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Synthesis, biological evaluation and structural determination of β-aminoacyl-containing cyclic hydrazine derivatives as dipeptidyl peptidase IV (DPP-IV) inhibitors

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Abstract—Inhibitors of dipeptidyl peptidase IV (DPP-IV) have been shown to be effective treatments for type 2 diabetes. A series of β-aminoacyl-containing cyclic hydrazine derivatives were synthesized and evaluated as DPP-IV inhibitors. One member of this series, (*R*)-3-amino-1-(2-benzoyl-1,2-diazepan-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one (**10f**), showed potent in vitro activity, good selectivity and in vivo efficacy in mouse models. Also, the binding mode of compound **10f** was determined by X-ray crystallography.
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Glucagon-like peptide-1 (GLP-1)[1] is an incretin hormone secreted from the L cells of the small intestine in response to food intake. This hormone plays several biological roles including the stimulation of insulin secretion, inhibition of glucagon secretion, retardation of gastric emptying, induction of satiety and stimulating the regeneration and differentiation of islet β-cells.[2] However, GLP-1 (GLP-1[7-36]amide) is rapidly degraded in vivo (lifetime: about 1 min) through the action of dipeptidyl peptidase IV (DPP-IV), which cleaves a dipeptide from the N-terminus to give the inactive GLP[9-36]amide.[3]

DPP-IV is a serine protease cleaving the N-terminal dipeptide with a preference for L-proline or L-alanine at the penultimate position.[4] This protease is expressed in many tissues and body fluids, and exists as either a membrane-bound or a soluble enzyme. Inhibition of DPP-IV increases the level of circulating GLP-1 and thus increases insulin secretion,[5] which can ameliorate hyperglycaemia in type 2 diabetes. A number of small molecule inhibitors of DPP-IV have been described[6] and several of these, including Vildagliptin (LAF237),[7a] Saxagliptin (BMS477118)[7b] and Sitagliptin (MK-0431),[8] are in late-stage of clinical development or approved by the U.S. Food and Drug Administration (Fig. 1).

Since DPP-IV is a dipeptidase that selectively binds substrates with proline at the P1 position, many of its inhibitors investigated to date possess 5-membered heterocyclic rings (e.g., pyrrolidine, thiazolidine, cyano-pyrrolidine and cyano-thiazolidine) that serve as proline mimics. A number of DPP-IV inhibitors contain cyano-pyrrolidine ring.[6] We have recently described several novel 5-membered proline surrogates that are based on pyrazolidine[9,10] and pyrazoline.[11] Our compounds containing the pyrazolidine template showed moderate in vivo efficacy as well as in vitro activity against DPP-IV.[9a,10] A group at Merck recently described a series of structurally novel β-amino amide derivatives that have good in vitro potency and in vivo efficacy.[8] Among these, MK-0431, which is approved by FDA, shows excellent selectivity and in vivo efficacy. This

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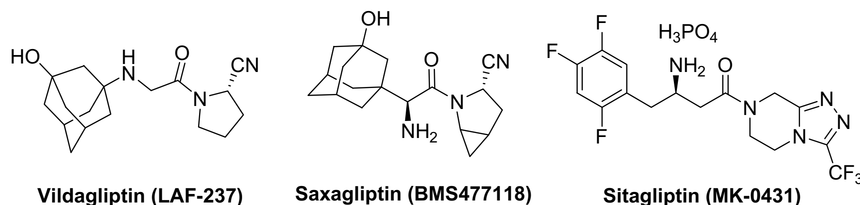


Figure 1. Molecular structures of representative DPP-IV inhibitors.

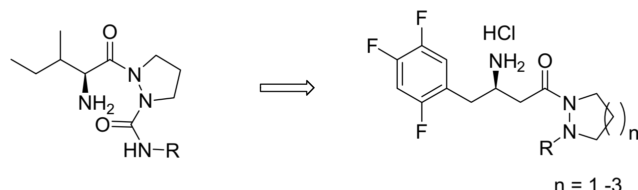
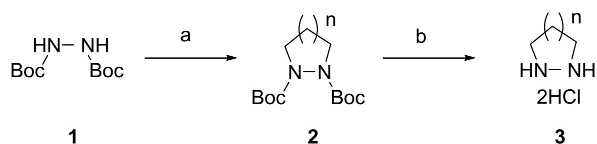


Figure 2. The design of new DPP-IV inhibitor, β-aminoacyl-containing cyclic hydrazine derivatives.

