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Discovery of new chemotype dipeptidyl peptidase IV inhibitors having (*R*)-3-amino-3-methyl piperidine as a pharmacophore

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ABSTRACT

Structures containing the (*R*)-3-amino-3-methyl piperidine unit as a new pharmacophore moiety have been shown to possess moderate inhibitory activity for DPP-4 with good pharmacokinetics profile. One of these compounds was found to have good oral bioavailability and PK/PD profile in ZF-rat.

Patients suffering from type 2 diabetes are on the increase worldwide. Although a number of therapies are available for this condition, recent efforts have been focusing on dipeptidyl peptidase IV (DPP-4) inhibitors as a new class of therapeutic agents for type 2 diabetes.[1] Indeed, a number of clinical studies have already confirmed the efficacy and good tolerance of DPP-4 inhibitors. DPP-4, a serine protease distributed throughout the body, cleaves a wide range of peptides to modulate their biological activity. One of these peptides is glucagon-like peptide-1 (GLP-1), which plays an important role in the regulation of blood glucose level.[2] GLP-1 is released after food ingestion and through its receptor stimulates insulin biosynthesis and secretion. GLP-1 also inhibits glucagon release, delays gastric emptying, and induces pancreatic β-cell proliferation.[3]

A number of DPP-4 inhibitors, such as **1** (sitagliptin)[4], **2** (vildagliptin),[5] and **3** (alogliptin)[6], have already been approved, while others, for example, **4** (linagliptin),[7] are still under development (Fig. 1). In our search for potent DPP-4 inhibitors, we have identified a series of compounds represented here by **6**, which have a pyrolo[3,2-*d*]pyrimidine structure.[8] The scaffold of these compounds was derived from our HTS hit compound **5**, which possesses a xanthine scaffold like that of **4**.[7] Based on **5**, other series of compounds were also identified and their SARs will be disclosed elsewhere in the near future.

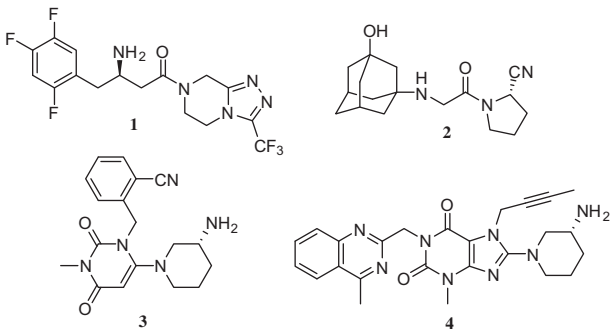

Figure 1. Structures of small molecule DPP-4 inhibitors.

Reports describing compounds **3**[6] and **4**[7] indicate that the (*R*)-3-amino piperidine group is essential for activity as this group interacts with DPP-4 (the enzyme). This finding is based on X-ray crystal analysis of the identified DPP-4 inhibitors complexed with hDPP-4. In our study, compound **6**, which has the same piperidine unit as **3** and **4**, was found to have an excellent DPP-4 inhibitory activity, but showed modest bioavailability (B.A.) (37%). Based on the structure of **6**, we assumed that the primary amine moiety, which has high hydrophilicity, is one of the main reasons for **6** modest B.A. From this point of view, we considered introducing substituents at the piperidine part of **6** to increase lipophilicity, and consequently identified (*R*)-3-amino-3-methyl piperidine unit

