Bioorganic & Medicinal Chemistry Letters 20 (2010) 3521–3525

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Tang Peng Cho [a,*], Lin Zhi Gang [a], Yang Fang Long [a], Wang Yang [a], Wang Qian [a], Zhang Lei [a], Luo Jing Jing [a], Feng Ying [b], Yan Pang Ke [a,b], Leng Ying [b], Feng Jun [a]

[a] Shanghai Hengrui Pharmaceuticals Co., Ltd, 279 Wenjing Road, Shanghai 200245, China
[b] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, China

ARTICLE INFO

Article history:
Received 22 February 2010
Revised 30 March 2010
Accepted 28 April 2010
Available online 18 May 2010

Keywords:
Bicyclo[3.3.0]octane derivatives
Dipeptidyl peptidase 4
Inhibitors
Oral glucose tolerance tests
Type 2 diabetes

ABSTRACT

A series of novel bicyclo[3.3.0]octane derivatives have been synthesized and found to be dipeptidyl peptidase 4 (DPP-4) inhibitors. Compounds **10a** and **10b** demonstrate good efficacies in oral glucose tolerance tests.

Type 2 diabetes mellitus (T2DM) is the most common form of the diabetes disease, accounting for about 90–95% of all diagnosed North American cases of diabetes.[1] T2DM may be effectively treated by agents that induce the biosynthesis and secretion of insulin during periods of hyperglycemia. Two endogenous peptides that stimulate glucose-dependent insulin secretion are the incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP).[2] Continuous infusion of GLP-1 in patients with type 2 diabetes has resulted in the lowering of plasma glucose.[3,4] However, active GLP-1 is rapidly converted to inactive GLP-1 by the serine protease dipeptidyl peptidase 4 (DPP-4),[5] thus limiting its therapeutic practicality. A straightforward solution is to inhibit DPP-4 to increase the level of endogenous intact GLP-1. Much attention has been paid to DPP-4 inhibitors as effective medicaments for the treatment of T2DM. Various classes of structurally different DPP-4 inhibitors have been reported.[6–8] Sitagliptin (Januvia®, MK-0431),[9] Vildagliptin (Galvus®, LAF-237)[10] and Saxagliptin (Onglyza™, BMS-477118)[11,12] were approved for the treatment of T2DM (Fig. 1).

Continued SAR studies of Vildagliptin and Saxagliptin in our laboratory led to the discovery of difluorocyclobutyl pyrrolidine-2-carbonitrile compound.[13] Retaining this pyrrolidine-2-carbonitrile fragment, a series of novel bicyclo[3.3.0]octane derivatives was designed, synthesized and evaluated as DPP-4 inhibitors. According to the retrosynthetic analysis, the derivatives **1** can be prepared from carbonyl compounds **2** and amine **3** as shown in Figure 1.

As shown in Scheme 1, *cis*-bicyclo[3.3.0]octane-3,7-dione (**4**)[14] was mono-protected with ethylene glycol in the presence of catalytic amount of *p*-TsOH to afford acetal **5**. Stereoselective reduction of monoketone **5** with $NaBH_4$ gave 5β-hydroxy isomer **6** and 5α-hydroxy isomer **7** in a 90:10 ratio. A higher diastereoselectivity (**6:7** = 99:1) could be achieved when bulky $LiAlH(O\text{-}t\text{-}Bu)_3$ was employed.[15] The key intermediate **6** was alkylated to ethers **8b–c** and reacted with various carbamic acid chlorides or acid anhydrides to give derivatives **8d–i**. Deprotection and followed by reductive amination of these compounds afforded the desired bicyclo[3.3.0]octane derivatives **10a–i** as their hydrochloride salts.[16]

Stereoisomers of compound **10b** were prepared in order to probe the SAR as related to stereochemistry. The 5β-secondary alcohol **6** was efficiently converted to 5α-hydroxy isomer **7** through a Mitsunobu reaction. The synthesis of desired compound **13** was accomplished in three steps from **7** following the same synthetic route for **10b** (Scheme 2). To synthesize stereoisomer **18**, the monoketone **12** was reduced to 2β-hydroxy isomer **14**, which was mesylated and inverted to 2α-azide **15** with NaN_3. Subsequent hydrogenation of **15** followed by alkylation with (*S*)-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile (**17**)[17] under KI catalyzed conditions afforded the isomer **18** (Scheme 3). Under the similar conditions, compound **21** was prepared from compound **9a** (Scheme 4).