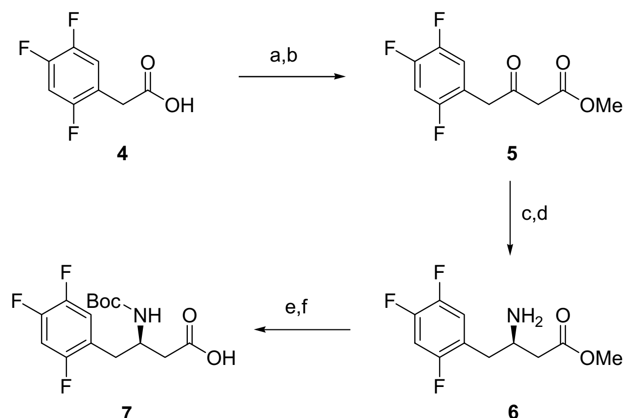
observation prompted us to explore the properties of new DPP-IV inhibitors that possess our basic pyrazolidine structure and contain appended β-amino acid moiety (Fig. 2, $n = 1$). In this effort, we also investigated related compounds that have 6- and 7-membered cyclic hydrazine core structures (Fig. 2, $n = 2$ or 3). We now wish to report the synthesis of β-aminoacyl-containing cyclic hydrazine derivatives and their biological evaluation as DPP-IV inhibitors (Fig. 2).

A series of β-aminoacyl-containing cyclic hydrazine derivatives were synthesized by using the route shown in Schemes 1–3. Cyclic hydrazines (**3**, $n = 1$–3), including pyrazolidine 2HCl ($n = 1$), were produced by employing a procedure we developed earlier (Scheme 1).[12] Di-*tert*-butyldihydrazodiformate (**1**) was treated with dibromoalkanes in the presence of Et_4NBr in toluene under reflux, followed by deprotection of Boc using 4 M HCl in dioxane to produce the cyclic hydrazine as their bis-HCl salts (**3**).

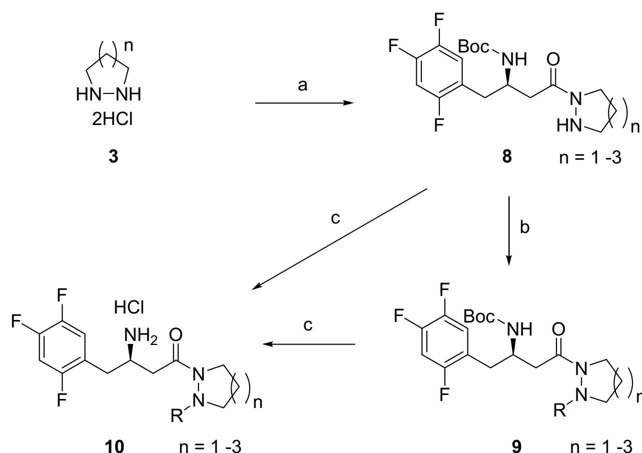
(*R*)-3-BocNH-4-(2,4,5-trifluorophenyl)butanoic acid (**7**)[8] was synthesized according to a modification of one described by the Merck group[13] (Scheme 2). 2-(2,4,5-Trifluorophenyl)acetic acid (**1**) was coupled with 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) using pivaloyl chloride as an activating reagent to produce the coupling product, followed by methanolysis to yield the β-keto ester (**5**). Reaction of **5** with ammonium acetates produced the corresponding β-enamino-ester, which was converted to the chiral β-(*R*)-amino ester (**6**) by asymmetric catalytic hydrogenation with the



Scheme 1. Reagents and conditions: (a) 50% NaOH, dibromopropane ($n = 1$), dibromobutane ($n = 2$), dibromopentane ($n = 3$), Et_4NBr, toluene, reflux, 6 h, 78–95%; (b) 4M HCl, dioxane, rt, 12 h, 89–98%.



