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Synthesis and biological evaluation of azobicyclo[3.3.0] octane derivatives as dipeptidyl peptidase 4 inhibitors for the treatment of type 2 diabetes

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ABSTRACT

A series of novel azobicyclo[3.3.0]octane derivatives were synthesized and evaluated as dipeptidyl peptidase 4 (DPP-4) inhibitors. The effort resulted in the discovery of inhibitor **2a**, which exhibited excellent efficacies in an oral glucose tolerance test. Introduction of methyl group (**2j**) could prolong the inhibition of serum DPP-4 activity.

Inhibition of dipeptidyl peptidase (DPP-4) has been a promising new approach to the treatment of type 2 diabetes mellitus (T2DM) since three drugs have been approved, Sitagliptin (Januvia®, MK-0431),[1] Vildagliptin (Glavus®, LAF-237),[2] and Saxagliptin (Onglyza™, BMS-477118).[3–5] (Fig. 1). DPP-4 is a key regulatory enzyme and a signaling factor of insulin-stimulating hormones, glucagon-like peptide (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP).[6–8] Due to rapid degradation of the both incretin hormones by DPP-4, the half-lives of active GLP-1 and GIP are extremely short.[9] Inhibition of plasma DPP-4 enzyme leads to prolong the actions of endogenous GLP-1 and GIP, which ultimately decreases blood glucose levels and glucagon levels, and improves glucose homeostasis with a low risk of hypoglycemia and potential for disease modification.[10]

Bicyclo[3.3.0]octane derivative **1** was previously reported to be a potent DPP-4 inhibitor,[11] we now report the synthesis, biological evaluation and SAR of this series of azobicyclo[3.3.0]octane compounds (**2**) which possess less stereocenters and can be synthesized more conveniently.

As shown in Scheme 1, Boc-protected cyclopenta[*c*]pyrrol-5(1*H*)-one **3**[12] was first deprotected and then treated with various acyl chlorides to afford key intermediates **4a–g**, which underwent reductive amination with (*S*)-1-(2-aminoacetyl)-pyrrolidine-2-carbonitrile (**5**) to give 5β substituted compounds **2a–g**.[13]

The 5α isomer of urea **2a** was synthesized as shown in Scheme 2. Ketone **4a** was stereoselectively reduced to 5β-alcohol **6**, which was mesylated and inverted to 5α-amine **9** by employing potassium phthalimide displacement followed by hydrolysis. Compound **9** was further substituted by (*S*)-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile (**10**) to afford the desired 5α-substituted isomer **2h**.[14]

In order to discourage intramolecular cyclization of amine to nitrile so as to improve stability of compounds **2a** and **2h**,[15] 5-methyl derivatives were also synthesized (Schemes 3 and 4). Reaction of ketone **4a** with tosylmethyl isocyanide gave a mixture of diastereomeric carbonitriles **11**, which was selectively methylated to give 5α-methyl derivative **12**. Acid hydrolysis of the carbonitrile **12** followed by Curtis rearrangement afforded the corresponding 5α-methyl-5β-amine **14**, which was substituted with **10** to give 5α-methyl isomer **2i**. In order to obtain 5β-methyl isomer **2j**, another synthetic approach was applied. Wittig methylenation of ketone **4a** gave methylene **15**[12], which was treated with $AgClO_4$ and trimethylsilyl cyanide followed by hydrolysis to give desired tertiary 5β-methyl isocyanide **16** in a yield of 18%.[16] Isocyanide **16** was hydrogenated and alkylated to give 5β-methyl isomer **2j**. The stereochemistry of 5-methyl groups for **2i** and **2j** was determined by 2D NOESY.

