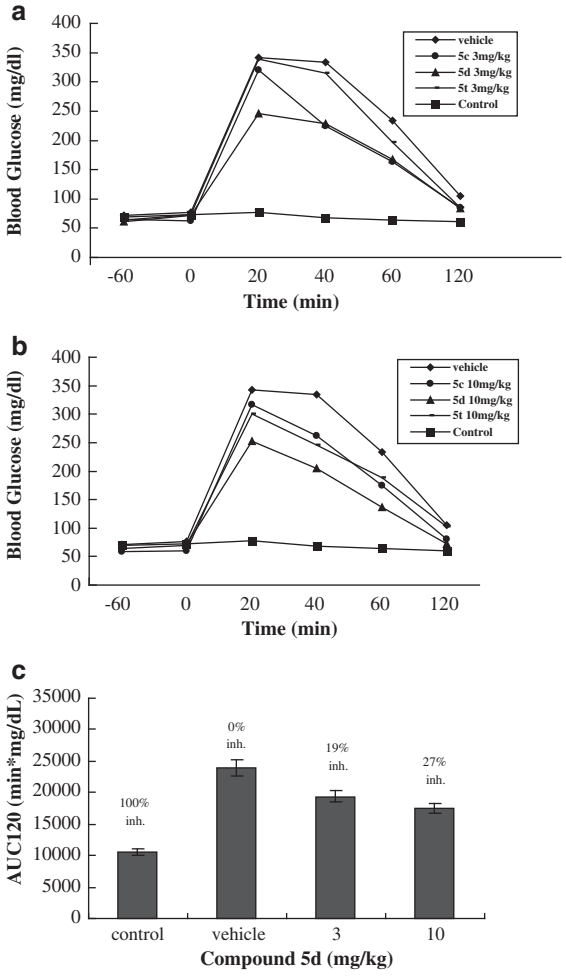


Figure 3. (a,b) Effect of compounds **5c**, **5d** and **5t** (3, 10 mg/kg) on glucose levels after an oral glucose tolerance test; (c) the suppression of glucose AUC was calculated from 0 to 120 min. Data are represented as mean ± SEM ($n = 7–10$/group).

5d with potent in vitro activity against DPP-4 and good pharmacokinetic profiles exhibited pronounced in vivo efficacy in an oral glucose tolerance test (OGTT) in ICR mice. On the base of these properties, compound **5d** was selected as a potential new candidate for the treatment of type 2 diabetes, and further study of compound **5d** against a panel of related peptidases, including DPP7, DPP8, DPP9, POP and FAP is underway in our laboratory.

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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.2011.01.086.

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15. (a) All new compounds were characterized by ^{1}H NMR and LC-MS prior to submission for biological evaluation. (b) Compound **5d**: ^{1}H NMR (400 MHz, CD_3OD, 5d fumarate salt): δ 7.33–7.39 (m, 1H), 7.19–7.25 (m, 1H), 6.66 (s, 1H), 5.04–5.10 (d, 1H), 4.90–4.93 (d, 1H), 4.53–4.58 (m, 1H), 4.47–4.50 (m, 1H), 4.10–4.14 (m, 1H), 3.99–4.04 (m, 1H), 3.85–3.90 (m, 1H), 2.98–3.1 (m, 2H), 2.82–2.98 (m, 2H). ESI-MS: 408.3 $[M+1]^+$. Elemetal analysis: Anal. Calcd for $C_{16}H_{15}F_6N_5O{\cdot}C_4H_4O_4$: C, 45.90; H, 3.66; N, 13.38. Found: C, 46.20; H, 3.99; N, 13.03.