Scheme 2. Reagents and conditions: (a) 2,2-dimethyl-1,3-dioxane-4,6-dione, DMAP, *N*,*N*-diisopropylethylamine, trimethylacetylchloride, acetonitrile, rt, 95%; (b) MeOH, toluene, reflux, 89%; (c) MeOH, ammonium acetate, reflux, 92%; (d) chloro(1,5-cyclooctadiene)rhodium (I) dimer, (*R*)-(−)-1-[(*S*)-2-(bis(4-trifluoromethylphenyl)phosphino) ferrocenyl ethyl-di-*tert*-butylphosphine, H_2 (100 psi), trifluoroethanol, 68%; (e) Boc_2O, CH_2Cl_2, rt, 93%; (f) LiOH, THF, MeOH, H_2O, rt, 99%.



Scheme 3. Reagents and conditions: (a) compound **7**, triethylamine, EDCI, CH_2Cl_2, rt, 47–96%; (b) electrophiles phenylisocyanate, *p*-toluenesulfonyl chloride, and benzoyl chloride, CH_2Cl_2, triethylamine, rt; (c) HCl, dioxane, rt, 75–90%.

chloro(1,5-cyclooctadiene)rhodium (I) dimer and (*R*)-(−)-1-[(*S*)-2-(bis(4-trifluoromethylphenyl)phosphino) ferrocenyl ethyl-di-*tert*-butylphosphine under H_2 (100 psi) in trifluoroethanol. Protection of the chiral β-(*R*)-amino ester (**6**) with Boc_2O, and hydrolysis with LiOH produced the desired (*R*)-3-BocNH-4-(2,4,5-trifluorophenyl)butanoic acid (**7**).

Table 1. Inhibitory activity of cyclichydrazine derivatives with β-aminoacyl group against DPP-IV

Compound	Structure	IC_{50}[a] (nM)
10a		3850
10b		990
10c		500
10d		350
10e		50
10f		70
MK-0431		50

[a] IC_{50} values were determined from direct regression curve analysis.

The synthesis of the β-aminoacyl cyclic hydrazine derivatives (**10**) was achieved by using the 3-step procedure shown in Scheme 3. The cyclic hydrazines (**3**, $n = 1$–3) were coupled with β-amino acid (**7**) in the presence of EDCI to produce the coupling product (**8**) which upon reaction with electrophiles yielded the corresponding *N*-substituted derivatives (**9**). Boc-deprotection with HCl transformed these substances into the desired β-aminoacyl-containing cyclic hydrazine derivatives (**10**).[14]

The β-aminoacyl-containing cyclic hydrazine derivatives (**10a–10f**) were evaluated in vitro for their inhibition of DPP-IV. MK-0431 was used as a reference compound. Unsubstituted pyrazolidine (R = H, **10a**) showed weak inhibitory activity (IC_{50} = 3.85 μM). However, *N*-substituted pyrazolidines (**10b–10d**) exhibited improved in vitro activities, among those pyrazolidines, benzoyl-substituted pyrazolidine (**10d**, IC_{50} = 350 nM) showed a nearly 10-fold more potency than that of the parent pyrazolidine (R = H, **10a**). Changing the pyrazolidine ring to the larger 6- and 7-membered cyclic hydrazines