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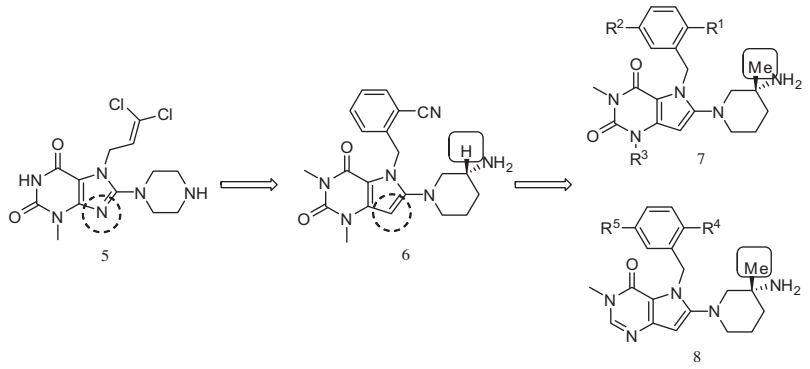
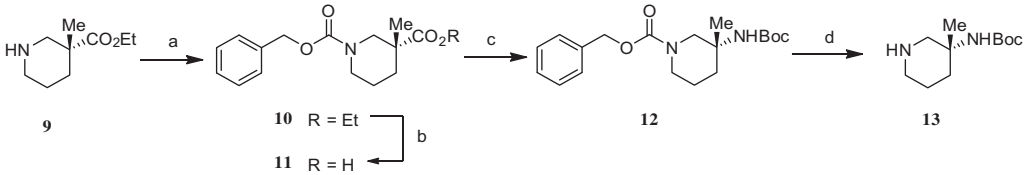


Figure 2. Synthesis of new chemotype DPP-4 inhibitors from HTS hit **5**.

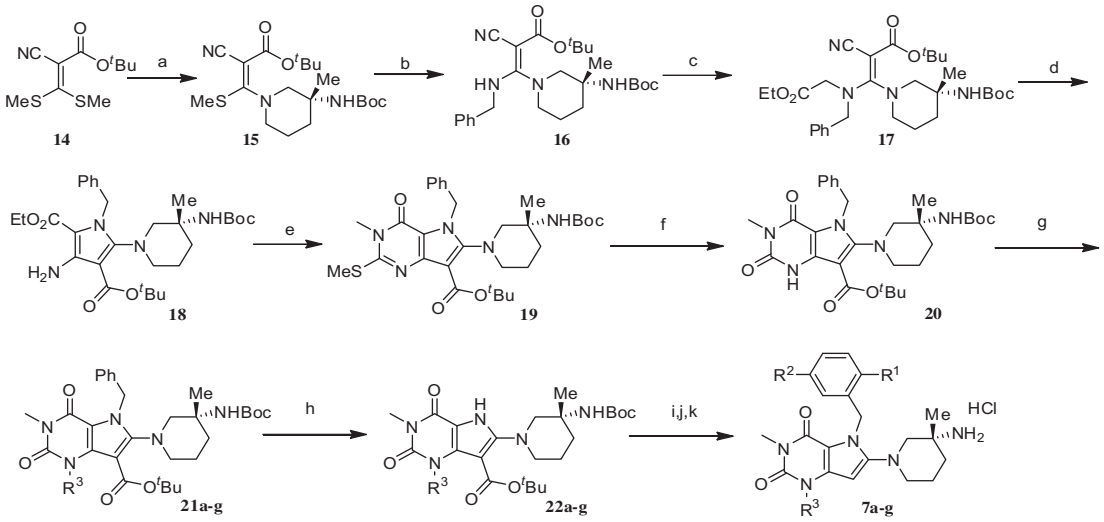


Scheme 1. Reagents and conditions: (a) CbzCl (1.1 equiv), Et_3N (1.2 equiv), THF, rt, 10 h; (b) NaOHaq (2.0 equiv), THF, MeOH, rt, overnight; (c) DPPA (1.02 equiv), Et_3N (1.2 equiv), toluene, 100 °C, 2 h then *t*-BuOK (0.1 equiv), *t*-BuOH (10 equiv), 80 °C, 2 h, 57% from **9**; (d) Pd/C (10 wt % vs **12**), H_2, MeOH, rt, 8 h, 95%.