* Corresponding author. Tel.: +86 21 54752877; fax: +86 21 54759072.
E-mail address: tangpc@shhrp.com (T.P. Cho).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.04.138

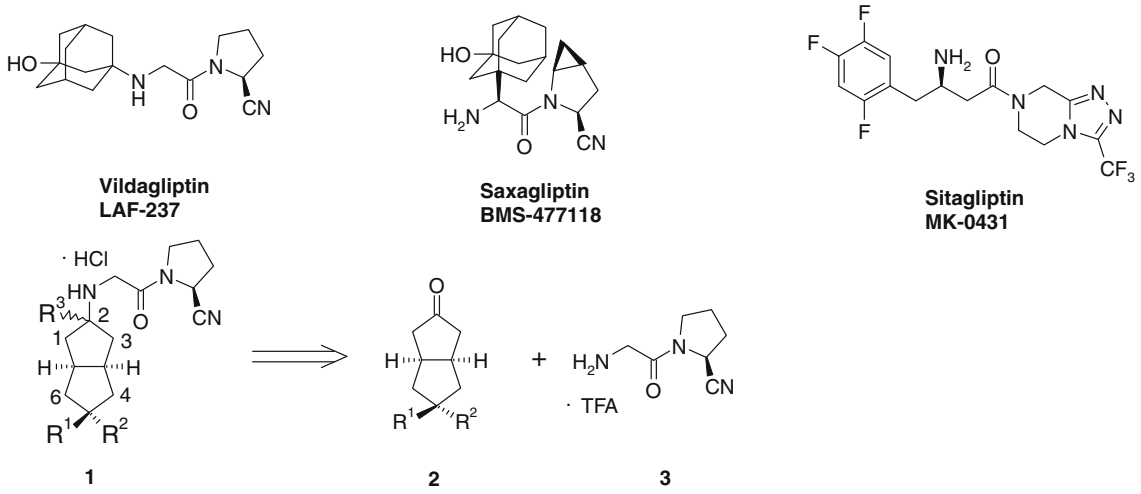
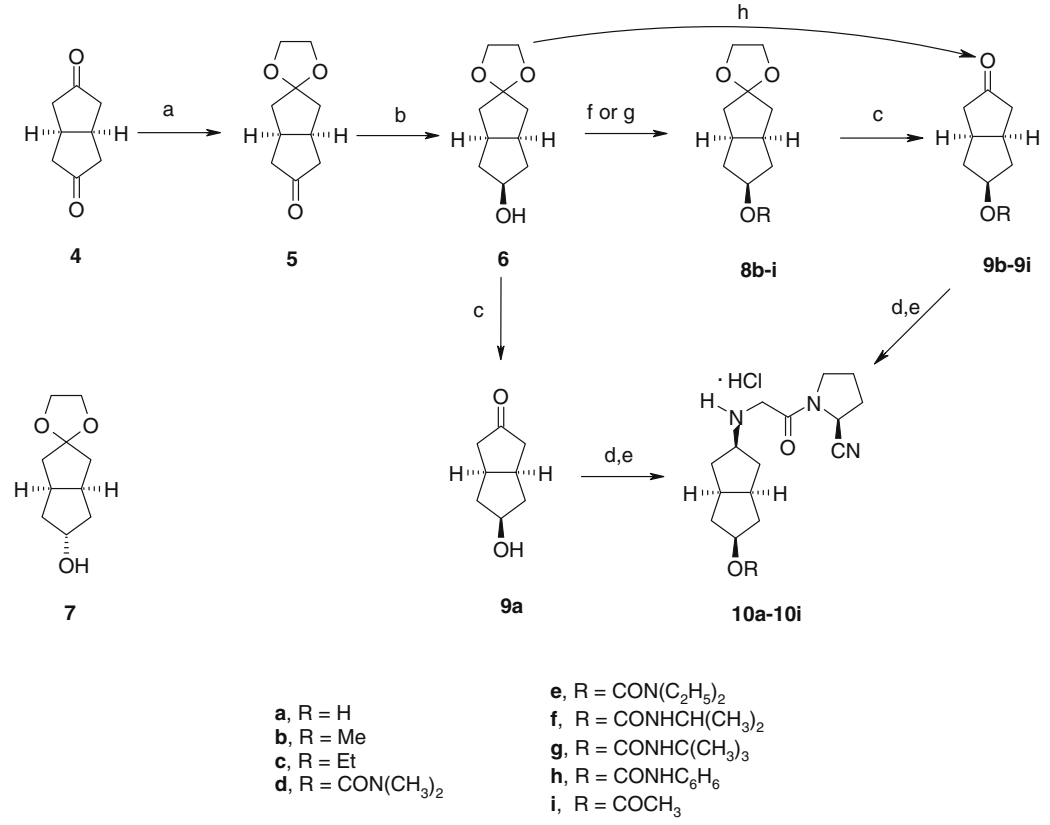
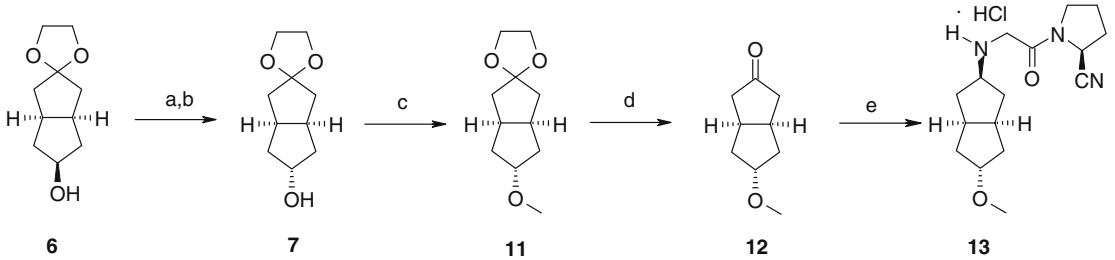