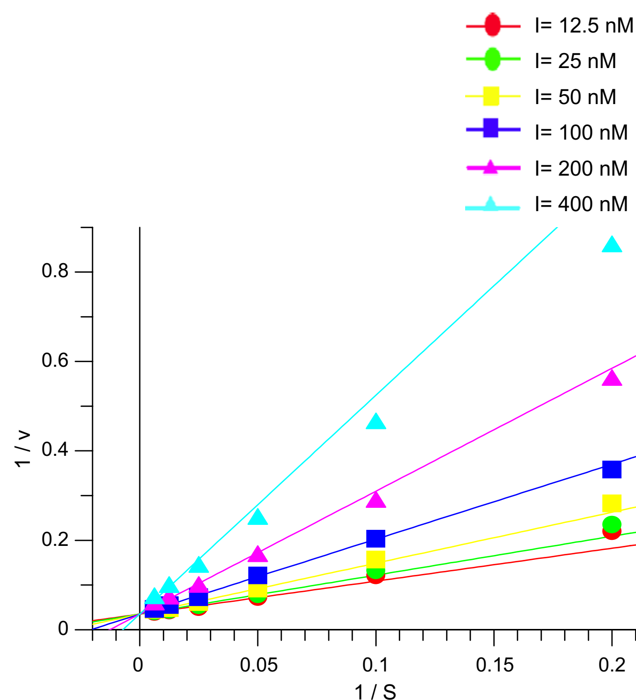


Figure 3. Inhibition kinetics of DPP-IV by **10f**. Different concentrations of **10f** were incubated in the presence of various concentrations of Ala-Pro-AFC. The initial rates of the reaction were measured, and the results were expressed as a Lineweaver–Burk plot. The data are represented as means of three separated experiments.

Table 2. Selectivity of Compounds **10e** and **10f** towards DPP-IV related enzymes

Compound	DPP-IV IC_{50}[a] (nM)	DPP-2 (nM)	DPP-8 (nM)	DPP-9 (nM)
10e	50	72,000	>100,000	>100,000
10f	70	18,190	89,120	48,660
	APN (nM)	POP (nM)	Trypsin (nM)	Elastase (nM)
10e	>100,000	>100,000	>100,000	>100,000
10f	>100,000	>1,000,000	>1,000,000	>100,000

[a] IC_{50} values were determined from direct regression curve analysis.

(**10e** and **10f**), found in **10e** and **10f**, resulted in improved inhibition. Compounds **10e** and **10f** were the most potent DPP-IV inhibitors (IC_{50} = 50 and 70 nM, respectively) in this series, showing 5–7 times greater activity than the 5-membered pyrazolidine (**10d**). Thus, we used those compounds (**10e** and **10f**) as representative compounds for further development (Table 1).

Compounds **10e** and **10f** were investigated for their selectivity towards a variety of DPP-IV related peptidases, including DPP-2, DPP-8, DPP-9, APN, POP, trypsin and elastase. Selectivity over DPP-8 and DPP-9 was considered particularly important because inhibition of these enzymes has been ascribed to toxicity in preclinical species.[15] The results of this effort (Table 2) showed that **10e** displayed a greater than 1400-fold selectivity over all the enzymes tested including DPP-8 and DPP-9. Also, compound **10f** showed good to excellent selectivity against most of the isozymes tested.

To identify the enzyme-inhibition kinetic patterns of compound **10f**, we carried out inhibition kinetic analyses with porcine kidney DPP-IV. As shown in Figure 3, compound **10f** showed a competitive inhibition pattern well fitted to a Lineweaver–Burk plot and had a K_i value of 56.2 nM. Similar experiments showed that **10e** is also a competitive inhibitor (K_i = 44 nM, data not shown).

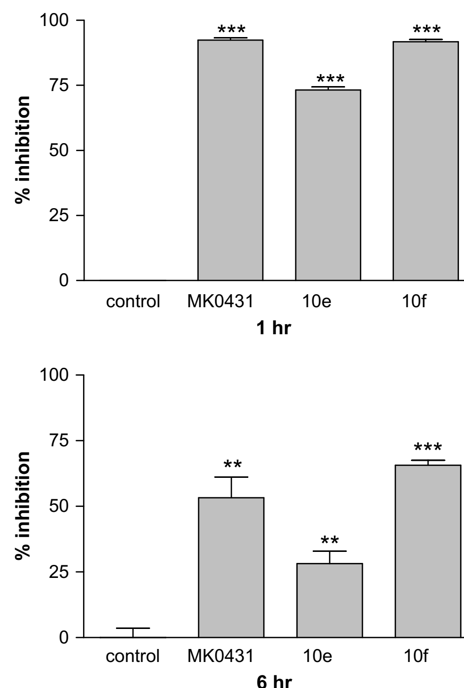


Figure 5. In vivo inhibitory effect of compounds **10e** and **10f** in C57BL/6J mice. Each compound was orally administered in a single dose of 50 mg/kg. The data are represented as means ± SEM (n = 7). $^{**}P < 0.01$ versus control; $^{***}P < 0.005$ versus control.