Enzymatic inhibitions of DPP-4, DPP-8, and DPP-9 of azobicyclo[3.3.0]octane derivatives were outlined in Table 1.[17] The selectivity of DPP-4 against DPP-8 and DPP-9 is critical as the inhibition of these two enzymes may be associated with profound toxicities.[18] Analog **2a** showed an IC_{50} of 9 nM against DPP-4. The selectivity ratio

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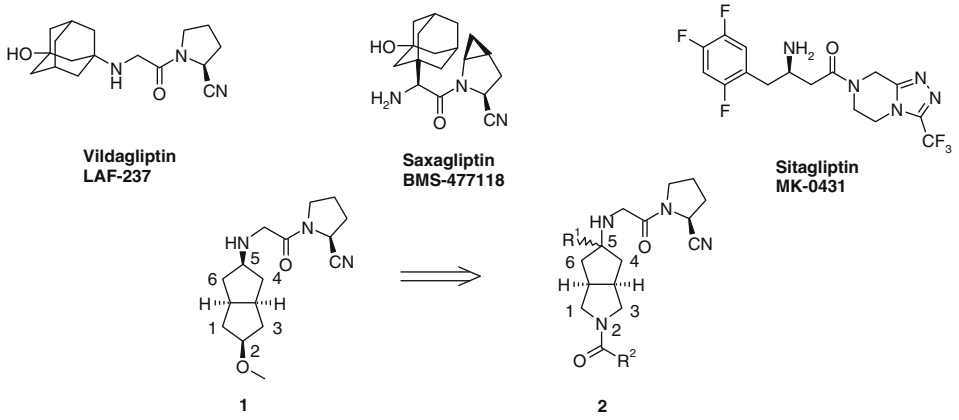
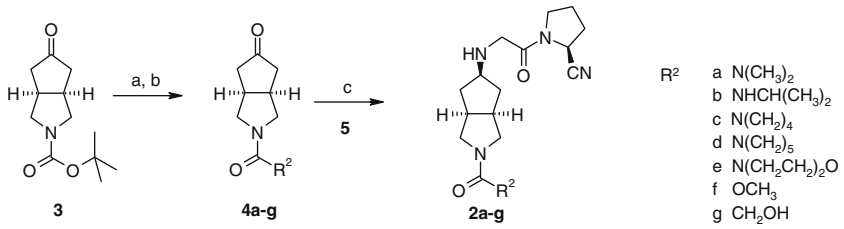
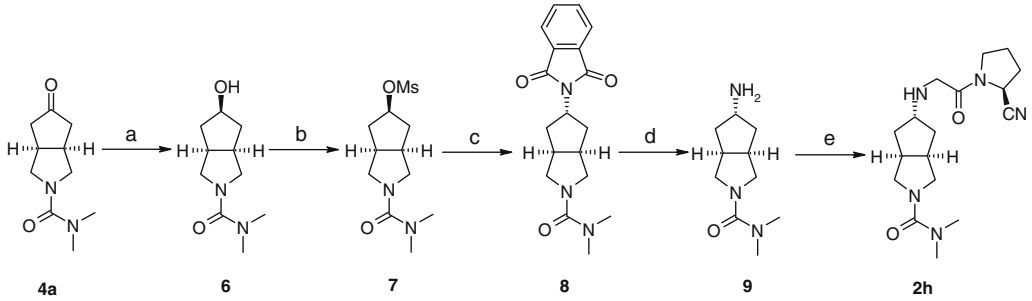


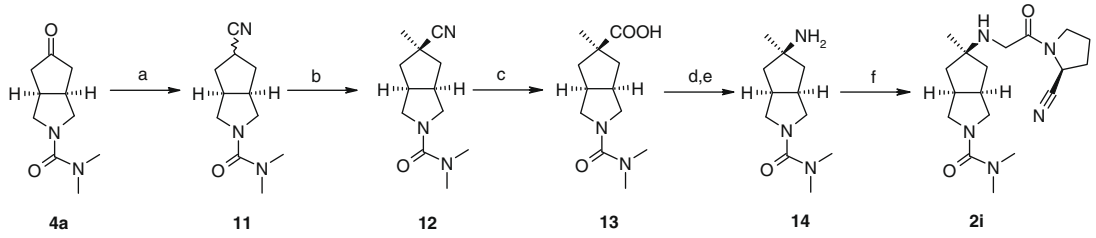
Figure 1. Structures of selected DPP-4 inhibitors and design of new azobicyclo[3.3.0]octane derivatives as DPP-4 inhibitors.