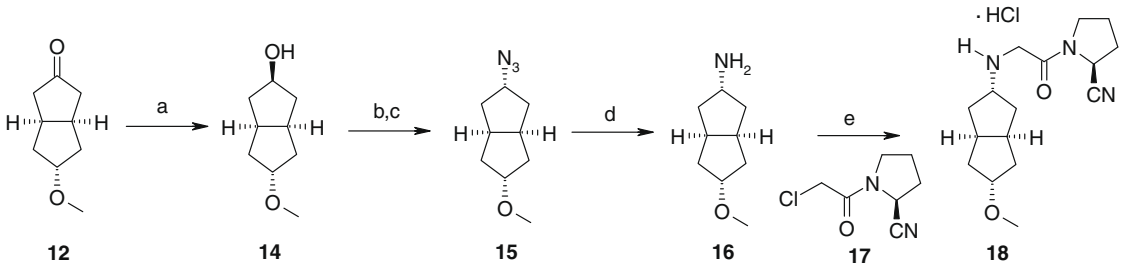
Figure 1. Structures of selected DPP-4 inhibitors and bicyclo[3.3.0]octane derivatives.



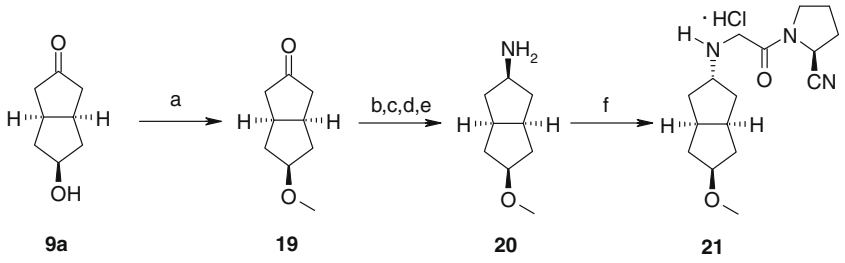
Scheme 1. Reagents and conditions: (a) $HOCH_2CH_2OH$, *p*-TsOH (Cat.), benzene, reflux, 56%; (b) $LiAlH(O\text{-}t\text{-}Bu)_3$, rt, 92%; (c) oxalic acid, ethyl acetate/water, 75%; (d) (*S*)-1-(2-aminoacetyl)pyrrolidine-2-carbonitrile TFA salt (**3**), $NaBH(OAc)_3$, Et_3N, rt 30–50%; (e) 0.5 N hydrochloric acid ether solution, 40–80%; (f) 50% NaH, RI, THF, rt, 88–95%; (g) carbamic acid chloride or acetic anhydride, DMAP, reflux, 67–88%; (h) triphosgene, RNH_2, toluene, reflux, 56%.