a — GLP-1 only

b — GLP-1 + DPP-IV

c — GLP-1+DPP-IV +10 M**10f**

d — GLP-1+DPP-IV+ 1 M**10f**

Figure 4. Effect of compound **10f** on GLP-1 degradation by DPP-IV in vitro. GLP-1 (15 μM) was incubated with porcine kidney DPP-IV (1 μU) in the presence of 10 or 1 μM **10f**.

The effect of compound **10f** on DPP-IV-mediated degradation of GLP-1 was probed in vitro by using GLP-1 peptide (NH_2-7-36) and DPP-IV. The extent of degradation of GLP-1 was analyzed by employing MALDI-TOF mass spectrometry. The results (Fig. 4) showed that compound **10f** blocks 85% and 46% DPP-IV-mediated degradation of GLP-1 over a 24 h period when used in concentrations of 10 and 1 μM, respectively.

Compounds **10e** and **10f** were evaluated in vivo for their ability to reduce DPP-IV activity in normal C57BL/6J mice (Fig. 5). Oral administration of compounds **10e** and **10f**, at high dose (50 mg/kg), resulted in ca 75% and 90% inhibition of plasma DPP-IV activity after 1 h, respectively. After 6 h, compound **10f** exhibited better and longer inhibition of DPP-IV activity than MK-0431 or **10e** (ca. 65% DPP-IV inhibition).

On the basis of its in vivo DPP-IV inhibition in normal mice, compound **10f** was subjected to in vivo evaluation by assessing its ability to improve glucose intolerance in TallyHo mice.[16] The results (Fig. 6) showed that glucose levels were significantly reduced in a dose-dependent manner from 10 mg/kg (52% reduction) to 50 mg/kg (77% reduction) in treated animals when **10f** was administered 30 min before an oral glucose challenge (2 g/kg).

The positive results arising from studies with **10f** stimulated an investigation aimed at determining the crystal structure of recombinant human DPP-IV, complexed with this inhibitor.