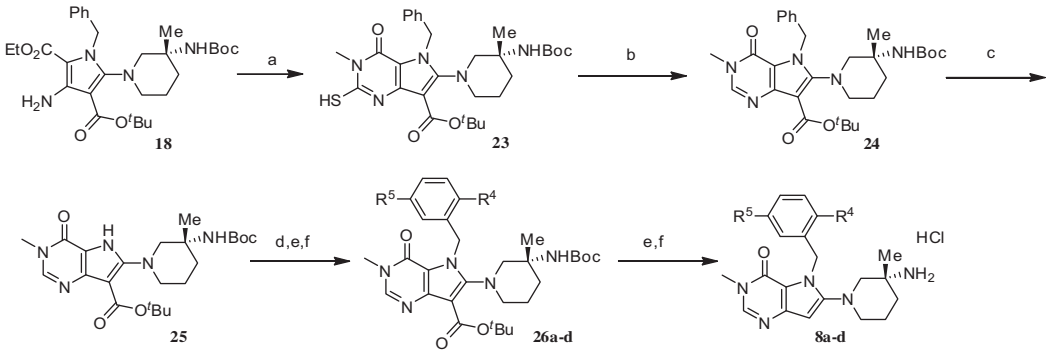
as acceptable for good B.A. and appropriate DPP-4 inhibitory activity. In this Letter we describe the structure–activity relationship, pharmacokinetics, and pharmacological evaluation of the newly synthesized structures, which are represented here as **7** and **8** (Fig. 2).

As shown in Scheme 1, the (*R*)-3-amino-3-methyl piperidine **13** was synthesized in five steps from the known compound **9**.[9] Following protection of the amine by a carbobenzyloxy (Cbz), hydrolysis of **10** gave the carboxylic acid **11**. After Curtius rearrangement using diphenylphosphoric azid (DPPA), the solution containing the intermediate isocyanate was first washed with water to remove by-products, and then treated with excess amount of *tert*-butyl alcohol and catalytic amount of potassium *tert*-butoxide to generate the *tert*-butoxy carbonyl (Boc) protected amine **12**. The target amine unit **13** was obtained by hydrogenation of **12**.

Compounds **7a–g** having a pyrolo[3,2-*d*]pyrimidine were synthesized as shown in Scheme 2.[8] Reaction of **13** with **14** without use of any base gave **15**, but the next reaction from **15** to **16** required DBU to proceed. Reaction of **16** with ethyl bromoacetate afforded **17**, which underwent cyclization in the presence of lithium *tert*-butoxide generated in situ from lithium amide and *tert*-butyl alcohol to produce the common intermediate **18**. The next intermediate **19** was obtained by methylation following reaction of methyl isothiocyanate (MeNCS) with **18**. The next reaction,



Scheme 2. Reagents and conditions: (a) **13** (1.0 equiv), toluene, 60 °C, 4 h, quant.; (b) benzylamine (1.2 equiv), DBU (2.0 equiv), CH_3CN, 80 °C, 5 h, 72%; (c) ethyl bromoacetate (1.1 equiv), K_2CO_3 (1.2 equiv), DMF, 50 °C, 1 h, quant.; (d) $LiNH_2$ (2.0 equiv), *t*-BuOH (20 equiv), CH_3CN, heptane, rt, 1 h, 72%; (e) MeNCS (2.0 equiv), K_2CO_3 (2.0 equiv), pyridine, 120 °C, 6 h then MeI (1.2 equiv), K_2CO_3 (2.0 equiv), acetone; (f) $Na_2WO_4{\cdot}5H_2O$ (1.1 equiv), H_2O_2 aq (10 equiv), MeOH–AcOH–H_2O (9/3/1), 50 °C, 5 h, 50% from **18**; (g) MeI (1.2 equiv) for **21a–c**, EtI (1.2 equiv) for **21d**, $ClCH_2CONMe_2$ (1.2 equiv) for **21e**, $Br(CH_2)_3OTHP$ (1.2 equiv) for **21f**, $Br(CH_2)_3OEt$ (1.2 equiv) for **21g**, K_2CO_3 (1.5 equiv), DMF, 50 °C, 3 h; (h) Pd/C (200 wt %), HCO_2NH_4 (300 equiv), MeOH, reflux, 4 h, **22a–c** 62%, **22d** 60%, **22e** 72%, **22f** 58%; (i) appropriate benzyl bromide (1.1 equiv), K_2CO_3 (2.0 equiv), DMF, 70 °C, 3 h; (j) $PhSO_3H$ monohydrate (2.0 equiv), 70 °C, 3 h; (k) HCl–Et_2O (1.1 equiv), $CHCl_3$, **7a** 21%, **7b** 18%, **7c** 23%, **7d** 30%, **7e** 12%, **7f** 21%, **7g** 25% from **22a–g**.