Scheme 2. Reagents and conditions: (a) benzoic acid, DEAD, Ph_3P, Et_2O, rt, 50%; (b) KOH, CH_3OH, 93%; (c) 50% NaH, CH_3I, THF, rt; (d) oxalic acid, ethyl acetate/water; (e) (*S*)-1-(2-aminoacetyl)pyrrolidine-2-carbonitrile TFA salt (**3**), $NaBH(OAc)_3$, Et_3N, then 0.5 N HCl/Et_2O, rt 33%.

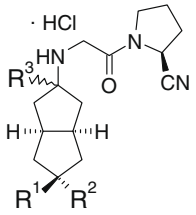


Scheme 3. Reagents and conditions: (a) $NaBH_4$, rt; (b) MsCl, Et_3N, CH_2Cl_2; (c) NaN_3, DMF, 65 °C,57%; (d) H_2, 10%Pd/C, CH_3OH; (e) (*S*)-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile (**17**), K_2CO_3, KI, CH_2Cl_2, then HCl/Et_2O, 31%.



Scheme 4. Reagents and conditions: (a) 50% NaH, CH_3I, THF, rt; (b) $NaBH_4$, rt; (c) MsCl, Et_3N, CH_2Cl_2; (d) NaN_3, DMF, 65 °C; (e) H_2, 10%Pd/C, CH_3OH, 92%; (f) (*S*)-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile (**17**), K_2CO_3, KI, CH_2Cl_2, then HCl/Et_2O, 28%.

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



Compd	R^1	R^2	R^3	IC_{50}[a,b] (μM)			SR[c]
				DPP-4	DPP-8	DPP-9	(DPP-8 IC_{50}/DPP-4 IC_{50})
10a	OH	H	H(α)	0.012	7.93	3.22	661
10b	OMe	H	H(α)	0.008	3.57	0.43	446
10c	OEt	H	H(α)	0.059	3.74	1.40	64
10d	$OCON(CH_3)_2$	H	H(α)	0.065	8.42	6.86	130
10e	$OCON(C_2H_5)_2$	H	H(α)	0.080	12.74	7.75	159
10f	$OCONHCH(CH_3)_2$	H	H(α)	0.204	ND	ND	—
10g	$OCONHC(CH_3)_3$	H	H(α)	0.326	ND	ND	—
10h	$OCONHC_6H_6$	H	H(α)	0.121	ND	ND	—
10i	$OCOCH_3$	H	H(α)	0.032	1.43	1.38	45
13	H	OMe	H(β)	0.027	ND	ND	—
18	H	OMe	H(β)	0.027	0.235	0.037	11
21	OMe	H	H(α)	0.047	0.023	0.037	0.5

ND: not determined.
[a] Average values (at least two experiments).
[b] In vitro activities of LAF-237[7]: DPP-4 IC_{50} = 0.009 μM, DPP-8 IC_{50} = 3.82 μM, DPP-9 IC_{50} = 0.23 μM, SR = 424.
[c] Selectivity ratio(SR) = DPP-8 IC_{50}/DPP-4 IC_{50}.

As shown in Table 1, these compounds were evaluated in vitro for their inhibitions of DPP-4, DPP-8 and DPP-9.[18] 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.[19] Compound **10a** (5β-OH) was found to be a potent and selective inhibitor (IC_{50} 0.012 μM, selectivity ratio: SR 661). Introduction of methyl group (**10b,** 5β-OCH_3) displayed slightly improvement in DPP-4 inhibitory potency (IC_{50} 0.008 μM) but not in selectivity (SR 446). Ethyl analog **10c** neither improved DPP-4 inhibitory potency nor enhanced selectivity against DPP-8 and DPP-9. Substitution with carbamates or carbonates (**10d–i**) gave less active compounds. Increasing the steric bulk at 5β position reduced the potency. Of all of stereoisomers of **10b**, compound **10b** was the most potent and selective DPP-4 inhibitor.