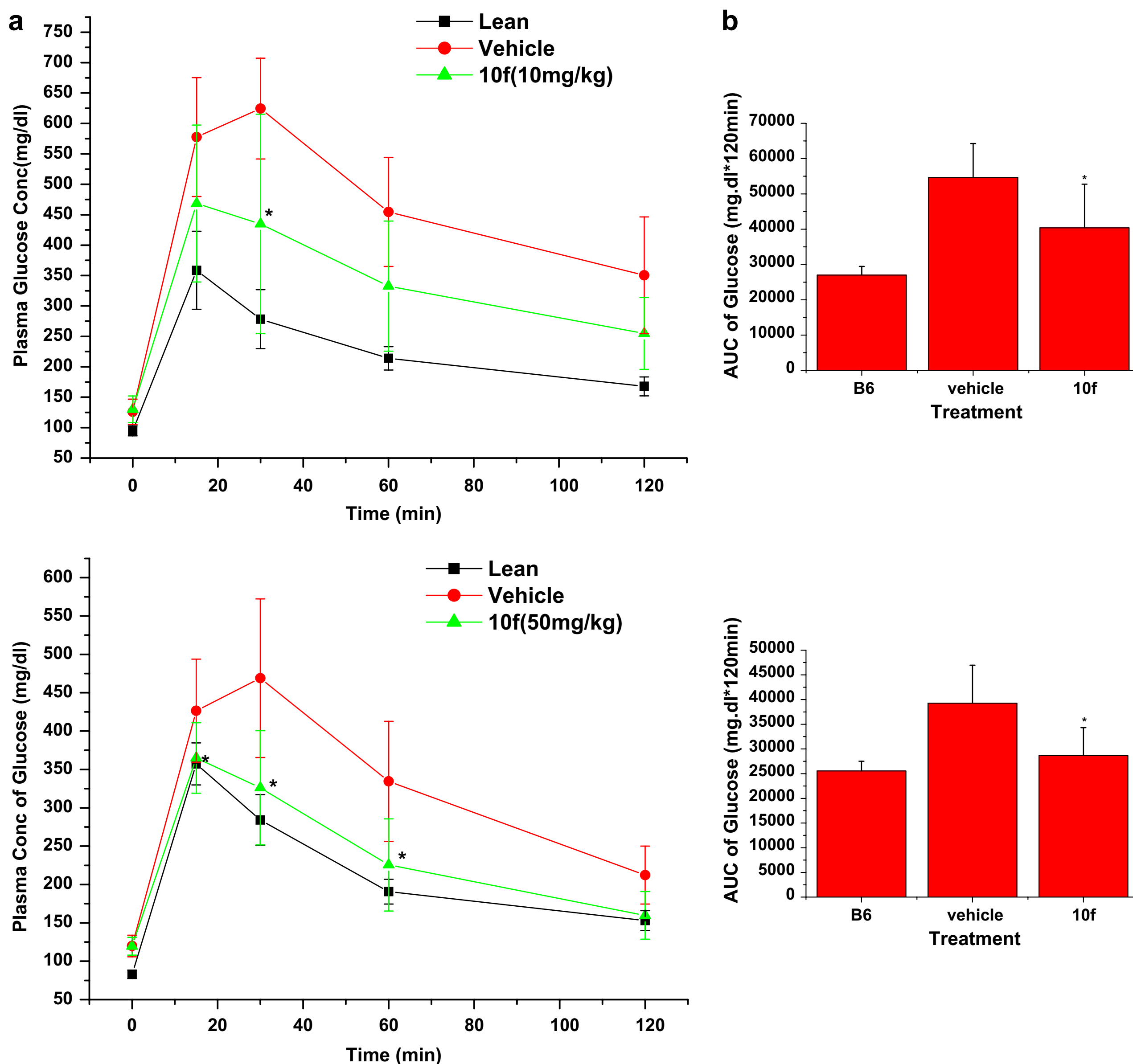


Figure 6. (a) Effects of compound **10f** on plasma glucose levels in oral glucose tolerance test in TallyHo mice. (b) Glucose AUC was determined from 0 to 120 min. The percent-inhibition values for each treatment were generated from the AUC data normalized to the 0.5% CMC (carboxyl methyl cellulose) challenged controls. The data are represented as mean ± SEM ($n = 7$). $^{*}P < 0.05$ versus vehicle in Student's t test.

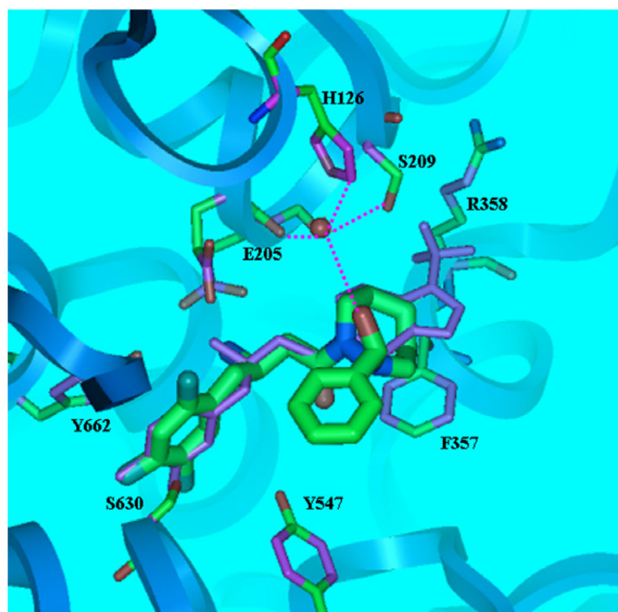


Figure 7. Compound **10f** bound to DPP-IV. The overlay of **10f** (green) and MK-0431 (purple) shows the different binding mode. Coordinate for the structure of DPP-IV in complex with **10f** has been deposited with the RCSB protein databank, PDB ID 2OLE.