Scheme 1. Reagents and conditions: (a) HCl, Et_2O, rt; (b) R^2COCl, K_2CO_3, CH_3CN, rt, 40–77%, over two steps; (c) (*S*)-1-(2-aminoacetyl)-pyrrolidine-2-carbonitrile TFA salt (**5**), $NaBH(OAc)_3$, THF, 15–43%.

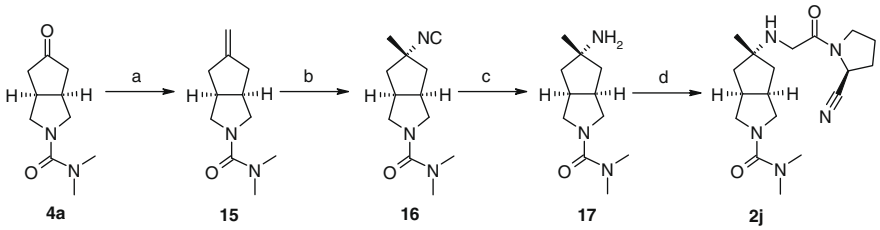


Scheme 2. Reagents and conditions: (a) $LiAl(t\text{-}BuO)_3H$, THF, −30 °C, 90%; (b) MsCl, Et_3N, CH_2Cl_2, 0 °C to rt, 75%; (c) phthalimide potassium, DMF, 60 °C, 90%; (d) N_2H_4, EtOH, reflux, 50%; (e) (*S*)-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile (**10**), K_2CO_3, KI, CH_2Cl_2, rt, 45%.



Scheme 3. Reagents and conditions: (a) tosylmethyl isocyanide, *t*-BuOK, 51%; (b) CH_3I, LHMDS, THF, rt; (c) concd HCl solution, 50 °C, 92%; (d) $ClCOOC_2H_5$, NaN_3, −5 °C; (e) toluene, reflux, then 8 N HCl solution; (f) (*S*)-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile (**10**), K_2CO_3, KI, CH_2Cl_2, rt, 55%.

(SR) of **2a** for DPP-4 versus DPP-8 was 1931-fold. Previous work from our laboratories indicated that increasing the steric bulk at 2-position of bicyclo[3.3.0]octane derivatives reduced the potency.[11] This trend was also observed in the series of derivatives **2** with compound **2b** (IC_{50} 0.12 μM), **2c** (IC_{50} 0.039 μM), **2d** (IC_{50} 0.069 μM), and **2e** (IC_{50} 0.050 μM). When compared to compound **2a**, carbamate **2f** showed a threefold decrease and amide **2g** exhibited twofold decrease in DPP-4 inhibitory potency. The inverted isomer **2h** was



Scheme 4. Reagents and conditions: (a) methyltriphenylphosphonium iodide ($CH_3IP(C_6H_5)_3$), *t*-BuOK, 80%; (b) TMSCN, $AgClO_4$, CH_2Cl_2, rt, 18%; (c) 6 N HCl solution, rt, 76%; (d) **10**, K_2CO_3, KI, CH_2Cl_2, rt, 87%.

Table 1
Potency and selectivities of azobicyclo[3.3.0]octane derivatives

Compd	R^2	R^1	IC_{50}[a,b] (μM)		
			DPP-4	DPP-8	DPP-9
2a	$N(CH_3)_2$	H(α)	0.009	17.38	5.70
2b	$NHCH(CH_3)_2$	H(α)	0.120	ND	ND
2c	$N(CH_2)_4$	H(α)	0.039	ND	ND
2d	$N(CH_2)_5$	H(α)	0.069	ND	ND
2e	$N(CH_2CH_2)_2O$	H(α)	0.050	ND	ND
2f	OCH_3	H(α)	0.024	18.24	5.04
2g	CH_2OH	H(α)	0.014	ND	ND
2h	$N(CH_3)_2$	H(β)	0.083	ND	ND
2i	$N(CH_3)_2$	CH_3(α)	0.013	4.89	16.90
2j	$N(CH_3)_2$	CH_3(β)	0.013	38.48	2.43

[a] Average values (at least two experiments).
[b] In vitro activities of LAF-237[2], DPP-4 IC_{50} = 0.009 μM; DPP-8 IC_{50} = 3.82 μM; DPP-9 IC_{50} = 0.23 μM; selectivity ratio (SR, DPP-8 IC_{50}/DPP-4 IC_{50}) = 424.
ND, not determined.