Therefore, compounds **10a** and **10b** were chosen for in vivo evaluation by assessing for their ability to improve glucose tolerance in lean mice (ICR mice) and type 2 diabetic mice (KKAy mice).[20] Administration of **10a** or **10b** with different doses (0.3,

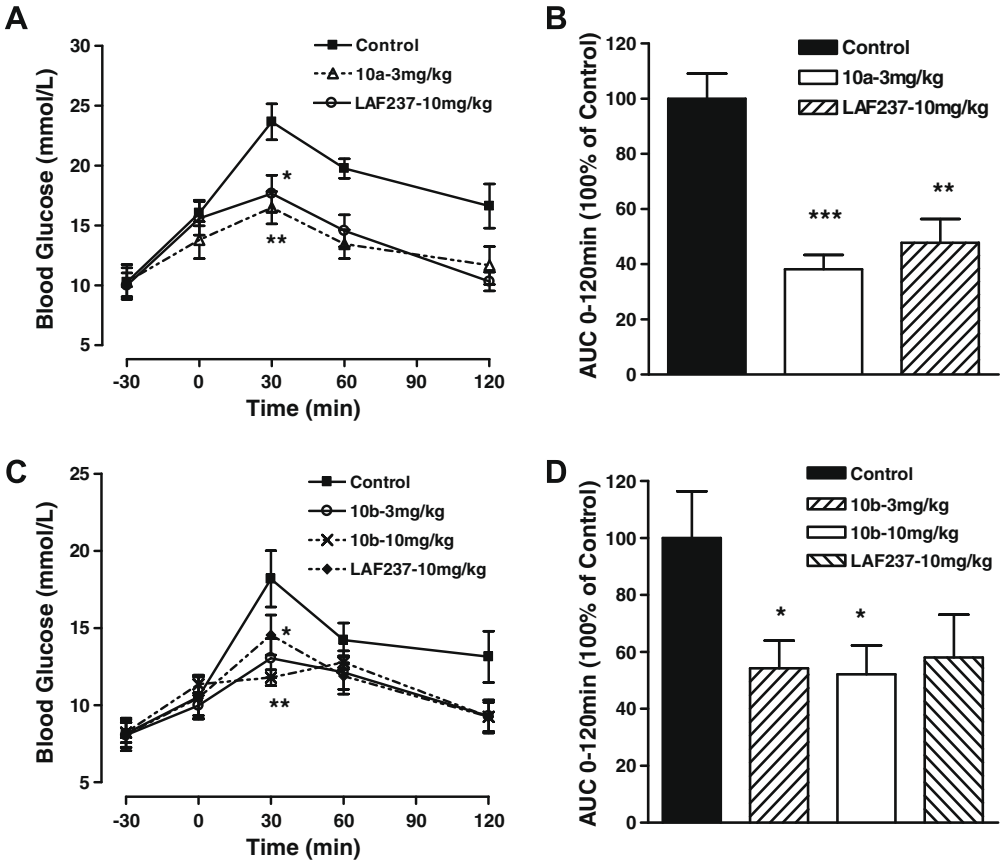


Figure 2. Glucose responses (A, C) and $AUC_{0-120min}$ change rate (B, D) during an oral glucose tolerance test (OGTT) in KKAy mice following treatment with **10a** or **10b**. Data are represented as Mean ± SEM ($n = 5$). * P <0.05, ** P <0.01, *** P <0.001 versus control.

1, 3 and 10 mg/kg) to ICR mice 0.5 h before an oral glucose challenge produced a significant decrease in glucose excursion, which suggested the improvement of glucose tolerance (data not shown). Moreover, the same results were observed on KKAy mice. After glucose load, the blood glucose level of KKAy mice increased significantly. Administration of 3 mg/kg **10a** 0.5 h before glucose challenge reduced the $AUC_{0-120min}$ value of blood glucose by 61.9%, while **LAF237** need 10 mg/kg to show comparable effect with the decrease rate of 52.2%. Compound **10b** also induced a significant decrease in $AUC_{0-120min}$ values by 45.7% and 47.1% at the dose of 3 and 10 mg/kg (Fig. 2).

In conclusion, a series of novel bicyclo[3.3.0]octane derivatives was synthesized and found to have inhibitory activities against DPP-4 and selectivities over DPP-8 and DPP-9. Compounds **10a** and **10b** showed good in vitro activities and moderate selectivities and demonstrated beneficial effects on oral glucose tolerance. Further studies are underway to optimize this class of compounds for the treatment of T2DM.