Scheme 3. Reagents and conditions: (a) MeNCS (2.0 equiv), K_2CO_3 (2.0 equiv), pyridine, 120 °C, 6 h; (b) $Na_2WO_4{\cdot}5H_2O$ (1.1 equiv), H_2O_2aq (10 equiv), MeOH–AcOH–H_2O (9/3/1), 50 °C, 5 h, 72% from **18**; (c) Pd/C (200 wt %), HCO_2NH_4 (300 equiv), MeOH, reflux, 4 h, 82%; (d) appropriate benzyl bromide (1.1 equiv), K_2CO_3 (2.0 equiv), DMF, 70 °C, 3 h; (e) $PhSO_3H$ monohydrate (2.0 equiv), 70 °C, 3 h; (f) HCl–Et_2O (1.1 equiv), $CHCl_3$, **8a** 14%, **8b** 20%, **8c** 21%, **8d** 15% from **26a–d**.

which included oxidation and hydrolysis of **19** was carried out under the same conditions and constructed the pyrolo[3,2-*d*]pyrimidine scaffold **20**. Alkylation of **20** with appropriate alkyl halides gave **21a–g**. As removal of benzyl protection in **21** did not proceed under usual hydrogenation conditions, that is, hydrogen atmosphere with a catalytic amount of palladium/carbon (Pd/C), excess amount of Pd/C, and ammonium formate were used under reflux. After benzylation of **22a–g**, Boc protection and substitution of the *tert*-butyl ester at the 7-position of the pyrolo[3,2-*d*]pyrimidine structure were cleaved by benzensulphonic acid monohydrate, and the purified free amines were converted to **7a–g** by 1 N HCl–ether solution.

As for compounds **8a–d** having a deazahypoxanthine, they were synthesized by almost the same route as that for **7a–g** (Scheme 3). The only difference was at the first step from the common intermediate **18–23**, which did not include methylation like in the case of **20**. The rest was the same as shown in Scheme 2.

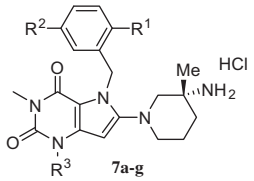
In vitro activity of the synthesized compounds listed in Table 1 was examined using human plasma DPP-4. As expected, introducing steric hindrance at the 3-position of the piperidine moiety decreased the activity (**6**: IC_{50} = 1.8 nM vs **7a**: IC_{50} = 36 nM) and increased the value of log P^* (0.1 for **6** and 0.28 for **7a**). However, compound **7a** retained acceptable DPP-4 inhibitory activity.

Variation of substituents at the benzyl position is reported in Table 1, that is, **7a–c**. Changes at the R^1 and R^2 positions did not come to improve the inhibitory activity for DPP-4. As for R^3, the activity was kept even in the case of a polar substituent (**7e**: IC_{50} = 13 nM, **7f**: IC_{50} = 23 nM), but there seems to be limitation to the extend of the linker (**7g**: IC_{50} = 122 nM). While the series of compounds **7a–f** exhibited good DPP-4 inhibitory activity, almost all compounds shown in Table 2 gave less satisfactory results (IC_{50} >50 nM), except for **8c** (IC_{50} = 23 nM).

Selectivity against DPP-8[10] and DPP-9[11], both of which belong to the same gene family as DPP-4, was also assessed. Although the biological roles of DPP-8 and DPP-9 are still unclear, it is important to avoid potential side effects associated with these off-target enzymes. All compounds disclosed in this Letter showed good selectivity (IC_{50} >10 μM) against DPP-8/9.