Table 2
Pharmacokinetic properties of selected DPP-4 inhibitors

Compd	CL_z/F (L/h/kg)	$t_{1/2}$ (h)	AUC_{0-t} (ng h/mL)	C_{max} (ng/mL)
2a	11.40 ± 3.40	0.94 ± 0.40	68.7 ± 20.2	39.4 ± 18.9
2i	50.87 ± 26.19	3.25 ± 1.65	266.3 ± 59.2	164.7 ± 91.3
2j	33.81 ± 20.13	0.90 ± 0.41	120.5 ± 72.0	146.4 ± 101.3

CL_z/F, apparent total plasma clearance; $t_{1/2}$, half life; AUC, area under the plasma concentration–time; C_{max}, peak plasma concentration. Oral administrations of 3.0 mg/kg to Sprague-Dawley rats (n = 6).

ninefold less potent than compound **2a** in DPP-4 inhibitory potency. Introduction of methyl group in 5-position (**2i** or **2j**) displayed slightly reduction in DPP-4 inhibitory potency.

The rat pharmacokinetic studies of selected compound**s**, as shown in Table 2, revealed that 5α-methyl derivative **2i** exhibited best pharmacokinetic profiles. Introduction of methyl group in 5-position has increased the half life in buffered solution (pH 7.2) from 25.7 h to about 50 days.[19]

In pharmacodynamic studies, oral glucose tolerance tests (OGTT) in lean mice (ICR mice) were conducted to determine the efficacy of **2a**.[11] Oral administration of **2a** with indicated doses to ICR mice 0.5 h before an oral glucose challenge produced significant decrease in glucose excursion. Compared to **LAF-237**, which reduced the $AUC_{0-120\ min}$ values 35.8%, 41.7%, and 51.4% at the dose of 1, 3, and 10 mg/kg, compound **2a** showed comparable effect with the decrease rate of 39.7%, 42%, and 45%, respectively (Fig. 2).

At the same time, the efficacy of **2a**, **2i**, and **2j** on the inhibition of serum DPP-4 activity was examined in cynomolgus monkey (*Macaca mulatta*). Oral dosing of 20 mg/kg **2j** inhibited serum DPP-4 activity by >75% within 9 h post-dose, whereas inhibition of >75% persisted only for 5 h post-dose by the treatment of **2a** and **2i** with the same dose. Moreover, at 12 h post-dose, 20 mg/

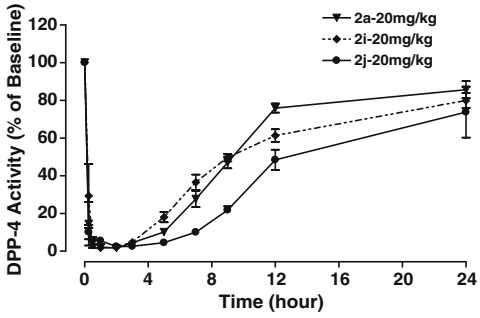


Figure 3. Effect of **2a**, **2i** or **2j** on serum DPP-4 activity in cynomolgus monkey. Data are represented as mean ± SEM (n = 4).