Acknowledgements

The authors would like to thank Mr. Sun Piao Yang for his vision and support in new drug discovery in China, and Mr. Wu Qian and Li Chun Fa of Analytical Chemistry Group of Shanghai Hengrui Pharmaceuticals Co., Ltd for ^{1}H NMR and Mass Spectroscopy services.

References and notes

1. Zimmet, P.; Alberti, K. G. M. M.; Shaw, J. *Nature* **2001**, *414*, 782.
2. Gautier, J. F.; Fetita, S.; Sobngwi, E.; Salaün-Martin, C. *Diabetes Metab.* **2005**, *31*, 233.
3. Nauck, M. A.; Wollschläger, D.; Werner, J.; Holst, J. J.; Ørskov, C.; Creutzfeldt, W.; Willms, B. *Diabetologia* **1996**, *39*, 1546.
4. Nauck, M. A. *Acta Diabetol.* **1998**(3), :117.
5. Sebokova, E.; Christ, A. D.; Boehringer, M.; Mizrahi, J. *Curr. Top. Med. Chem.* **2007**, *7*, 547.
6. Rasmussen, H. B.; Branner, S.; Wiberg, F. C.; Wagtmann, N. *Nat. Struct. Biol.* **2003**, *10*, 19.
7. Longenecker, K. L.; Stewart, K. D.; Madar, D. J.; Jakob, C. G.; Fry, E. H.; Wilk, S.; Lin, C. W.; Ballaron, S. J.; Stashko, M. A.; Lubben, T. H.; Yong, H.; Pireh, D.; Pei, Z.; Basha, F.; Wiedeman, P. E.; von Geldern, T. W.; Trevillyan, J. M.; Stoll, V. S. *Biochemistry* **2006**, *45*, 7474.
8. Qiao, L.; Baumann, C. A.; Crysler, C. S.; Ninan, N. S.; Abad, M. C.; Spurlino, J. C.; DesJarlais, R. L.; Kervinen, J.; Neeper, M. P.; Bayoumy, S. S.; Williams, R.; Deckman, I. C.; Dasgupta, M.; Reed, R. L.; Huebert, N. D.; Tomczuk, B. E.; Moriarty, K. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 123.
9. Kim, D.; Wang, L.; Beconi, M.; Eiermann, G. J.; Fisher, M. H.; He, H.; Hickey, G. J.; Kowalchick, J. E.; Leiting, B.; Lyons, K.; Marsilio, F.; McCann, M. E.; Patel, R. A.; Petrov, A.; Scapin, G.; Patel, S. B.; Roy, R. S.; Wu, J. K.; Wyvratt, M. J.; Zhang, B. B.; Zhu, L.; Thornberry, N. A.; Weber, A. E. *J. Med. Chem.* **2005**, *48*, 141.
10. 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.
11. Augeri, D. J.; Robl, J. A.; Betebenner, D. A.; Magnin, D. R.; Khanna, A.; Robertson, J. G.; Wang, A.; Simpkins, L. M.; Taunk, P.; Huang, Q.; Han, S.-P.; Abboa-Offei, B.; Cap, M.; Xin, L.; Tao, L.; Tozzo, E.; Welzel, G. E.; Egan, D. M.; Marcinkeviciene, J.; Chang, S. Y.; Biller, S. A.; Kirby, M. S.; Parker, R. A.; Hamann, L. G. *J. Med. Chem.* **2005**, *48*, 5025.
12. Peters, J.-U. *Curr. Top. Med. Chem.* **2007**, *7*, 579.
13. Tang, P. C.; Lin, Z. G.; Wang, Y.; Yang, F. L.; Wang, Q.; Fu, J. H.; Zhang, L.; Gong, A. S.; Luo, J. J.; Dai, J.; She, G. H.; Si, D. D.; Feng, J. *Chin. Chem. Lett.* **2009**, *21*, 253.
14. Bertz, S. H.; Cook, J. M.; Gawish, A.; Weiss, U. *Org. Synth.* **1990**, *Coll. Vol. 7*, 50.
15. The stereoisomeric ratio was determined by gas chromatography (GC) analysis.
16. All new compounds were characterized by ^{1}H NMR and MS prior to biological evaluation. Preparation of compound **10a** is described herein. A stirred solution of (*S*)-1-(2-aminoacetyl)-pyrrolidine-2-carbonitrile trifluoroacetic acid salt (**3**) (3.6 mmol) in 50 mL methanol was added with triethylamine (10.7 mmol), 5β-hydroxy-hexahydro-pentalen-2-one (**9a**) (0.5 g, 3.6 mmol) and sodium triacetoxy borohydride (14.3 mmol). The reaction mixture was stirred overnight at room temperature until the start material disappeared as monitored by TLC. The mixture was poured into saturated sodium carbonate