Among the compounds listed in Tables 1 and 2, **7b–f** and **8c**, which showed IC_{50} values <30 nM in in vitro activity assessment, were selected for in vitro pharmacokinetics evaluation. In terms

Table 1
In vitro activity of the synthesized DPP-4 inhibitors

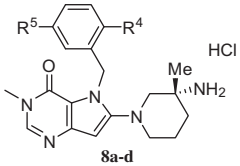


7a-g

Compounds	R_1	R_2	R_3	DPP-4[a] IC_{50} (nM)
6	—	—	—	1.8
7a	CN	H	Me	36
7b	Cl	F	Me	28
7c	Me	F	Me	27
7d	CN	F	Et	12
7e	Cl	F	CH_2CONMe_2	13
7f	Cl	F	$(CH_2)_3OH$	23
7g	Cl	F	$(CH_2)_3OEt$	122

[a] DPP-4 inhibitory activity is given as the mean of at least three experiments.

Table 2
In vitro activity of the synthesized DPP-4 inhibitors



8a-d

Compounds	R_4	R_5	DPP-4[a] IC_{50} (nM)
8a	Cl	F	103
8b	CN	F	87
8c	Me	F	23
8d	CN	H	151

[a] DPP-4 inhibitory activity is given as the mean of at least three experiments.

Table 3
In vitro pharmacokinetic parameter of **7b–f** and **8c**

	CYP inhibition[a] IC_{50} (μM)			MS[b] (ml/min/mg protein)	
	1A2	2D6	3A4	Human	Rat
7b	>40	3.4	10.2	0.082	0.046
7c	>40	>40	20.2	0.056	0.013
7d	26.8	0.4	9.4	0.021	<0.01
7e	26.3	<0.165	13.4	0.019	<0.01
7f	>40	0.2	7.7	0.016	0.017
8c	>40	14.3	11.9	0.048	0.264

[a] CYP inhibition was evaluated after incubation for 10 min with human liver microsomes and NADPH. Initial concentration of each compound was 10 μM.
[b] MS means metabolic stability, which was evaluated after incubation for 30 min with hepatic microsomes and NADPH. Initial concentration of each compound was 10 μM.

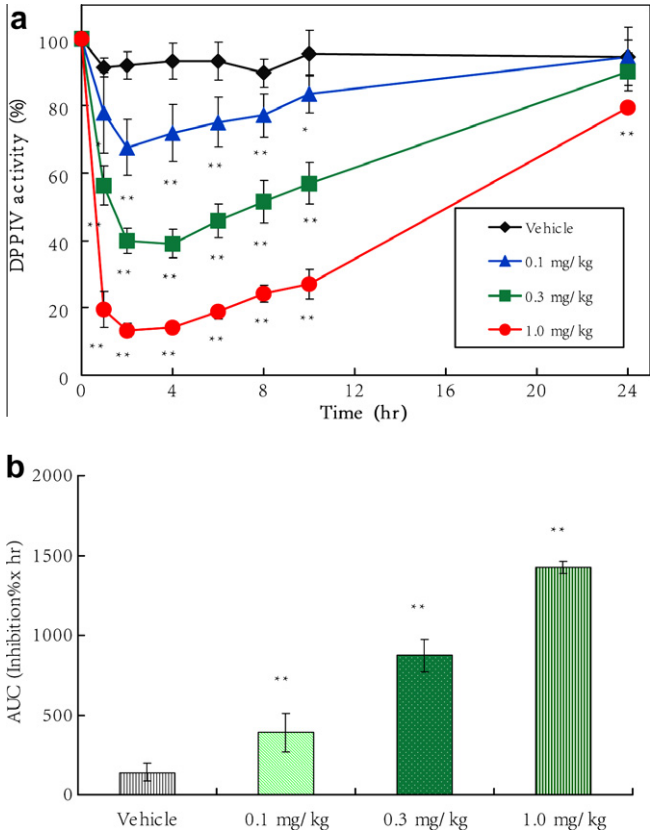


Figure 3. (a) Time-dependent activity of **7c** in plasma after oral single administration in ZF rats; $^{*}p$ <0.05, $^{**}p$ <0.01 versus the vehicle-treated group (Dunnett's multiple comparison test). (b) Dose-dependent increase in AUC of plasma DPP-4 inhibition (inhibition% × h) in ZF rats (test of linearity (lack of fit)); $^{**}p$ <0.01, compound **7c**-treated Zucker-fatty rats versus vehicle-treated Zucker-fatty rats; Dunnett multiple comparison with two-sided significance level of 5%. Data are given as the mean ± SEM (n = 5).