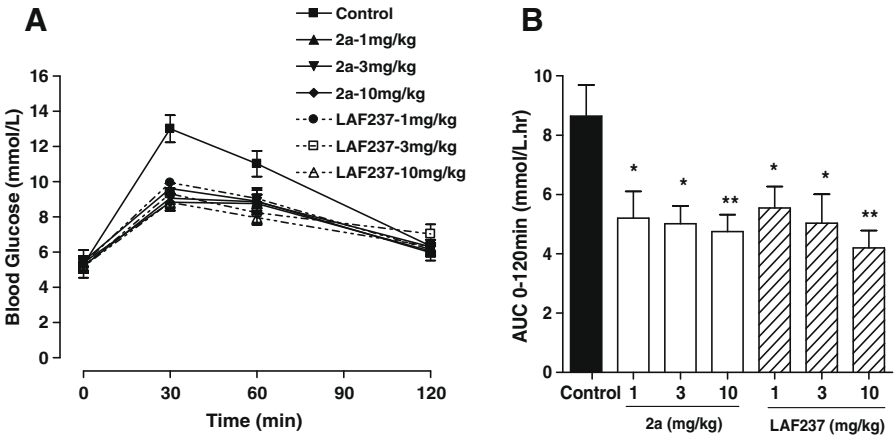


Figure 2. Glucose responses (A) and $AUC_{0-120\ min}$ change rate (B) during an oral glucose tolerance test (OGTT) in ICR mice following treatment with **2a**. Data are represented as mean ± SEM (n = 5). $^*P$ <0.05, $^{**}P$ <0.01 versus control.

kg **2j** and **2i** inhibited serum DPP-4 activity by 51% and 38%, separately, whereas the same dose of **2a** showed much lower inhibition rate with 24%, which also suggested that introduction of methyl group could prolong the inhibition of serum DPP-4 activity. (Fig. 3)

In summary, we have identified a series of novel azobicyclo[3.3.0]octane derivatives as potent DPP-4 inhibitors. Compound **2a** possesses good DPP-4 activity, high selectivity over other related enzymes, moderate pharmacokinetic profiles, and excellent in vivo efficacy in an OGTT in lean mice. Its 5α-methyl analog **2i** showed better pharmacokinetic profiles and 5β-methyl analog **2j** showed better inhibition activity of serum DPP-4. Further studies of 5-methyl azobicyclo[3.3.0]octane derivatives are in process.

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13. All new compounds were characterized by ^{1}H NMR and MS. Where noted, compounds for evaluation were determined to be >95% pure by analytical reverse-phase HPLC. Data for compound **2a** (HCl salt): ^{1}H NMR (CD_3OD, 400 MHz, ppm) δ 4.82 (m, 1H), 4.02 (m, 2H), 3.62–3.25 (m, 7H), 2.76 (s, 6H), 2.51–1.49 (m, 10H). MS (ESI) m/z: 334.5 $[M+1]^+$. Data for compound **2i**: ^{1}H NMR (DMSO-d_6, 400 MHz, ppm) δ 4.82 (m, 1H), 3.97 (s, 2H), 3.79 (m, 1H), 3.49 (m, 2H), 3.21 (m, 4H), 2.75 (s, 6H), 2.62 (m, 2H), 2.19 (m, 2H), 2.06 (m, 2H), 1.92 (m, 2H), 1.61 (m, 2H), 1.20 (s, 3H). MS (ESI) m/z: 348.2 $[M+1]^+$. Data for compound **2j**: ^{1}H NMR (DMSO-d_6, 400 MHz, ppm) δ 4.82 (m, 1H), 4.07 (s, 2H), 3.80 (m, 1H), 3.67 (m, 1H), 3.50 (m, 1H), 3.21 (m, 4H), 2.76 (s, 6H), 2.63 (m, 2H), 2.23–2.18 (m, 2H), 2.08–2.00 (m, 2H), 1.96–1.92 (m, 2H), 1.68–1.63 (m, 2H), 1.24 (s, 3H). MS (ESI) m/z: 348.2 $[M+1]^+$.
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19. Half life in aqueous buffered solution. $t_{1/2}$ for **2a**, 25.7 h; $t_{1/2}$ for **2i**, >50 d; $t_{1/2}$ for **2j**, >50 d. The stability of the inhibitors in buffered, aqueous solution (pH 7.2) was monitored by reverse-phase HPLC. The inhibitors with half-lives of less than 48 h decompose to multiple products which have not been characterized (a) Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Burkey, B. F.; Dunning, B. E.; Prasad, K.; Mangold, B. L.; Russell, M. E.; Hughes, T. E. *J. Med. Chem.* **2003**, *46*, 2774; (b) Ashworth, D. M.; Atrash, B.; Baker, G. R.; Baxter, A. J.; Jenkins, P. D.; Jones, D. M.; Szelke, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2745.