As can be seen by viewing the binding site region in the complex (pictured in Fig. 7), the β-aminoacyl group has a similar conformation as that of MK-0431.[8] However, the cyclic hydrazine moiety in **10f** has different binding modes with the carbonyl oxygen of its benzoyl moiety forming water-bridged hydrogen bonding interactions with the side chains of His 126 and Ser 209 and the carbonyl group of Glu205.

In conclusion, this investigation has led to the design, synthesis and biological evaluation of a series of β-aminoacyl-containing cyclic hydrazine derivatives. In this series, **10e** and **10f** were found to be competitive inhibitors of DPP-IV and to display excellent selectivity over related isozymes. The in vivo efficacy of **10f** was demonstrated by its inhibition of plasma DPP-IV activity and its suppression of blood glucose elevation. Also, the binding mode of **10f** in the binding site of DPP-IV was determined by using X-ray crystallography. The cyclic hydrazine derivatives evaluated in this effort are members of an interesting chemical class that possess two nitrogens suitable for diverse derivatization. Further studies are underway to optimize this compound class for the treatment of diabetes.

Acknowledgments

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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.2007.01.111.

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14. Compound **10e**; A mixture of 3-(*R*)-*tert*-butoxycarbonylamino-4-(2,4,5-trifluorophenyl)butyric acid (2 g, 6 mmol), hexahydro pyridazine·2HCl (2.86 g, 18 mmol), EDCI (3.45 g, 18 mmol) and triethylamine (8.36 mL, 60 mmol) in CH_2Cl_2 (50 mL) was stirred for 12 h at room temperature. The reaction mixture was diluted with brine and CH_2Cl_2. The organic layer was dried and evaporated. The residue was purified by silica gel column chromatography to give [3-oxo-3-(tetrahydropyridazin-1-yl)-1-(2,4,5-trifluorobenzyl)propyl]carbamic acid *tert*-butyl ester (1.6 g, 66%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.11–7.02 (m, 1H), 6.92–6.83 (m, 1H), 5.53 (d, J = 8.5 Hz, 1H), 4.18–4.11 (m, 1H), 3.68–3.12 (m, 3H), 2.88–2.67 (m, 6H), 1.72–1.58 (m, 4H), 1.37 (s, 9H). To a mixture of (*R*)-[3-oxo-3-(tetrahydropyridazin-1-yl)-1-(2,4,5-trifluorobenzyl) propyl]carbamic acid *tert*-butyl ester (300 mg, 0.747 mmol) and Et_3N (208 μL, 1.495 mmol) in CH_2Cl_2 (10 mL) was added benzoyl chloride (158 mg, 1.121 mmol) and the mixture was stirred for 2 h at room temperature. The solvents were evaporated, and the residue was purified by silica gel column chromatography to give (*R*)-[3-(2-benzoyltetrahydropyridazin-1-yl)-3-oxo-1-(2,4,5-trifluorobenzyl)propyl] carbamic acid *tert*-butyl ester (375 mg, 99%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.61–7.40 (m, 5H), 7.11–7.02 (m, 1H), 6.92–6.83 (m, 1H), 5.30–5.50 (m, 1H), 4.70–4.50 (m, 1H), 4.13–3.95 (m, 2H), 3.10–2.40 (m, 5H), 1.90–1.54 (m, 4H), 1.37 (s, 9H); LC-MS *m*/*e* (relative intensity) 506 $(M+H)^+$. To a solution of (*R*)-[3-(2-benzoyltetrahydropyridazin-1-yl)-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamic acid *tert*-butyl