Table 4
Pharmacokinetic parameters of **6** and **7c** in rat

		Dose (mg/kg)	CL (ml/min/kg)	V_{dss} (L/kg)	$T_{1/2}$ (h)	B.A (%)
6	iv	1	52.7	4.2	—	
	po	10	—	—	1.30	37
7c	iv	1	51.5	14.4	—	
	po	10	—	—	4.69	69.3

of CYP enzymes inhibition, especially 2D6, **7c** and **8c** were clearly better than the other compounds (Table 3). As for metabolic stability in rats, **7c**[12] was selected as the most suitable for pharmacokinetic (PK) study in rat.

Compound **7c** showed longer half-life ($T_{1/2}$) than **6** with almost the same clearance (CL). This result is in good correlation with that of human serum protein binding (95.1% for **7c** and 71.1% for **6**), which is believed to be associated with the value of log P^{*} (1.17 for **7c** and 0.1 for **6**). Compound **7c** distribution volume (V_{dss}) increased, suggesting improved B.A., and its maximum concentration time (T_{max}) was observed at 3 h after administration. These findings indicate that **7c** possesses favorable oral B.A. and is therefore suitable for PK/PD evaluation in Zucker-Fatty (ZF) rat (Table 4).

To assess the potency of single oral administration of **7c** (0.1–1.0 mg/kg), the inhibitory activity of this compound for DPP-4 in ZF rats was evaluated over a time-course of 0–24 h (Fig. 3). Compound **7c** showed the strongest inhibition against DPP-4 at 1.0 mg/kg around 2 h after administration and remained active for up to 10 h. In addition, AUC of plasma DPP-4 inhibition by **7c** increased in a dose dependent manner. These results indicate that **7c** acts as a potent inhibitor in ZF rats.

In conclusion, we found that compounds having (*R*)-3-amino-3-methyl piperidine moiety, as represented here by compound **7c**, are potent DPP-4 inhibitors with good bioavailability and PK/PD profiles. Unfortunately, compound **7c** showed modest inhibition against hERG (IC_{50} = 6 μM), making further evaluation of this compound was suspended. To search for the more desirable compounds, additional optimization study of these series of compounds is currently in progress.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.101.

References and notes

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12. Analytical data of **7c** as a HCl salt: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.20 (3H, s), 1.48–1.61 (2H, m), 1.65–1.90 (2H, m), 2.35 (3H, s), 2.63–2.72 (1H, m), 2.75–2.82 (1H, m), 2.90 (1H, d, J = 11.8 Hz), 3.05 (1H, d, J = 12.1 Hz), 3.12 (3H, s), 3.39 (3H, s), 5.42 (2H, s), 5.93 (1H, dd, J = 10.2, 2.3 Hz), 6.06 (1H, s), 6.94 (1H, td, J = 8.4, 2.7 Hz), 7.22 (1H, dd, J = 8.4, 6.0 Hz), 8.19 (3H, br s). ^{13}C NMR (100 MHz, $CDCl_3$-d_3) δ: 18.7, 21.1, 23.0, 27.9, 31.7, 33.3, 45.8, 53.4, 54.1, 59.5, 83.9, 106.1, 112.0 (^{2}J(C, F) = 22.9 Hz), 113.3 (^{2}J(C, F) = 21 Hz), 130.3 (^{4}J(C, F) = 2.7 Hz), 131.5 (^{3}J(C, F) = 7.6 Hz), 135.6, 139.3 (^{3}J(C, F) = 6.5 Hz), 149.8, 151.38, 154.8, 161.6 (^{1}J(C, F) = 241 Hz). IR (ATR): 2942, 1685, 1635 cm^{-1}. HRMS (ESI+): m/z 414.2299 (calcd m/z 414.2300 for $C_{22}H_{28}FN_5O_2$ + H). Anal. Calcd for $C_{22}H_{28}FN_5O_2$ HCl 3/2H_2O: C, 55.40; H, 6.76; N, 14.68. Found: C, 55.53; H, 6.59; N, 14.59.