solution (20 mL) and extracted with ethyl acetate (3 × 80 mL). The combined organic extracts were washed with saturated sodium chloride aqueous solution, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure, the residue is purified by column chromatography to give (*S*)-1-[2-(5β-hydroxyl-octahydro -pentalen-2-ylamino)acetyl] pyrrolidine-2-carbonitrile (**10a**) (200 mg, yield 20.4%) as a white powder. Compound **10a** (192 mg, 0.686 mmol) was suspended in 10 mL ether. The mixture was added with a solution of 0.5 N hydrochloric acid in ether in an ice-water bath. The resulting solid was concentrated to give compound **10a** hydrochloride salt (80 mg, white powder, yield 40%). Compound **10a**: ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm) 4.49(m, 1H), 3.98(m, 1H), 3.36(m, 2H), 3.20(m, 1H), 3.07(s, 1H), 2.99(m, 1H), 2.77(m, 2H), 1.98(m, 5H), 1.79(m, 2H), 1.31(m, 3H), 1.05(m, 2H). MS *m/z* (ESI): 278.2 $[M+1]^+$. Compound **10b**: ^{1}H NMR (400 MHZ, $CDCl_3$) δ (ppm) 4.79(m, 1H), 3.82(m, 2H), 3.67(m, 2H), 3.48(m, 1H), 3.26(s, 3H), 2.40(m, 2H), 2.36–1.98(m, 6H), 1.85(m, 3H), 1.62(m, 2H), 1.53–1.14(m, 2H). MS *m/z* (ESI): 292.7 $[M+1]^+$.

17. Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Dunning, B. E.; Mangold, B. L.; Mone, M. D.; Russell, M. E.; Weldon, S. C.; Hughes, T. E. *J. Med. Chem.* **2002**, *45*, 2362.
18. IC_{50} values for DPP-4, DPP-8 and DPP-9 were obtained by chemical Luminescent assay using Glo™ Protease Assay Kit (cat. G 8350). For assay conditions, see: Deng, B. C. (Tang, P. C.); Yang, F. L.; Fan, J.; Feng, H.; Wang, Y.; Yang, T. CN101468988A.
19. Lankas, G. R.; Leiting, B.; Roy, S. R.; Eiermann, G. J.; Beconi, M. G.; Biftu, T.; Chan, C.-C.; Edmondson, S.; Feeney, W. P.; He, H.; Ippolito, D. E.; Kim, D.; Lyons, K. A.; Ok, H. O.; Patel, R. A.; Petrov, A. N.; Pryor, K. A.; Qian, X.; Reigle, L.; Woods, A.; Wu, J. K.; Zaller, D.; Zhang, X.; Zhu, L.; Weber, A. E.; Thornberry, N. A. *Diabetes* **2005**, *54*, 2988.
20. Oral Glucose tolerance test on ICR and KKAy mice: KKAy and ICR mice were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences (Shanghai, China). The mice were provided with a normal diet and water ad libitum. The KKAy mice were housed individually. All the animals were kept under conventional conditions of controlled temperature, humidity, and lighting. All procedures were approved by the Animal Care and Use committee, Shanghai Institute of Materia Medica, Chinese Academy of Sciences. The effects of **10a** and **10b** on blood glucose after an oral glucose challenge were observed on ICR and KKAy mice. Indicated dose of **10a**, **10b**, LAF237 or vehicle (distilled water) was orally administered to 6 h fasting ICR or KKAy mice 0.5 h prior to oral glucose load (2.5 g/kg). Blood glucose values were measured with ONE TOUCH BASIC *Plus* blood glucose meter (LIFESCAN Inc., USA) at 0, 30, 60, 120 min after the glucose load. The area under the concentration–time curve from 0 to 120 min ($AUC_{0-120min}$) of blood glucose after challenge was calculated by the trapezoidal rule. All date were expressed as the mean ± SEM. The statistical analysis between two groups was performed using an unpaired Student's *t* test. $P < 0.05$ was considered to be statistically significance.