ester (360 mg, 0.712 mmol) in EtOAc (2 mL) was added 4M-HCl/1,4-dioxane (2 mL) and the mixture was stirred for 12 h at room temperature. The solvents were evaporated, and the residue was crystallized with ether to give (*R*)-3-amino-1-(2-benzoyltetrahydropyridazin-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one HCl (290 mg, 92%). ^{1}H NMR (DMSO-d_3, 300 MHz) δ 8.16 (br s, 3H), 7.60–7.21 (m, 7H), 4.40–4.20 (m, 1H), 3.80–3.60 (m, 2H), 2.96–2.86 (m, 6H), 2.09–1.68 (m, 4H); HRMS (free base, $C_{21}H_{22}F_3N_3O_2$): calcd, 405.1664 found, 405.1638. Analytical HPLC method A: t_R = 3.46 min (99.1% purity); method B: t_R = 6.06 min (95.8% purity).
Compound **10f**; A mixture of 3-(*R*)-*tert*-butoxycarbonylamino-4-(2,4,5-trifluorophenyl)butyric acid (2 g, 6 mmol), [1,2]diazepan·2HCl (3.36 g, 18 mmol), EDCI (3.45 g, 18 mmol) and triethylamine (8.36 mL, 60 mmol) in CH_2Cl_2 (50 mL) was stirred for 12 h at room temperature. The reaction mixture was diluted with brine and CH_2Cl_2. The organic layer was dried and evaporated. The residue was purified by silica gel column chromatography to give [3-[1,2]diazepan-1-yl-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamic acid *tert*-butyl ester (2.2 g, 90%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.07–7.03 (m, 1H), 6.71–6.87 (m, 1H), 5.63 (d, J = 8.4 Hz, 1H), 4.15–4.13 (m, 1H), 3.64 (t, J = 5.0 Hz, 1H), 3.46 (t, J = 5.0 Hz, 1H), 3.93–3.89 (m, 4H), 2.74 (dd, J = 13.1, 5.4 Hz, 1H), 2.58 (dd, J = 19.8, 4.6 Hz, 1H), 1.71–1.56 (m, 6H), 1.36 (s, 9H). To a mixture of (*R*)-[3-[1,2]diazepan-1-yl-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamic acid *tert*-butyl ester (200 mg, 0.482 mmol) and Et_3N (97 mg, 0.962 mmol)in CH_2Cl_2 (10 mL) was added benzoyl chloride (101 mg, 0.722 mmol) and the mixture was stirred for 3 h at room temperature. The solvents were evaporated, and the residue was purified by silica gel column chromatography to give (*R*)-*tert*-butyl 4-(2-benzoyl-1,2-diazepan-1-yl)-4-oxo-1-(2,4,5-trifluorophenyl)butan-2- ylcarbamate (210 mg, 84%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.61–7.37 (m, 5H), 7.20–7.02 (m, 1H), 7.01–6.83 (m, 1H), 5.50-5.20 (m, 1H), 4.45–3.99 (m, 3H), 3.98–2.40 (m, 5H), 1.90–1.50 (m, 6H), 1.37 (s, 9H). To a solution of (*R*)-*tert*-butyl 4-(2-benzoyl-1,2-diazepan-1-yl)-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-ylcarbamate (220 mg, 0.423 mmol) in EtOAc (2 mL) was added 4M-HCl/1,4-dioxane (2 mL) and the mixture was stirred for 16 h at room temperature. The solvents were evaporated, and the residue was crystallized with ether to give (*R*)-3-amino-1-(2-benzoyl-1,2-diazepan-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one hydrochloride (143 mg, 75%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.41–8.20 (br s, 2H), 7.70–7.22 (m, 7H), 4.25–3.89 (m, 1H), 3.83–3.68 (m, 2H), 3.55–3.30 (m, 2H), 3.18–2.67 (m, 4H), 1.82–1.46 (m, 6H); HRMS (free base, $C_{22}H_{24}F_3N_3O_2$): calcd, 419.1821 found, 419.1837. Analytical HPLC method A: t_R = 3.17 min (99.9% purity); method B: t_R = 6.31 min (98.4% purity).

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